ESCV 2024

18-21 September 2024
Frankfurt/Main, Germany

26th Annual Conference of the European Society for Clinical Virology

FINAL PROGRAM AND ABSTRACT BOOK

www.escv2024.org

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18-21 September 2024
Frankfurt/Main, Germany

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WELCOME MESSAGE

ESCV 2024
18-21 September 2024
Frankfurt/Main, Germany

Welcome to 26th ESCV Conference

Dear Colleagues and Friends,

On behalf of the Conference Organizing Committee of the European Society for Clinical Virology it is our great pleasure to invite you all to the 26th Annual Meeting of the ESCV which will take place in Frankfurt/Main, Germany, 18-21 September 2024. The Conference will be dedicated to various aspects of clinical virology and comprise presentations, symposia, discussions, and various workshops. It will cover state of the art as well as the most exciting developments in clinical virology. Be ready to prepare and discuss your latest research with fellow colleagues and top experts in the field. This will be an outstanding opportunity especially for young researchers attending the ESCV for the first time. It will be also a great chance to meet friends, renew old acquaintances and build new networks.

Topics of the 26th ESCV Conference will be focused on latest discoveries and innovations in clinical virology and will include: respiratory viruses, gastrointestinal viruses, neurovirology, viruses, tumors and immunocompromised hosts, hepatitis and HIV, viral infections in pregnancy, emerging and re-emerging viruses, immune response and vaccines, development of new antiviral strategies, advancements in diagnostics & clinical cases, NGS and virus discovery, one health approach and patient & staff perspectives / health services research.

High scientific profile industry-sponsored workshops will round up the programme. Finally, most prestigious scientific awards such as the Heine-Medin, the Gardner Lecture and the Abbott Diagnostic award will be assigned. The Conference will be held at Westend Campus of Goethe University, Frankfurt. It is located in the heart of Frankfurt and the old buildings, and the parks are sites of special cultural interest. Here, outstanding facilities and versatile exhibition areas welcome every event with the perfect ambience and quick connections to the city public transportation network.

The city of Frankfurt itself is conveniently located in the heart of Europe. The city is one of the most important international business and financial hubs. It is home to the most famous skyline in Germany but also has a very attractive old town. Beyond its role as a financial capital, it boasts a broad range of exceptional cultural sites and museums like the Städel Museum and Schirn Kunsthalle. The Opera Frankfurt recently became “Opera of the year” for the seventh time. Frankfurt is easily accessible by countless national and international high-speed railway connections and Frankfurt Int’l Airport is easy to reach from all across the globe.

We look forward to welcoming you to ESCV 2024 in Frankfurt!

Sandra Ciesek & Marcus Panning
Conference Presidents

Pamela J. Vallely
ESCV President

COMMITTEES

PROF. PAMELA J. VALLELY
PRESIDENT

ASSOCIATE PROFESSOR MARIET C.W. FELTKAMP
PAST PRESIDENT

PROF. MARCUS PANNING
VICE PRESIDENT

PROF. MARIA SÃO JOSÉ ALEXANDRE
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DR. XAVIER LÓPEZ LABRADOR
COMMUNICATION OFFICER

PROF. GEORGE SOURVINOS
TREASURER

ASSOCIATE PROF. HELI J. HARVALA
MEETINGS SECRETARY

SCIENTIFIC COMMITTEE

- Pam Vallely
- Mariet Feltkamp
- Marcus Panning
- Maria Sao Jose Alexandre
- Catherine Moore
- Xavier Lopez
- George Sourvinos
- Heli Harvala
- Sandra Ciesek
- Holger Rabenau
- Annemarie Berger
- Niko Kohmer
- Benjamin Marx
- Tuna Toptan-Grabmair
- Katharina Grikscheit
- Marek Widera
- Denisa Bojkova
- Loraine Busetto
- Daniela Huzly

ABOUT ESCV

The European Society for Clinical Virology (ESCV) is a non-profit organization founded in 1997. Since then, it has provided a forum for virologists, scientists and physicians interested in all aspects of Clinical Virology and human viral diseases.

AIMS OF THE SOCIETY

The aims of the European Society for Clinical Virology are to promote knowledge of viruses, particularly as it relates to the clinical diagnosis and management of viral diseases, their pathogenesis, natural history, treatment and prevention.
In addition, the Society promotes viral epidemiological studies and good contacts with relevant organizations, such as the World Health Organization (WHO), other official organizations and other virological groups.

CONFERENCE DATES | DESTINATION | WEBSITE

18-21 September 2024 |
Frankfurt/Main, Germany | https://escv2024.org/

CONFERENCE HOURS

Registration Desk Opening Hours

September 17, 2024 – Tuesday (Only Pre-Conference Workshop)	| 08:30 – 15:00
September 18, 2024 – Wednesday	| 12:00 – 18:30
September 19, 2024 – Thursday	| 07:45 – 17:00
September 20, 2024 – Friday	| 07:45 – 17:00
September 21, 2024 – Saturday	| 08:30 – 12:00

Exhibition Opening Hours

September 18, 2024 – Wednesday (Until the end of the Welcome Reception)	| 13:00 – 19:00
September 19, 2024 – Thursday	| 08:30 – 19:00
September 20, 2024 – Friday	| 08:30 – 18:30
September 21, 2024 – Saturday	| 09:00 – 13:00

Language of the Conference

The official language of the ESCV 2024 Conference is English. No translations will be available during the sessions.

Dress Code for Conference Participants

The dress code for the ESCV 2024 Conference is business casual for all occasions unless stated otherwise.

CME ACCREDITATION

The 26th Annual Conference of the European Society for Clinical Virology has been accredited by the European Accreditation Council for Continuing Medical Education (EACCME®) with 18.5 European CME credits (ECMEC®s).

Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

A post-conference survey will be sent to all participants after the Annual Conference. In order to get your CME Credits, you will be required to fill in this survey and submit it.

INFORMATION FOR SPEAKERS & PRESENTERS

All invited speakers, oral abstract presenters and industry symposium speakers are kindly requested to upload their presentations in the Speakers' Ready Room at least 3 hours prior to the start time of their sessions. If the session is the first session in the morning, please upload your presentation the day before.

Speakers' Ready Room Opening Hours:

September 18, 2024 – Wednesday	| 12:00 – 18:00
September 19, 2024 – Thursday	| 08:00 - 17:30
September 20, 2024 – Friday	| 08:00 - 17:30
September 21, 2024 – Saturday	| 08:30 – 12:00

CONFERENCE MATERIALS FOR PARTICIPANTS

Name Badges

The name badges will be distributed from the Registration Area. All participants are kindly requested to wear their name badges throughout the Conference.

Conference Bags & Inclusions

The conference bags including the final program book, conference notepads, pens, invitations for industry symposia as well as inserts from industry partners will be distributed from the Registration Area.

Certificates of Attendance

The certificates of attendance will be sent to all participants as a PDF file after the Annual Conference via e-mail.

Certificates of Presentation

The presenters of oral and poster abstracts will be eligible to get a certificate of presentation which will be sent to the presenters after the Annual Conference via e-mail.

PRACTICAL DETAILS ABOUT FRANKFURT & GERMANY

Time Zone
Frankfurt, Germany operates Central European Time (CET) Zone during the conference period.

Electricity
In Frankfurt, Germany the power plugs and sockets are of type C and F.

Climate
In September, Frankfurt experiences mild and comfortable weather as the city transitions from summer to autumn. Average daytime temperatures range between 18 to 24 degrees Celsius (64 to 75 degrees Fahrenheit). The lingering warmth from summer creates pleasant conditions for outdoor activities, with cooler evenings warranting a light jacket or sweater.

Currency & Exchange
Germany, being a member of the Eurozone, utilizes the euro (€) as its official currency. The euro is available in various denominations, including coins (1 and 2 euros, as well as 1, 2, 5, 10, 20, and 50 cents) and banknotes (5, 10, 20, 50, 100, 200, and 500 euros).

Currency exchange is straightforward in Germany, and major cities have numerous banks, currency exchange offices, and ATMs where travelers can obtain euros. Credit and debit cards are widely accepted in most establishments, including restaurants, shops, and hotels. However, it's advisable to carry some cash for smaller purchases or in places that may not accept cards.

Foreign visitors should be aware that the exchange rates offered by banks and currency exchange offices may vary, and it's wise to compare rates to get the best deal. Additionally, many ATMs in Germany allow cash withdrawals using major international credit and debit cards, providing a convenient way for travelers to access euros.

DISCLAIMER

Liability & Insurance
The Conference Organising Secretariat and Organisers cannot accept liability for personal accidents or loss of or damage to private property of participants. Upon registration, participants agree that neither the ESCV, the Local Organising Committee nor the Conference Organising Secretariat assumes any liability. Participants are advised to take out their own personal travel and health insurance for their trip.

Safety and Security
Bags and/or suitcases must not be left unattended at any time whether inside or outside the Conference venue and session hall(s). As all hotels do, we strongly recommend our participants to use their safety deposit boxes for their valuables.

CONFERENCE ORGANISING SECRETARIAT

KENES GROUP

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children,
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University of Pittsburgh,
United States

Prof. Bert Niesters
University Medical Center
Groningen,
Netherlands

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UK Health Security
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United Kingdom

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Medical Unievrsity
Vienna,
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University of Torino
(Emeritus),
Italy

Prof. Harald Schwalbe
Goethe-University
Frankfurt,
Germany

ESCV
2024

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SCIENTIFIC PROGRAMME

SEPTEMBER 18, 2024 - WEDNESDAY

13:30 - 14:30 **SCIENTIFIC SESSION 1: Enteric viruses & Neurovirology**
Co-Chairs: Heli J. Harvala, Mario Hönemann

Keynote Lecture: **Enteroviruses - so much more than polio in the 21st century,** ***Thea Fischer,*** *Denmark*

Offered talk 1: One year of Europe-wide surveillance pilot for Enteroviral CNS infections, ***Caroline Klint Johannesen***, *Denmark*
Offered talk 2: Organotypic Intestinal Cultures as Promising Model for Personalized Antiviral Treatment in Immunocompromised Patients, ***Katja Wolthers***, *Netherlands*
Offered talk 3: Association between natural killer cell genetics and the development of Long COVID associated- and prepandemic small fiber neuropathy, ***Marianne Graninger***, *Austria*

14:30 - 15:30 **SCIENTIFIC SESSION 2: Pandemic preparedness and health services research**
Co-Chairs: Loraine Busetto, Jonas Schmidt-Chanasit

Keynote Lecture: **Pandemic preparedness and health services research**, ***Richard Pebody***, *UK*

Offered talk 4: Unlocking the full potential of wastewater-based epidemiology for pandemic preparedness: realistic quality assurance measures for the reliable detection of circulating and emerging viruses in wastewater, ***Marek Widera***, *Germany*
Offered talk 5: AI-Optimized vaccination strategies to mitigate smallpox outbreaks in urban settings: A case study of Frankfurt, ***Kevin Yeo***, *UK*
Offered talk 6: Wastewater Surveillance Aids Real-time Tracking of Community Transmission of SARS-CoV-2 and Influenza, ***Debasis Biswas***, *India*

15:30 - 16:00 **COFFEE BREAK**

16:00 - 17:30 **SCIENTIFIC SESSION 3: Respiratory viruses**
Co-Chairs: Catherine Moore, Denisa Bojkova

Keynote Lecture: **Emerging viruses - when public health, diagnostics and translational research meets**, ***Isabella Eckerle***, *Switzerland*

Offered talk 7: Characterization of RSV full-length viral genomes after Nirsevimab breakthrough infections in a large national observational real-world study conducted in France, ***Marie Anne Rameix Welti***, *France*
Offered talk 8: Age- and cell-type-specific metabolic programming shapes respiratory and systemic immune responses against the Influenza A virus, ***Maike Willers***, *Germany*
Offered talk 9: Characterization of antigenic relations of SARS-CoV-2 variants and population immunity using antigenic cartography, ***Janine Kimpel***, *Austria*
Offered talk 10: The role of RSV viral load and co-infection on disease severity in a very large cohort of paediatric patients, ***Rossana Scutari***, *Italy*
Offered talk 11: Coronaviruses impair resolution phase in monocytes via aberrant interferon signalling, ***Joshua D Kandler***, *Germany*

17:30 - 18:00 **Opening Ceremony**

18:00 - 19:00 **WELCOME RECEPTION**

SEPTEMBER 19, 2024 - THURSDAY

08:30 – 10:00 **SCIENTIFIC SESSION 4: Viromics and virus discovery**
Co-Chairs: Tuna Toptan-Grabmair, Jutte J.C. de Vries

Keynote Lecture: **Are there any more human cancer viruses left to be found? *Patrick Moore***, *US*

Offered talk 12: Multicenter benchmarking of short and long read wet lab protocols for clinical viral metagenomics, ***Xavier Lopez-Labrador***, *Spain*
Offered talk 13: Tracing the origin, spread and molecular evolution of Dengue type 1 cases occurred in Northern Italy in 2023, ***Greta Romano***, *Italy*
Offered talk 14: Metagenomic and transcriptomic investigation of paediatric acute liver failure cases, ***Jutte J.c. De Vries***, *Netherlands*
Offered talk 15: Evaluating metagenomics and targeted approaches for diagnosis and surveillance of viruses, ***Oscar Enrique Torres Montaguth***, *United Kingdom*
Offered talk 16: Detection of clinically relevant viral pathogens using antibody capture and metagenomic sequencing, ***Leonard Schuele***, *Netherlands*

10:00 – 10:30 **COFFEE BREAK**

10:30 – 12:00 **SCIENTIFIC SESSION 5: Viral infections in immunocompromised patients**
Co-Chairs: George Sourvinos, Holger Rabenau

Gardner Lecture: **Viruses, Cancer and Immunocompromised Hosts, *Hans Hirsch***, *Switzerland*

Offered talk 17: HCMV-specific T-cell response as a tool for predicting late HCMV reactivation in hematopoietic stem cell transplant recipients after letermovir prophylaxis,
Irene Cassaniti, *Italy*
Offered talk 18: Characterization of the Blood Virome in Liver Transplant Patients,
Alessandra Franze, *Spain*
Offered talk 19: Intraindividual evolution of noroviral sequences during protracted infections,
Dieter Hoffmann, *Germany*
Offered talk 20: Viral infections in Stem Cell Transplant recipients with posttransplant lymphoproliferative disorder: Torque Teno Virus as a marker of immune function,
Martyna Sylwia Pociupany, *Belgium*
Offered talk 21: Investigating nosocomial BK Polyomavirus infections in paediatric hematopoietic stem cell transplantation recipients: challenges and prospects,
Aurélien Aubry, *France*

12:00 – 13:30 **LUNCH BREAK**

12:30 - 13:30 **INDUSTRY SYMPOSIUM 1**

SEPTEMBER 19, 2024 - THURSDAY

13:30 – 15:00 **SCIENTIFIC SESSION 6: Advancements in diagnostics & Clinical cases**
Co-Chairs: Marcus Panning, Benjamin Marx

Keynote Lecture: **Innovative diagnostic approaches towards persistent viruses and associated diseases, *Elisabeth Puchhammer-Stöckl***, *Austria*

Offered talk 22: Transient biopsy-proven progressive multifocal leukoencephalopathy-immune reconstitution inflammatory syndrome in an elderly woman without known immunodeficiency, ***Christine Hanssen Rinaldo***, *Norway*
Offered talk 23: National-scale environmental and clinical surveillance of Poliovirus strains using novel quantitative PCR assays, ***Oran Erster***, *Israel*
Offered talk 24: Genomic studies of adeno-associated virus hepatitis,
Sarah Buddle, U*nited Kingdom*
Offered talk 25: Impact of inherited human herpesvirus 6 in transplantation,
Leo Hannolainen, *Finland*
Offered talk 26: A Novel Next Generation Sequencing Approach for Detecting Antiviral Resistance Mutations in Cytomegalovirus, ***Maria Arnedo Muñoz***, *Spain*

15:00 – 15:30 **COFFEE BREAK**

15:30 – 17:00 **SCIENTIFIC SESSION 7: Diagnostic stewardship and test accuracy**
Co-Chairs: Benjamin Pinsky, Niko Kohmer

Keynote Lecture: **Diagnostic Stewardship in Clinical Virology as Strategy!**
Hubert G.M. Niesters, *Netherlands*

Offered talk 27: Performance of a novel dual marker RT-qPCR- based whole-blood assay for the detection of SARS-CoV-2-reactive T cells, ***Franziska M. Kanis***, *Germany*
Offered talk 28: Are we using syndromic meningitis testing rationally? Five-year experience of a university hospital laboratory, ***Zeynep Ceren Karahan***, *Turkey*
Offered talk 29: Prospective comparison of cytomegalovirus quantification in whole blood and plasma samples among hematopoietic stem cell transplant and kidney transplant recipients,
Linda Feghoul, *France*
Offered talk 30: Dengue virus shedding kinetics in body fluids of infected patients, Rome, Italy, 2018-2023, ***Giulia Matusali***, *Italy*
Offered talk 31: Diagnosing acute Epstein-Barr Virus infections: do all roads lead to Rome?
Jos Van Acker, *Belgium*

17:00 - 18:00 **ENNGS Meeting**

17:00 - 19:00 **POSTER SESSION 1**

SEPTEMBER 20, 2024 - FRIDAY

08:30 – 10:00 **SCIENTIFIC SESSION 8: Development of new antiviral strategies**
Co-Chairs: Maria São José Alexandre, Sandra Ciesek

Keynote Lecture: **Development of antiviral inhibitors of low molecular weight targeting the frameshift element of SARS-CoV-2**, ***Harald Schwalbe***, *Germany*

Offered talk 32: Safety, tolerability and immunogenicity of PRV-101, a multivalent vaccine targeting coxsackie B viruses associated with type 1 diabetes: a double-blind randomised placebo-controlled Phase 1 trial, ***Heikki Hyoty***, *Finland*
Offered talk 33: Development and characterisation of a novel SARS-CoV-2 macrodomain (Mac1) targeting inhibitor, ***Susanne Pfefferle***, *Germany*
Offered talk 34: Prevention of BK virus DNAemia by IVIG administration in at-risk kidney transplant recipients, ***Morgane Solis***, *France*
Offered talk 35: Brincidofovir sensitivity testing of vaccinia virus containing potential resistance associated mutations, ***Kevin Yeo***, *US*
Offered talk 36: Emerging host-targets for anti-mpox therapy, ***Denisa Bojkova***, *Germany*

10:00 – 10:30 **COFFEE BREAK**

10:30 – 12:00 **SCIENTIFIC SESSION 9: Immune response and viral vaccines**
Co-Chairs: Mariet C.W. Feltkamp, Daniela Huzly

Keynote Lecture: **Human Papillomavirus immunisation and the path to cervical cancer elimination?** ***Kate Cuschieri***, *UK*

Offered talk 37: T-cell immunity to a conserved coronavirus epitope correlates with improved outcome of SARS-CoV-2 infection, ***David M. Florian***, *Austria*
Offered talk 38: IL-12 is a key responsive element to corticosteroid treatment in COVID-19, ***Benedikt Strunz***, *Sweden*
Offered talk 39: Specific long-term changes in anti-SARS-CoV-2 IgG modifications and antibody functions in mRNA, adenovector, and protein subunit vaccines, ***Sebastian Reinig***, *Taiwan*
Offered talk 40: Individual T-cell Cytokine patterns and B-cell reactions after HPV vaccination elucidated by multi-colour EliSpot, ***Rosemarie Preyer***, *Germany*
Offered talk 41: A Combined Cross-Sectional Analysis and Case-Control Study Evaluating Tick-Borne Encephalitis Vaccination Coverage, Disease and Vaccine Effectiveness in Children 0-17 in Switzerland, 2005-2022, ***Kyra Zens***, *Switzerland*

12:00 – 13:30 **LUNCH BREAK**

12:00 - 13:30 **POSTER SESSION 2**

12:15 - 13:15 **ENPEN Meeting**

SEPTEMBER 20, 2024 - FRIDAY

13:30 – 15:00 **SCIENTIFIC SESSION 10: Viral infections in pregnancy**
Co-Chairs: Pamela J. Vallely, Annemarie Berger

Keynote Lecture: **Cytomegalovirus infection screening in pregnancy how far are we?** ***Marianne Leurez-Ville***, *France*

Offered talk 42: Sequelae of congenital cytomegalovirus following maternal primary infections are limited to those acquired before 12 weeks, ***Jacques Fourgeaud***, *France*
Offered talk 43: Universal neonatal screening for congenital Cytomegalovirus, nine years after implementation and still going strong, ***Jos Van Acker***, *Belgium*
Offered talk 44: Exploring the urinary metabolomic fingerprint of human cytomegalovirus: a 1H-NMR study on congenitally infected newborns, ***Valentina Delloste***, *Italy*
Offered talk 45: Pichinde virus infection of pregnant guinea pigs as a comparative model of human Lassa virus infection during pregnancy, ***Hinh Ly***, *United States*
Offered talk 46: Valacyclovir in Pregnancy To Prevent and Treat Congenital Cytomegalovirus Infection: Results from a Large Tertiary Care Hospital in Rome, ***Rosaria Santangelo***, *Italy*

15:00 – 15:30 **COFFEE BREAK**

15:30 – 17:00 **SCIENTIFIC SESSION 11: Emerging and re-emerging viral infections & One health**
Co-Chairs: Elisabeth Puchhammer-Stöckl, Katharina Grikscheit

Keynote Lecture: **A virologist's lookback on COVID-19**, ***Christian Drosten***, *Germany*

Offered talk 47: High West Nile virus infection rates in mosquitoes from Berlin, 2023, ***Corinna Patzina Mehling***, *Germany*
Offered talk 48: A 10 year long-lived cellular and humoral MERS-CoV immunity cross-recognizing the Wild-type and Variants of SARS-CoV-2: a potential one-way MERS-CoV cross-protection towards a pan-coronavirus vaccine, ***Bandar Alosaimi***, *Saudi Arabia*
Offered talk 49: Seroprevalence and vaccination coverage of tick-borne encephalitis in Sweden, ***Bo Albinsson***, *Sweden*
Offered talk 50: Identifying gaps in population immunity in light of large measles outbreaks in Austria in 2023 and 2024, ***David Niklas Springer***, *Austria*
Offered talk 51: Comparison of neutralizing and binding antibodies levels against different orthopoxviruses in mpox patients and healthy vaccinated individuals, ***Concetta Castilletti***, *Italy*

17:00 - 17:30 **ESCV GENERAL ASSEMBLY**

19:30 – 23:00 **CONFERENCE NETWORKING DINNER**

SEPTEMBER 21, 2024 - SATURDAY

09:00 - 10:30 **SCIENTIFIC SESSION 12: Hepatitis viruses and HIV**
Co-Chairs: Xavier López Labrador, Marek Widera

Keynote Lecture: **Hepatitis D Virus infection; present status**, ***Mario Rizzetto***, *Italy*

Offered talk 52: HDV infection in the UK, ***Will Irving***, *United Kingdom*
Offered talk 53: HIV-2 infections in Southern France and genotypic assessment though genome next generation sequencing, ***Philippe Colson***, *France*
Offered talk 54: Heterogeneity in the diagnostic performances of HDV RNA quantification assays used in clinical practice in Italy: data from the first national quality control multicenter study, ***Romina Salpini***, *Italy*
Offered talk 55: Large Hepatitis E virus Genotype 3 outbreak in New Caledonia Island, ***Florence Abravanel***, *France*
Offered talk 56: Evaluation of a Sanger sequencing-based kit for genotyping drug resistance mutations in various HIV-1 subtypes using clinical and analytical samples, ***Ontlametse T Bareng***, *Austria*

10:30 - 11:00 **COFFEE BREAK**

11:00 - 12:40 **SCIENTIFIC SESSION 13: Prizes session**
Co-Chairs: Maria São José Alexandre, Pamela J. Vallely

Biomérieux Award Lecture: The good and the bad; update on polyoma- and anelloviruses, ***Mariet Feltkamp***, *Netherlands*
European Diagnostic Virology Award Lecture: Metagenomics for the diagnosis of infection, ***Julianne Brown***, *UK*
Heine -Medin Award Lecture: From Endemic to Pandemic: The promise and realization of real-time phylogenetics, ***Emma Hodcroft***, *Switzerland*
Offered talk 57: Genomic diversity of cytomegalovirus in congenital infections in France, ***Jacques Fourgeaud***, *France*

12:40 - 13:00 **CLOSING REMARKS & HANDOVER TO ESCV 2025**

Molecular Diagnostics: Harmonising Measurement for Patient Benefit

Workshop Date / Time:
September 17, 2024 / 10:00 – 17:00

Workshop Organizer(s):
Neil Almond & Jacqueline Fryer

Workshop Programme:

10:00 – 10:10	Welcome and introduction ***Neil Almond***

Session 1

10:10 – 10:30	Influenza - Challenges for Molecular Diagnostics ***Catherine Moore***
10:30 – 10:50	Calibration of Molecular Diagnostic Assays - Lessons learned from other pathogens ***Emma Bentley***
10:50 – 11:10	Molecular Detection of Respiratory Pathogens - Data from EQA ***Sanjiv Rughooputh & Martin Kammel***
11:10 – 11:30	A proposal for developing an International Standard calibrant for Influenza virus NAT assays ***Carmelina Vinci***
11:30 – 12:00	Discussion - What reference materials are required to assure the performance of influenza molecular diagnostics?
12:00 – 13:00	Lunch

Session 2

13:00 – 13:20	Next Generation Sequencing - The promises and the challenges for obtaining reproducible sequence data ***Jutte de Vries***
13:20 – 13:40	Next Generation sequencing Developing and Using reference materials ***Denise O'Sullivan***
13:40 – 14:00	EQA - what is the material; what does it tell us? ***Bert Niesters***
14:00 – 14:30	Discussion - Scientific framework for assuring the quality of NGS-based diagnostics
14:30 – 14:50	Coffee Break

Session 3

14:50 – 15:10	INSTAND EQA scheme for genome detection of West Nile virus: assessment of laboratory performance based on dPCR quantifications ***Martin Kammel***
15:10 – 15:30	Digital PCR and its role in harmonizing of qPCR calibrated assays for Hepatitis D Virus ***Esmeralda Valiente***
15:30 – 16:00	CMV - Production of WHO International Standard for CMV DNA and proposals for replacement ***Jacqueline Fryer***
16:00 – 16:50	Discussion - Prioritisation of programme for future standards development
16:50 – 17:00	Round up ***Neil Almond***
17:00	Close of workshop

Industry Symposium 1:

Viral Respiratory Surveillance Unveiled

Date & Time : Thursday, September 19, 2024 at 12:30 – 13:30
Hall : Main Hall
Moderator : Jelena Feenstra
Speakers : Respiratory Viruses – from Wastewater to Clinical Samples, research study from Leuven, Belgium, **Dr. Elke Wollants**
Evolve your respiratory testing to improve future patient care, **Dr. Gustav Ferdinand Quade**

WELCOME RECEPTION

Date | Time : Wednesday, 18 September 2024 | 18:00 – 19:30
Venue : Johann Wolfgang Goethe-Universität Frankfurt
Address : Campus Westend, Hörsaalzentrum
Theodor-W.-Adorno-Platz 5
60323 Frankfurt

The Welcome Reception – a get together & welcoming reception for the participants – will be held on the opening day of the Annual Conference, in the exhibition area at the conference venue.

All Annual Conference participants are cordially invited to attend the Welcome Reception.

CONFERENCE DINNER

Date | Time : Friday, 20 September 2024 | 19:30 – 23:30
Venue : Casino Building – Goethe-Universität Frankfurt
Address : Nina-Rubinstein-Weg 1, 60323 Frankfurt

The Conference Networking Dinner is a ticket event for participants of ESCV 2024.

ESCV
2024

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Frankfurt/Main, Germany

POSTERS

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-001	Poster Rotation 1	(Re-) emerging viral infections and one health	Correlation of circulating levels of free secreted dengue nonstructural 1 (sNS1) antigen with severity of disease in adult patients admitted with dengue	Arjun Bhugra	United Kingdom
PP-002	Poster Rotation 1	(Re-) emerging viral infections and one health	The 2024 Parvovirus B19 epidemic analyzed through blood donors in the Netherlands	Boris Michiel Hogema	Netherlands
PP-003	Poster Rotation 1	(Re-) emerging viral infections and one health	Re-emergence of measles - adults outbreak: experience of a Romanian tertiary hospital	Corneliu Petru Petru Popescu	Romania
PP-004	Poster Rotation 1	(Re-) emerging viral infections and one health	Dengue circulation in early 2024: are Italian autochthonous cases and Latin America epidemiology having an affect?	Davide Mileto	Italy
PP-005	Poster Rotation 1	(Re-) emerging viral infections and one health	Seroprevalence of Crimean-Congo hemorrhagic fever virus in small ruminants, northern Greece	Dimitrios Chatzidimitriou	Greece
PP-006	Poster Rotation 1	(Re-) emerging viral infections and one health	Six pediatric cases of entervirus EV-C105: experience of a hospital located in northeast Italy	Elena Pomari	Italy
PP-007	Poster Rotation 1	(Re-) emerging viral infections and one health	First two Oropouche fever cases diagnosed in Italy in epidemiologically non-related travellers from Cuba	Elena Pomari	Italy
PP-008	Poster Rotation 1	(Re-) emerging viral infections and one health	Detection of measles virus genotype D8 in wastewater of the Brussels capital region, Belgium, March 2024	Elke Wollants	Belgium
PP-009	Poster Rotation 1	(Re-) emerging viral infections and one health	(Re)Emerging respiratory infections for patients in a regional university hospital: towards combined molecular virological diagnosis with wastewater-based epidemiology	Evelyne Schvoerer	France
PP-010	Poster Rotation 1	(Re-) emerging viral infections and one health	Analysis of the monophyletic lineage of avian Influenza H5N1 which circulated in Venezuelan birds during the 2022-2023 outbreak	Flor Helene Pujol	Venezuela
PP-011	Poster Rotation 1	(Re-) emerging viral infections and one health	The Origin of SARS-CoV-2 and its Furin Cleavage Site, a Parsimonious 5-Stage Evolutionary Model	Jonathan Latham	United States
PP-012	Poster Rotation 1	(Re-) emerging viral infections and one health	Post-Pandemic Measles Re-Emergence in a High Vaccinated Country – Portugal 2024	Paula Palminha	Portugal
PP-013	Poster Rotation 1	Advancements in diagnostics & clinical cases	Whole genome sequencing of RNA viruses on clinical samples using RNA-seq technology	Catherine Gaudy Graffin	France
PP-014	Poster Rotation 1	Advancements in diagnostics & clinical cases	A case of non conventional clinical presentation of Parvovirus B19 infection in a healthy baby	Cristina Russo	Italy
PP-015	Poster Rotation 1	Advancements in diagnostics & clinical cases	Comparison of Cytomegalovirus (CMV) Polymerase Chain Reaction (PCR) Test Results Run on Fully Automated Systems: Qiagen NeuMoDx Experience with Roche Cobas 6800	Dilek Colak	Turkey
PP-016	Poster Rotation 1	Advancements in diagnostics & clinical cases	"Optimizing Diagnostic Workflows: Machine Learning-Aided Result Interpretation	Dominik Danso	Germany
PP-017	Poster Rotation 1	Advancements in diagnostics & clinical cases	TTVGuideIT: validation of and quantitative use of a PCR assay across 13 different sites	Edmund J Gore	Netherlands
PP-018	Poster Rotation 1	Advancements in diagnostics & clinical cases	Identification of the new herpes simplex virus 2 variant in three patients suffering from recurrent infections unresponsive to antiviral therapy	Hanna Helena Schalkwijk	Belgium
PP-019	Poster Rotation 1	Advancements in diagnostics & clinical cases	Integrating Hepatitis C virus whole-genome sequencing into clinical diagnostics using Oxford Nanopore Technology	Hayley Joanne Cassidy	Netherlands
PP-020	Poster Rotation 1	Advancements in diagnostics & clinical cases	Enhancing mutation analysis with minMutFinder: a versatile solution for accurate viral population characterisation	Ignasi Prats Méndez	Spain
PP-021	Poster Rotation 1	Advancements in diagnostics & clinical cases	High-throughput DNA sequencing of human cytomegalovirus genomes in formalin-fixed paraffin-embedded tissues	Kathy K Li	United Kingdom
PP-022	Poster Rotation 1	Advancements in diagnostics & clinical cases	Enterovirus RNA testing in cerebrospinal fluid and respiratory samples: consideration of feasibility across different age groups	Maja M. Lunar	Slovenia
PP-023	Poster Rotation 1	Advancements in diagnostics & clinical cases	Is the quantification of CMV DNA in patients on letermovir prophylaxis affected by the presence of lysed cellular material in plasma samples?	Maja M. Lunar	Slovenia
PP-024	Poster Rotation 1	Advancements in diagnostics & clinical cases	Detection of Acyclovir-Resistant Herpes Simplex Virus-1 Without Prior Long-term Acyclovir Treatment	Sharon Isralls	United Kingdom
PP-025	Poster Rotation 1	Development of new antiviral strategies	Evaluation of Antiviral Efficacy and Validation of EN 14476 Standard Test for Virucidal Activity	Ana Maria Tiplea	United Kingdom
PP-026	Poster Rotation 1	Development of new antiviral strategies	New antiviral therapies against paramyxoviruses: Boosting innate immunity by delivery of defective interfering RNA and targeting of adenosine deaminase acting on RNA 1	Christian Karl Pfaller	United States
PP-027	Poster Rotation 1	Development of new antiviral strategies	Shedding light on the mechanism of action of an antiviral compound against Zika virus	Erika Plicanti	Italy
PP-028	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	No evidence for ONNV or MAYV infections in returning travelers diagnosed in Belgium, from 2006-2023: a retrospective study	Alexandra Vujkovic	Belgium
PP-029	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of multiplex reference control preparations to support the performance monitoring of rapid meningitis and encephalitis nucleic acid based diagnostic testing	Alistair Ritchie	United Kingdom
PP-030	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of Two Fully Automated PCR Assays for the Quantification of CMV and EBV DNA in Whole Blood	Anne Le Bouter	France
PP-031	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	First fully-automated human Adenovirus qPCR reported in IU/ml is accurate and reliable for timely monitoring of HAdV DNAemia in transplant recipients	Auet Asfaha	United Kingdom

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-032	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Proof of high stability and reproducibility of quantitative PCR results of HBV, HCV and HIV positive samples and the relevance for look back testing in hemovigilance	Benjamin Marx	Germany
PP-033	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Performance evaluation of a cytomegalovirus and an Epstein-Barr virus DNA real-time PCR assay in whole blood specimens on a fully automated platform	Birgit Reinhardt	Germany
PP-034	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	CMV IgG seroconverts two days earlier than CMV IgM on a commercial platform	Håkon Kinck Borén	Norway
PP-035	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Clinical performance evaluation of a CE-IVD diagnostic test for detection of Parvovirus B19 in patient plasma	Jelena Feenstra	Austria
PP-036	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of a multiplex real-time PCR-based CE-IVD test clinical performance for detection and differentiation of JC and BK viruses in urine samples	Jelena Feenstra	Austria
PP-037	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Comparisons of two syndromic multiplex and three miniplex panel tests for the detection of viruses causing upper respiratory tract infections	Katharina Grikscheit	Germany
PP-038	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of T cell responses detected by the Elecsys IGRA SARS-CoV-2 assay	Nina Germic	Switzerland
PP-039	Poster Rotation 1	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of the QIAstat-Dx meningitis-encephalitis syndromic test kit in a university hospital	Arzu Sayıner	Turkey
PP-040	Poster Rotation 1	Enteric viruses & Neurovirology	Enterovirus in children with acute respiratory infection (2022-2023)	Arzu Sayıner	Turkey
PP-041	Poster Rotation 1	Enteric viruses & Neurovirology	Detection of pegivirus and norovirus by viral metagenomics in suspected adult central nervous system infections of unknown etiology, Spain, 2023	Ana Donoso	Spain
PP-042	Poster Rotation 1	Enteric viruses & Neurovirology	Human Herpesvirus Type 6 (HHV6) as a Causal Agent of Encephalitis in Immunocompetent Patients: Case Study from 2019-2023	Devi Carolina Salas Olortegui	Spain
PP-043	Poster Rotation 1	Enteric viruses & Neurovirology	Epstein-Barr virus can infect rabbits and induce brain pathology similar to what has been observed in patients with multiple sclerosis	Gulfaraz Khan	United Arab Emirates
PP-044	Poster Rotation 1	Enteric viruses & Neurovirology	Molecular characterization of Enteroviruses in patients with acute respiratory infection in the Central African Republic	Joël Doté	Central African Republic
PP-045	Poster Rotation 1	Enteric viruses & Neurovirology	Molecular surveillance of enterovirus A71 in Spain after 2016 outbreak (2017-2023)	María Cabrerizo	Spain
PP-046	Poster Rotation 1	General virology	Host potential genetic risk factors related to Inflammatory and Degenerative Diseases of the CNS in ZIKV and CHIKV infected patients	Andreza Lemos Salvio	Brazil
PP-047	Poster Rotation 1	General virology	The Dynamic Gain-Loss Network of SARS-CoV-2 Spike Amino Acid Changes During Virus Evolution – United Kingdom, Jan. 2020-Aug. 2023	Cleo Anastassopoulou	Greece
PP-048	Poster Rotation 1	General virology	Frankfurt Virus Platform – acquisition & distribution of newest respiratory virus isolates and tools to enable high quality research	Denisa Bojkova	Germany
PP-049	Poster Rotation 1	General virology	A large outbreak of acute hemorrhagic conjunctivitis in Fujian, China in 2023	Dongmei Yan	China
PP-050	Poster Rotation 1	General virology	Deciphering Genetic Modulators of Peripheral Nervous System Involvement in Arbovirus Infections	Elielson Veloso Da Silva	Brazil
PP-051	Poster Rotation 1	General virology	HCMV encoded miRNAs in acute HIV infection and during one year of follow-up	Elisabetta Lazzari	Italy
PP-052	Poster Rotation 1	General virology	Circulation of enterovirus types in Europe between January 2018 and August 2023: a retrospective multi-center surveillance study	Emiel Vanhulle	Netherlands
PP-053	Poster Rotation 1	General virology	Respiratory viruses during the weeks 04-18/2024 in Northern Greece	Enada Leshi	Greece
PP-054	Poster Rotation 1	General virology	AAV2 is a common finding in adenovirus infections in children and adults	Gerbrig Berger	Netherlands
PP-055	Poster Rotation 1	General virology	An outbreak investigation of Hand Foot and Mouth Disease in North India	Gursimran Kaur Mohi	India
PP-056	Poster Rotation 1	General virology	Virus like particles (VLP) and ultrastructural cell morphology in human cervical carcinoma cells lines: (SiHa - HPV/16 and HeLa - HPV/18)	Rachel Siqueira De Queiroz Simões	Brazil
PP-057	Poster Rotation 1	General virology	Genotyping of high-risk human papillomavirus associated with predictor factors to cervical cancer in unvaccinated sexually active women	Rachel Siqueira De Queiroz Simões	Brazil
PP-058	Poster Rotation 1	(Re-) emerging viral infections and one health	Assessing Four Molecular Assays for Robust MPX Virus DNA Detection	Henar Alonso Ezcurra	Spain
PP-059	Poster Rotation 1	General virology	Bloody Herpesviruses – How the permanence after primary infection complicates molecular diagnosis	Henar Alonso Ezcurra	Spain
PP-060	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Revolutionizing Hepatitis B Detection: Cutting-edge Molecular Assay for Precise Virus Load Measurement in EDTA-plasma	Henar Alonso Ezcurra	Spain
PP-061	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Expanding Horizons: Dual-Purpose qPCR for Qualitative and Quantitative Detection of Hepatitis B Virus	Henar Alonso Ezcurra	Spain
PP-062	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Understanding why modelling studies by the Center for Disease Analysis Foundation fail to offer reliable estimates of Hepatitis C prevalence in Switzerland and Germany	Barbara Bertisch	Switzerland

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-063	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	HIV Detection and Viral Load Measurement in Cerebral Spinal Fluid (CSF) Using the Abbott Alinity M	Catherine Moore	United Kingdom
PP-064	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Microbiological infections in people living with HIV and relationship with CD4 cell count in the era of antiretroviral therapy	Chiara Nonne	Italy
PP-065	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Human leucocyte G expression after BKV infection	Deema Fallatah	Saudi Arabia
PP-066	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Evaluation of the diagnostic performance of two fully automated hepatitis E virus antibody test: the Ghent experience	Elizaveta Padalko	Belgium
PP-067	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Analysis of the nationwide HIV screening test data performed at Public Health Centers in Korea (2011-2022)	Gayeong Kim	South Korea
PP-068	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	The survey about implementing the national 2019 HIV diagnosis algorithm	Gülden Çelik	Turkey
PP-069	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Three-Year Analysis Of HIV Diagnostic Data	Gulden Celik(yilmaz)	Turkey
PP-070	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Reliable and sensitive quantification of plasma HDV RNA: A European multicenter study	Harald H Kessler	Austria
PP-071	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	A peculiar HBsAg isoforms composition characterizes chronic HDV coinfection respect to HBV mono-infection, with higher middle-/large-HBs levels paralleling HDV activity	Alessia Magnapera	Italy
PP-072	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	IgG avidity analysis reveals patterns of Hepatitis E virus reinfection in viremic patients	Amal Chaghouri	France
PP-073	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Chemiluminescent immunoassay for diagnosis of acute HEV infections	Ingrid Albrecht Walz	Germany
PP-074	Poster Rotation 1	Hepatitis, HIV and other blood borne viruses	Viral load measurements with results below detection limit in HBV samples with high or low Hepatitis B surface antigen titres	Robert Ehret	Germany
PP-075	Poster Rotation 1	Advancements in diagnostics & clinical cases	CMV specific T-cell activity determines the risk of clinically significant CMV reactivation following letermovir prophylaxis after allo-HSCT	Inga Tometten	Germany
PP-076	Poster Rotation 1	Immune response and vaccines	Comparison of immune responses to SARS-CoV-2 spike after Omicron infection and Omicron BA.4/5 vaccination in kidney transplant recipients	Inga Tometten	Germany
PP-077	Poster Rotation 1	Immune response and vaccines	Neutralization of emerging SARS-CoV-2 XBB and JN.1 lineages is improved by repeated Omicron exposures	David Niklas Springer	Austria
PP-078	Poster Rotation 1	Immune response and vaccines	Assessment of Antibody Responses in Patients With MERS-CoV Infection and SARS-CoV-2 Vaccination	Hadi M Yassine	Qatar
PP-079	Poster Rotation 1	Immune response and vaccines	Impact of obesity on the immune response to Measles-Mumps-Rubella vaccination in children	Aoife Murphy	Ireland
PP-080	Poster Rotation 1	Immune response and vaccines	Assessing a multivalent vaccine containing VP8* proteins from different rotavirus genotypes in mice	Javier Buesa	Spain
PP-081	Poster Rotation 1	Immune response and vaccines	Difference in sero-prevalence of CVA EV-C types in children from South Sudan and the Netherlands	Karen Couderé	Belgium
PP-082	Poster Rotation 1	Immune response and vaccines	High-risk HPV but not HPV16/18 are frequently detected in a French cohort of vaccinated women	Sandy Gross	France
PP-083	Poster Rotation 1	Immune response and vaccines	Humoral and T cell-mediated immune responses to the mRNA COVID-19 vaccine in lung and liver transplant recipients	Sara Uceda Renteria	Italy
PP-084	Poster Rotation 1	Immune response and vaccines	Antibody detection in the context of vaccination against tick-borne encephalitis virus using a newly designed ELISA	Susann Hohensee	Germany
PP-085	Poster Rotation 1	Pandemic preparedness and health services research	Automatization of CDC Influenza H5 assays into commercial integrated platforms: a strategic approach to pandemic preparedness	Chrishanthi Lowe	Germany
PP-086	Poster Rotation 1	Pandemic preparedness and health services research	Successful implementation of large-scale regular health checkups in a university in Japan during the COVID-19 pandemic	Hiroshi Kamano	Japan
PP-087	Poster Rotation 1	Pandemic preparedness and health services research	Transmission of SARS-CoV-2 among health care workers and patients in a hospital ward outbreak revealed by deep sequencing	Julia Widstrom	Sweden
PP-088	Poster Rotation 1	Respiratory viruses	Respiratory syncytial virus genetic diversity and lineage changes in Ireland pre- and post-COVID-19 pandemic	Alan M Rice	Ireland
PP-089	Poster Rotation 1	Respiratory viruses	FluReD: a new bioinformatic tool for enhancing reassortment and zoonosis detection in influenza surveillance	Alejandra González Sánchez	Spain
PP-090	Poster Rotation 1	Respiratory viruses	An updated molecular protocol to determine community prevalence of influenza C virus during the 2023/2024 influenza season in Wales	Anastasia Katrin Couzens	United Kingdom
PP-091	Poster Rotation 1	Respiratory viruses	Overview of Adenovirus respiratory infections: preliminary findings of a multicentre study in Italy, 2022-2023	Antonio Piralla	Italy
PP-092	Poster Rotation 1	Respiratory viruses	Comparative Analysis of Flu, COVID-19, and RSV Infections in Pediatric Admissions	Artur Sulik	Poland
PP-093	Poster Rotation 1	Respiratory viruses	Detection of causes of respiratory viral infections in animals in Kazakhstan in 2023-2024	Assem Baimukhametova	Kazakhstan
PP-094	Poster Rotation 1	Respiratory viruses	Severe influenza during the 2023/24 SARS-CoV-2 pandemic season. Restoration of the Influenza infections dynamics	Beatriz Mengual Chuliá	Spain

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PP-095	Poster Rotation 1	Respiratory viruses	Decrease in Severe SARS-CoV-2 cases in 2023-24 season, Valencia Hospital Surveillance Network for the Study of Influenza and Other Respiratory Viruses (VAHNSI), Spain	Beatriz Mengual Chuliá	Spain
PP-096	Poster Rotation 1	Respiratory viruses	Clinical Impact of Current Variants in COVID-19 Patients	Bedia Dinç	Turkey
PP-097	Poster Rotation 1	Respiratory viruses	Sequence Analysis of RSV Positive Samples in the Pediatric Patient Group at Ankara Bilkent City Hospital Following the COVID-19 Pandemic	Bedia Dinç	Turkey
PP-098	Poster Rotation 1	Respiratory viruses	Impact of the RSV passive immunisation campaign in infectivity and variant circulation	Carlos Davina Nunez	Spain
PP-099	Poster Rotation 1	Respiratory viruses	Impact of genetic diversity of influenza A in the effectiveness of the vaccine	Carlos Davina Nunez	Spain
PP-100	Poster Rotation 1	Respiratory viruses	Validation of a lab-developed Influenza-A HA typing multiplex-PCR assay for detection of H1pdm09, H3 and H5 on a high-throughput system	Dominik Sebastian Nörz	Germany
PP-101	Poster Rotation 1	Respiratory viruses	Detection and differentiation of influenza A/B virus, respiratory syncytial virus, and SARS-CoV-2 during 2021-2023, as part of integrated laboratory surveillance in Bosnia and Herzegovina	Edina Zahirović	Bosnia and Herzegovina
PP-102	Poster Rotation 1	Respiratory viruses	National surveillance for the epidemiology of respiratory viruses in Korean children before and after the COVID-19 pandemic	Eunhwa Choi	South Korea
PP-103	Poster Rotation 1	Respiratory viruses	Five-year (2017-2022) evolutionary dynamics of human coronavirus HKU1 in southern France with emergence of viruses harboring the spike H512R substitution	Hikmat Houmadi	France
PP-104	Poster Rotation 1	Respiratory viruses	Etiologic spectrum and clinical characteristics of respiratory viral infections and co-infections in Bulgaria during the first two seasons of the post-COVID-19 era	Ivelina Trifonova	Bulgaria
PP-105	Poster Rotation 1	Respiratory viruses	Development and Characterization of an Aerodynamic System for Pulmonary Delivery of Influenza Vaccine	Saurabh Bhargava	India
PP-106	Poster Rotation 1	Respiratory viruses	PlexPlus® mass multiplex assay detects 14 viral respiratory targets in a single well	Simon Bone	United Kingdom
PP-107	Poster Rotation 1	Respiratory viruses	Investigation of respiratory viruses, Greece, 2021-2024	Styliani Pappa	Greece
PP-108	Poster Rotation 1	Respiratory viruses	Validation of facial tissues as an alternative to nasopharyngeal swabs to diagnose viral respiratory infection	Vincent Thibault	France
PP-109	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Is it time to change the guidelines for preventing HSV infections in liver transplantation?	Vincent Thibault	France
PP-110	Poster Rotation 1	Viral infections in pregnancy	A cross-sectional survey of Congenital Cytomegalovirus Infection Awareness among Medical Faculty Students	Aylin Erman Daloglu	Turkey
PP-111	Poster Rotation 1	Viral infections in pregnancy	Viral load considerations in relation to sample type and age of a cytomegalovirus-infected group of patients	Irma Salimović Bešić	Bosnia and Herzegovina
PP-112	Poster Rotation 1	Viral infections in pregnancy	Increased incidence of Parvovirus B19 in Barcelona: comparative analysis from 2019 to 2024	Marta Bernat-Sole	Spain
PP-113	Poster Rotation 1	Viromics and virus discovery	Impact of micro-organisms RNA extraction from respiratory samples on microbiome analysis using Nanopore NGS technology	Alice Michel	France
PP-114	Poster Rotation 1	Viromics and virus discovery	Rapid development of drug-resistant associated mutations in herpes simplex viruses type 1 after single passage in the presence of Acyclovir	Lena Jaki	Germany
PP-115	Poster Rotation 1	Viromics and virus discovery	Evaluation of measles whole genome sequencing protocols and metagenomic pipelines using Nanopore and Illumina sequencing	Meik Dilcher	New Zealand
PP-116	Poster Rotation 1	Viromics and virus discovery	Discovering viruses in diverse settings: a public health perspective	Neta S Zuckerman	Israel
PP-117	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	A case report: Cytomegalovirus(CMV) meningoencephalitis in a patient receiving immunosuppressive therapy for treatment of malignant glioma	Elif Çalışkan	Turkey
PP-118	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Cytomegalovirus infection and emergence of resistance during letermovir salvage therapy in a pediatric patient with severe combined immunodeficiency	Fien Horsten	Belgium
PP-119	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Predictive value of EBV viremia for lymphoma diagnostic in kidney transplant patients?	Honorine Fenaux	France
PP-120	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Occurrence and frequency of Human Herpesviruses (HHV-1 to 6) in broncheoalveolar lavage fluid (BALF) from various kind of patients	Karin Rottengatter	Germany
PP-121	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Workflow and result comparison of alpha herpesvirus detection in cerebrospinal fluid (CSF) samples	Karin Rottengatter	Germany
PP-122	Poster Rotation 1	Viruses, tumors and immunocompromised hosts	Frequency of CMV and of co-infection of CMV and {P. jirovecii} in bronchoalveolar lavage fluid (BALF) samples	Karin Rottengatter	Germany
PP-123	Poster Rotation 2	(Re-) emerging viral infections and one health	Monitoring of the circulation Hepatitis A virus in France: tools and challenges	Honorine Fenaux	France
PP-124	Poster Rotation 2	(Re-) emerging viral infections and one health	Performance evaluation of a multiplex RT-PCR assay for detection and differentiation of Dengue, Zika and Chikungunya viral RNA in serum samples	Jelena Feenstra	Austria

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PP-125	Poster Rotation 2	(Re-) emerging viral infections and one health	One Health paradigm for Surveillance of Climate Sensitive Vector Borne Diseases in Yadadri Bhuvanagiri District Telangana, India	Lakshmi Jyothi Tadi	India
PP-126	Poster Rotation 2	(Re-) emerging viral infections and one health	Magnitude and Determinants of Zoonotic Enteric Disease in Under Five Children of Rural Telangana	Lakshmi Jyothi Tadi	India
PP-127	Poster Rotation 2	(Re-) emerging viral infections and one health	Measles whole genome phylogenetic analysis in the ongoing outbreak in Romania	Leontina Banica	Romania
PP-128	Poster Rotation 2	(Re-) emerging viral infections and one health	A rational approach for the selection of samples for testing for rat hepatitis E virus (Rocahepevirus) in a large virus diagnostics laboratory	Lisa Jasmin Mueller	Germany
PP-129	Poster Rotation 2	(Re-) emerging viral infections and one health	FlaviSeq: a combined RT-PCR and Nanopore sequencing approach for the rapid detection and determination of human flavivirus infections	Marcel Jonges	Netherlands
PP-130	Poster Rotation 2	(Re-) emerging viral infections and one health	Establishment of a hepatitis E virus genotype 3 (HEV-3)-specific target enrichment panel resolves complete genomes but still requires additional sequencing of the hypervariable region (HVR) to enable correct determination	Mathias Schemmerer	Germany
PP-131	Poster Rotation 2	(Re-) emerging viral infections and one health	Optimization of a whole genome amplicon sequencing protocol for Clade I Monkeypox Virus	Pedro Henrique	Belgium
PP-132	Poster Rotation 2	(Re-) emerging viral infections and one health	{Torque Teno Virus} (TTV) and {Cytomegalovirus} (CMV) Viral Load Dynamics: Which Emerges First During Immunosuppression?	Piergiorgio Roberto	Italy
PP-133	Poster Rotation 2	(Re-) emerging viral infections and one health	Emergence of B1c Genotype of Coxsackievirus A16 in Heilongjiang Province of China in 2022	Qian Yang	China
PP-134	Poster Rotation 2	(Re-) emerging viral infections and one health	Tick-Borne Encephalitis infection among patients with neurological acute and sub-acute disorders living in Saint-Etienne area in France between 2021 to 2023	Sylvie Pillet	France
PP-135	Poster Rotation 2	Advancements in diagnostics & clinical cases	EBV DNA detection in plasma of COVID 19 patients	Marija Kaevska	Czech Republic
PP-136	Poster Rotation 2	Advancements in diagnostics & clinical cases	Evaluating RT-LAMP Versus RT-PCR and WGS for Co-Detection of SARS-CoV-2 and Influenza in Respiratory Specimens	Ramyatha Battula	Germany
PP-137	Poster Rotation 2	Advancements in diagnostics & clinical cases	Human Papillomavirus Genotypes in Self-Sampling Women in Portugal	Raquel Rocha	Portugal
PP-138	Poster Rotation 2	Advancements in diagnostics & clinical cases	Multimodal Analysis of ADEM-Like Presentation as first clinic episode of Highly Active Multiple Sclerosis Following COVID-19 Vaccination	Renan Amphilophio Fernandes	Brazil
PP-139	Poster Rotation 2	Advancements in diagnostics & clinical cases	Simplified Respiratory Screening: Self-Collected Nasal and Saliva Samples with Flocked Swab	Simone Paghera	Italy
PP-140	Poster Rotation 2	Advancements in diagnostics & clinical cases	An unusual presentation of Seoul virus infection triggering hemophagocytic lymphohistiocytosis (HLH)	Styliani Pappa	Greece
PP-141	Poster Rotation 2	Advancements in diagnostics & clinical cases	Detection of immune response to Rubella antigen using an anti-Rubella IgM ELISA and a newly designed anti-Rubella IgG ELISA	Susann Hohensee	Germany
PP-142	Poster Rotation 2	Advancements in diagnostics & clinical cases	X-ray irradiation of SARS-CoV-2 Omicron BA.4 and comparison of the sensitivity of live and irradiated virus in a lateral flow test assay format	Teresa Ramalho	United Kingdom
PP-143	Poster Rotation 2	Advancements in diagnostics & clinical cases	Detection of antiviral antibodies in urine – noninvasive alternative for serodiagnostics	Visa Nurmi	Finland
PP-144	Poster Rotation 2	Advancements in diagnostics & clinical cases	Automating SARS-CoV-2 CPE Detection with Deep Learning to Simplify Post-Isolation Processes	Zeynep Akkutay Yoldar	Turkey
PP-145	Poster Rotation 2	Advancements in diagnostics & clinical cases	The biomolecular markers for the personalized prediction of the treatment effect in patients with head and neck cancer: pilot study	Zuzana Vojtěchová	Czech Republic
PP-146	Poster Rotation 2	Development of new antiviral strategies	RSV Treatment Beyond Direct Antivirals: Exploring the Host Cells for Potential Targets for Therapeutic Intervention	Fionn Daire Keogh	Germany
PP-147	Poster Rotation 2	Development of new antiviral strategies	The effectiveness of Marecipe AV therapeutics, an herbal compound prescription, for life-threatening viral infections in animals	Jiangnan Feng	China
PP-148	Poster Rotation 2	Development of new antiviral strategies	Identification of a small-molecule Inhibitor of KSHV Lytic Replication	Michael O. Okpara	South Africa
PP-149	Poster Rotation 2	Development of new antiviral strategies	Membrane Traps: towards a general, drug-free approach to inactivate viruses by engulfment	Nicola De Franceschi	Poland
PP-150	Poster Rotation 2	Development of new antiviral strategies	Identifying compounds to target host dependency factors of SARS-CoV-2	Rainer Koenig	Germany
PP-151	Poster Rotation 2	Development of new antiviral strategies	Cellular receptor tyrosine kinase signaling plays an important role in coronavirus infection	Shania Sanchez	United States
PP-152	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	In Silico Analysis: Key to Accurate RNA Virus Detection	Henar Alonso Ezcurra	Spain
PP-153	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Validating a Novel Molecular Assay for Precise Epstein-Barr Virus Load Quantification in EDTA-Plasma	Henar Alonso Ezcurra	Spain
PP-154	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Validation of cobas 6800 human papillomavirus test in comparison to the cobas HPV test on cobas 4800 system	Kun Lee Lim	Singapore
PP-155	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Analytical performance evaluation of Alinity-m quantitative PCR assays for HBV DNA, CMV DNA, HCV RNA, HIV RNA and correlation analysis with Cobas-6800	Muhammed Alper Özarslan	Turkey
PP-156	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	HSV 1 & 2 / VZV – a new qualitative multi-plex PCR test on the Alinity m platform	Robert Ehret	Germany
PP-157	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Viral load measurement in HIV-1 samples with highly mutated Integrases	Robert Ehret	Germany

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-158	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Evaluation of the performance of the NeuMoDx MPXV Test	Robert Ehret	Germany
PP-159	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Diagnosing viral gastro-enteritis using the fully automated sample-in, result-out STARlet All in One system (AIOS)	Roel Nijhuis	Netherlands
PP-160	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Lack of amplification of SARS-CoV-2 E-gene in a widely used real-time PCR assay	Roel Nijhuis	Netherlands
PP-161	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Rational use of meningitis/encephalitis (M/E) panel combined with clinical diagnostic criteria	Sidre Erganis	Turkey
PP-162	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Diagnosing acute Epstein-Barr Virus infections: This is the Way	Thomas Strypens	Belgium
PP-163	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Comparative evaluation of six commercialized RT-PCR Kits for measles virus detection	Tiffanie Belveyre	France
PP-164	Poster Rotation 2	Diagnostic stewardship & Diagnostic test accuracy	Does specific IgM immunoblot testing improve diagnosis of acute viral infection?	Vincent Thibault	France
PP-165	Poster Rotation 2	Enteric viruses & Neurovirology	Retrospective Evaluation of Five-Year Test Results of Meningitis/Encephalitis Panel	Bedia Dinç	Turkey
PP-166	Poster Rotation 2	Enteric viruses & Neurovirology	Etiology and diagnostic methods in viral acute CNS infections - 2 years in a Romanian tertiary hospital	Corneliu Petru Petru Popescu	Romania
PP-167	Poster Rotation 2	Enteric viruses & Neurovirology	Exploring non-A rotavirus infections in Spain: Are there age-specific trends?	Javier Buesa	Spain
PP-168	Poster Rotation 2	Enteric viruses & Neurovirology	Enterovirus 71 Infection: Five Years in Review (2019-2023)	Paula Palminha	Portugal
PP-169	Poster Rotation 2	Enteric viruses & Neurovirology	Infection study of human rotavirus on human intestinal enteroids	Sonja Jacobsen	Germany
PP-170	Poster Rotation 2	Enteric viruses & Neurovirology	Influence of COVID-19 Lockdown on Rotavirus Genotype Dynamics in Slovenia	Tina Mikuletič	Slovenia
PP-171	Poster Rotation 2	Enteric viruses & Neurovirology	Human parechovirus detection in a tertiary hospital over seven years: epidemiology and clinical features	Yoleidys Martínez Ysasis	Spain
PP-172	Poster Rotation 2	Enteric viruses & Neurovirology	Viral aetiology in acute gastroenteritis across all age groups in Ireland: an eight-year observational study	Zoe Yandle	Ireland
PP-173	Poster Rotation 2	General virology	Differential distribution of varicella-zoster virus (VZV) clades among patients: A retrospective study from two French tertiary care hospitals	José Fernandez	France
PP-174	Poster Rotation 2	General virology	Abortive infection fate of lumpy skin disease virus in white blood cells	Manoj Kumar	Israel
PP-175	Poster Rotation 2	General virology	Virological patterns in different parvovirus B19 related clinical situations	Nicolas Veyrenche	France
PP-176	Poster Rotation 2	General virology	Molecular detection and identification of human enteroviruses and parechoviruses in children and adults admitted to a university hospital	Nikolaos Siafakas	Greece
PP-177	Poster Rotation 2	General virology	Oral Combination vaccine against Hepatitis B & Influenza: Development and Characterization	Saurabh Bhargava	India
PP-178	Poster Rotation 2	General virology	Early Life Dynamics of Torque-teno Virus Infection: Insights from the PreventADALL Mother-Child Cohort Study	Sigve Aadalen	Norway
PP-179	Poster Rotation 2	General virology	Distribution of HPV Genotypes in Turkey and Factors Affecting Epidemiological Variation	Yeşim Tuyji Tok	Turkey
PP-180	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Retrospective evaluation of HIV diagnostic algorithm as a single center	Arzu Sayıner	Turkey
PP-181	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	HCV screening in vulnerable population	Carlos Davina Nunez	Spain
PP-182	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Characteristics of recent HIV infections and associated risk factors among newly diagnosed individuals in Korea (2016-2022)	Jin Sook Wang	South Korea
PP-183	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	IVDR-compliant establishment and validation of a laboratory-developed RT-qPCR for detection of Rocahepevirus ratti (ratHEV) using a fully automated workflow	Lisa Sophie Pflüger	Germany
PP-184	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	HBV cryptic activity is frequently revealed in anti-HBc positive/HBs negative HIV patients despite HBV-active therapy and can threaten HIV suppression	Lorenzo Piermatteo	Italy
PP-185	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Molecular diagnosis of hepatitis delta virus infections in Marseille university hospitals	Philippe Colson	France
PP-186	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	An evaluation of emerging Rat Hepatitis E Virus's risk to blood safety in England	Piya Rajendra	United Kingdom
PP-187	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Single-center evaluation of HDV-RNA quantification assays in a large cohort of Bulevertide-treated and untreated Chronic Hepatitis Delta patients	Sara Uceda Renteria	Italy
PP-188	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	First molecular characterization and phylogenetic analysis of goat hepatitis E virus in Portugal	Sérgio Santos Silva	Portugal
PP-189	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Contribution of HHV-6 Real-Time PCR in Hair Follicles and Nail Clippings for the Diagnosis of Chromosomally Integrated HHV-6 (ciHHV-6)	Sílvia Lopo	Portugal
PP-190	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	HTLV seroprevalence in blood donors from Romania – a 15 years longitudinal study	Simona Ruta	Romania

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-191	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	HBs isoforms can play a role in predicting virological response in patients with chronic hepatitis delta under bulevirtide monotherapy	Stefano Danna	Italy
PP-192	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Evaluation of the ACCESS anti-HBs Assay on the DxI 9000 ACCESS Immunoassay Analyzer, as an aid in the diagnosis of and determination of susceptibility to hepatitis B virus (HBV) infection	Stephanie Bord	France
PP-193	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Low rate of hepatitis Delta virus (HDV) coinfection among Dutch candidate blood donors with chronic hepatitis B virus (HBV) infection	Thijs J Van De Laar	Netherlands
PP-194	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Blood-based biomarkers for screening of Herpes Simplex Virus and HIV co-infections in outbreak-associated cases of Human Hepatitis Viruses	Rachel Siqueira De Queiroz Simões	Brazil
PP-195	Poster Rotation 2	Hepatitis, HIV and other blood borne viruses	Verification of a commercial anti-hepatitis E virus IgG and IgM immunoassay	Veerle Matheeussen	Belgium
PP-196	Poster Rotation 2	Immune response and vaccines	Novel candidate peptide vaccine against HCV infection	Rachel Siqueira De Queiroz Simões	Brazil
PP-197	Poster Rotation 2	Immune response and vaccines	Production of rabbit specific antibodies against conserved and potentially immunogenic peptides of the HCV envelope glycoprotein E2	Rachel Siqueira De Queiroz Simões	Brazil
PP-198	Poster Rotation 2	Immune response and vaccines	The investigation of immunogenic activity of the adenovirus vector	Anastasiya V Kokliushkina	Russia
PP-199	Poster Rotation 2	Immune response and vaccines	Anti-Dengue Virus-Specific IgM and IgG Antibodies in Acute and Convalescent Serum Samples from Patients with Confirmed Dengue disease, Jazan region, The South West of Saudi Arabia	Khalid Jebril Shrwani	Saudi Arabia
PP-200	Poster Rotation 2	Immune response and vaccines	Is the high-dose influenza vaccine better for everyone over 60 years of age?	Laura Colombo	Italy
PP-201	Poster Rotation 2	Immune response and vaccines	Economic benefits of a workplace vaccination program against influenza: a case study series for local public transportation, air- and railways companies across Europe	Laura Colombo	Italy
PP-202	Poster Rotation 2	Immune response and vaccines	Antigen specific VZV reactive T-cells –Effect of the antigen type depending on the clinical question	Rosemarie Preyer	Germany
PP-203	Poster Rotation 2	Immune response and vaccines	Adapting LIBRA-seq/BEAM to whole BK polyomavirus particles to identify broadly-specific human monoclonal antibodies	Sarah Marchand	France
PP-204	Poster Rotation 2	Immune response and vaccines	Baseline gene expression differentiates the T cell response kinetics to BNT162b2 vaccination	Vinaya Venkat	Finland
PP-205	Poster Rotation 2	Pandemic preparedness and health services research	Exploration of the SARS-CoV-2 pandemic from the perspective of nursing home staff in Hesse, Germany	Loraine Busetto	Germany
PP-206	Poster Rotation 2	Pandemic preparedness and health services research	How Venezuela faced the identification of variants and genomic surveillance of SARS-CoV-2	Rossana Celeste Jaspe González	Venezuela
PP-207	Poster Rotation 2	Pandemic preparedness and health services research	Monkeypox virus detection in Bulgaria	Stefka Krumova	Bulgaria
PP-208	Poster Rotation 2	Respiratory viruses	Common respiratory viruses dynamics during the last 5 years period: How has it changed?	Elif Çalışkan	Turkey
PP-209	Poster Rotation 2	Respiratory viruses	Phylogenetic analysis of influenza B virus strains circulating in northern Greece in the 2023-2024 season	Enada Leshi	Greece
PP-210	Poster Rotation 2	Respiratory viruses	RSVTyper: A multiplex tiling approach for amplification, sequencing and downstream analysis of Respiratory Syncytial Virus	Inga Tometten	Germany
PP-211	Poster Rotation 2	Respiratory viruses	Genomic biodiversity of SARS-CoV-2 strains identified during COVID-19 pandemic: single-center study, Bosnia and Herzegovina	Irma Salimović Bešić	Bosnia and Herzegovina
PP-212	Poster Rotation 2	Respiratory viruses	Surveillance insights of human Parechovirus (HPeV) respiratory infections among infants in Barcelona (2021-2022)	Jana Navarro Freixas	Spain
PP-213	Poster Rotation 2	Respiratory viruses	Monitoring the impact of SARS-CoV-2 variants on diagnostic cycle threshold values of target genes	Jaythoon Hassan	Ireland
PP-214	Poster Rotation 2	Respiratory viruses	Environmental surveillance of elevator floors detects influenza clinical trends	Jj Miranda	United States
PP-215	Poster Rotation 2	Respiratory viruses	Human Respiratory Syncytial virus (hRSV) and codetected viruses: characterisation and clinical related data from infected children	Julia Dina	France
PP-216	Poster Rotation 2	Respiratory viruses	Genetic characterization of adenoviruses in pediatric population, Greece, 2022-2023	Katerina Tsioka	Greece
PP-217	Poster Rotation 2	Respiratory viruses	Prevalence of respiratory viruses before and during the COVID pandemic both in primary care and in a hospital setting	Katherine Loens	Belgium
PP-218	Poster Rotation 2	Respiratory viruses	Rapid identification of Influenza and Respiratory Syncitial viruses with molecular Point-of-Care testing: impact on pediatric population management	Laura Squarzon	Italy
PP-219	Poster Rotation 2	Respiratory viruses	Genetic diversity of human Rhinovirus circulating in Portugal during 2023/2024 season	Licínia Gomes	Portugal
PP-220	Poster Rotation 2	Respiratory viruses	Investigation of SARS-CoV-2 IgG binding capability to variants of the SARS-CoV-2 virus	Lucy Johnson	Ireland
PP-221	Poster Rotation 2	Respiratory viruses	Genomic insights into respiratory syncytial virus during 2022-2024 in Catalonia's SIVIC program: focusing on neutralizing monoclonal antibodies	Maria Piñana	Spain

Poster Number	Poster Rotation	Topic	Title	Presenter	Country
PP-222	Poster Rotation 2	Respiratory viruses	Respiratory syncytial virus in adult patients at a tertiary care hospital in Germany: clinical features and molecular epidemiology of the fusion protein in the severe respiratory season of 2022/20	Mario Hönemann	Germany
PP-223	Poster Rotation 2	Respiratory viruses	Study of viral respiratory infections in an {in vitro} model of polarized primary human airway epithelial cells from children and adults	Mathilde Bellon	Switzerland
PP-224	Poster Rotation 2	Respiratory viruses	Virological characteristics of the SARS-CoV-2 XBB variants	Matthias Denkewitz	Germany
PP-225	Poster Rotation 2	Respiratory viruses	Low prevalence of nirsevimab resistance associated mutations (NRAMs) during acute RSV infection in children and immunocompromised patients in a tertiary hospital in Amsterdam, The Netherlands during the 2023/2024 respiratory season	Matthijs Rudolf Albert Welkers	Netherlands
PP-226	Poster Rotation 2	Respiratory viruses	Antiviral Susceptibility in Influenza virus isolates: Genotypic and phenotypic assessment during the 2023-2024 season in Catalonia	Narcís Saubi	Spain
PP-227	Poster Rotation 2	Respiratory viruses	The clinical burden of rhinoviruses compared to influenza and respiratory syncytial virus in an 8-year period at the National Influenza Centre in Slovenia	Natasa Berginc	Slovenia
PP-228	Poster Rotation 2	Respiratory viruses	Molecular Characterisation and Seasonal Circulation of Human Metapneumovirus from 2022-2024 in the North-Eastern Region of Slovenia	Nika Volmajer	Slovenia
PP-229	Poster Rotation 2	Respiratory viruses	To assess the role of community respiratory viruses in patients with bacteria confirmed hospital pneumonia	Peter Valentine Coyle	Qatar
PP-230	Poster Rotation 2	Respiratory viruses	Wastewater-based surveillance of respiratory viruses in Belgium: integration of RSV and influenza to SARS-CoV-2	Raphael Janssens	Belgium
PP-231	Poster Rotation 2	Respiratory viruses	Molecular epidemiology of respiratory syncytial virus in autumn-winter 2023-24 in Rome, Italy	Roberta Campagna	Italy
PP-232	Poster Rotation 2	Respiratory viruses	Elucidation of the conformational dynamics of SARS-CoV-2 Spike-protein through MD simulation	Snehal Balaso Bhongale	India
PP-233	Poster Rotation 2	Respiratory viruses	Enterovirus D68 Detection in Respiratory Specimens: Retrospective Study Over A Five-Month Period from July to November 2023	Ursula Morley	Ireland
PP-234	Poster Rotation 2	Respiratory viruses	Antibody avidity 10 weeks post BNT162b2 vaccination and risk of subsequent breakthrough infection: A nested case-control-study within the Shieldvacc vaccine trial	Wegene Borena	Austria
PP-235	Poster Rotation 2	Viral infections in pregnancy	Increased incidence of Parvovirus B19 infections in pregnant women in Germany, 2023/2024	Martin Enders	Germany
PP-236	Poster Rotation 2	Viral infections in pregnancy	Congenital CMV diagnosis using saliva and urine: A study in Region Zealand, Denmark	Rikke Lykke Johansen	Denmark
PP-237	Poster Rotation 2	Viral infections in pregnancy	Investigating host factors implicated in vertical transmission of SARS-CoV-2	Sulagna Mandal	India
PP-238	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	Surveillance of Cytomegalovirus viral load in Living Donor Liver Transplant (LDLT) recipients and its correlation with pre and post-transplant Immunological markers	Arjun Bhugra	United Kingdom
PP-239	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	High-risk human papillomavirus detection in cervical samples as a predictive factor for vaccine coverage	Edina Zahirović	Bosnia and Herzegovina
PP-240	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	SARS-CoV-2 intra-host evolution in immunocompromised patients	Kees Mourik	Netherlands
PP-241	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	The Genetic Variation of Human Papillomavirus Type 16 (HPV16) E6 and E7 gene and the Association with the Development of Cervical Cancer	Marwan Albuhtori	United Kingdom
PP-242	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	Frequency and context of HPV DNA detection below the clinical threshold of the commercial kit Alinity m HR-HPV Assay (Abbott)	Quentin Lepiller	France
PP-243	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	HHV-6 DNA is highly prevalent in the blood after haploidentical stem cell transplantation	Sandy Gross	France
PP-244	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	Review of Management of Herpes Simplex Virus (HSV) Viraemia Among Immunocompromised Patients in a London Hospital, United Kingdom	Tamsin McKinnon	United Kingdom
PP-245	Poster Rotation 2	Viruses, tumors and immunocompromised hosts	Role of human herpes viruses in the development of head and neck squamous cell carcinoma in turkish patients	Zeynep Ceren Karahan	Turkey

ESCV 2024

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ORAL PRESENTATION ABSTRACTS

OP-001
Enteric viruses & Neurovirology

One year of Europe-wide surveillance pilot for Enteroviral CNS infections

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AIM: Enteroviruses (EVs) are common viruses causing clinical manifestations ranging from mild fever to severe affection of the heart, liver, and central nervous system (CNS), and sometimes fatalities. Surveillance of enterovirus infections is crucial for understanding their epidemiology and guiding public health strategies. This study aimed to investigate the burden of CNS infections caused by enteroviruses in hospitals across Europe by implementing standardized surveillance protocols
METHODS: Prospective surveillance was conducted in 2023-2024 in six European hospitals. Standardized surveillance protocols were completed by physicians for each patient with CNS symptoms positive for enterovirus. Sites joined the CNS surveillance between 400 and 99 days before this summary was conducted.
RESULTS: A total of 35 patients were included in the study from the 6 hospitals, ranging from one to 16 patients per site. Neonates constituted 38% of the population, while 67% were ≤1 year old. Gender was reported for 34 patients, with 21 males (62%). Clinical diagnoses were reported for 27 patients (77%), with 20 cases (74%) diagnosed with meningitis and three cases (11%) with encephalitis. For 15 patients, enterovirus type was reported, the most common being coxsackievirus B5 (CV-B5, 3 patients). Echoviruses 5, 7, 9, 11, 18 and 21 were also detected, along with CV-B2 and CV-B3.
CONCLUSIONS: This study provides data from a comprehensive surveillance of severe infections due to EV in six European hospitals. It highlights the continued occurrence of EV infections leading to neurological complications. This pilot holds promise for continued surveillance efforts.
SOURCES OF FUNDING: This project has received a Research Grant from Nordsjællands Hospital
Keywords: Enterovirus, Central Nervous System Infections, Prospective Surveillance,

OP-002
Enteric viruses & Neurovirology

Organotypic Intestinal Cultures as Promising Model for Personalized Antiviral Treatment in Immunocompromised Patients

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AIM: Enteroviruses (EVs) are recognized to cause acute, mild self-limiting diseases. However, in immunocompromised patients EVs can lead to severe chronic infections. Here we explored treatment options for rare cases of severe chronic enterovirus-induced diarrhea in immunodeficient patients.
METHODS: We identified the rarely detected EV-C Coxsackievirus A1 (CVA1) in a patient with common variable immunodeficiency (CVID), and CVA19 in two hematological patients, all suffering from severe chronic diarrhea. EV-Cs are difficult to culture, but CVA1 could be isolated from patient stool and was tested in vitro against FDA-approved antivirals fluoxetine, itraconazole, ribavirin, and remdesivir (RDV).
RESULTS: In the RD cell-line, only RDV demonstrated a significant inhibition of CVA1 replication. All three patients were treated with a 10-day course of RDV. Treatment was successful in one patient, verified by clinical improvement and eradication of CVA19 RNA. However, in the other two patients symptoms endured and EV RNA remained detectable in stool after treatment. Next we used an organotypic intestinal co-culture of epithelial cells with fibroblasts, obtained from a healthy donor. Here we showed only a mild non-significant reduction in CVA1 viral load by RDV. Additional results with organoids from different donors suggested a donor-dependent antiviral response against RDV.
CONCLUSIONS: RDV efficacy against CVA1 tested in our organotypic model is in alignment with the differential responses observed in our patients, resulting in a more predictive antiviral result than obtained from the cell-line. Generation of patient-derived organoids is a promising next step towards a personalized medicine approach for determining antiviral drug efficacy.
SOURCES OF FUNDING: H2020 OrganoVIR(812673) and GUTVIBRATIONS(953201)
Keywords: Enterovirus, gastrointestinal viruses, immunocompromised, antiviral treatment, personalized medicine, organoids

OP-003
Enteric viruses & Neurovirology

Association between natural killer cell genetics and the development of Long COVID associated- and prepandemic small fiber neuropathy

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AIM: Small fiber neuropathy (SFN) can be confirmed by skin biopsy in a subset of Long COVID (LC) patients. NK cells play a crucial role in viral clearance as well as in nerve metabolism. The aim of this study was to identify associations between SFN development in LC patients as well as in prepandemic SFN patients, and genetic markers associated with specific NK cell functions.
METHODS: LC patients with clinical signs of SFN (neuropathy and autonomic dysfunction) were compared to a group of prepandemic SFN patients and a population control group. The following genetic NK cell markers were assessed in all cohorts: CD16A, G1m, HLA-E, NKG2C and rs9916629. Genotyping was performed by TaqMan assays and Sanger Sequencing. Cytomegalovirus (CMV) serostatus was assessed by a commercial IgG ELISA.
RESULTS: We included 50 LC-SFN patients, 27 prepandemic SFN patients and 320 control persons. Deletions of the NKG2C receptor occurred significantly more frequently in prepandemic SFN patients (24%) compared to the control cohort (p=<0.0001). When analyzed in combination with CMV serostatus, markers associated with NK cell deficiency, i.e. NKG2C deletion and CMV seronegativity, were observed significantly more often in prepandemic SFN patients (84%) compared to LC-SFN patients or population controls (p=0.0171 and 0.001, respectively).
CONCLUSIONS: In conclusion, the observed genotype distributions hint towards an involvement of CMV-related immune responses, especially via the NKG2C pathway, in the development of prepandemic SFN.
SOURCES OF FUNDING: This study was funded by the Medical and Scientific Fund of the Mayor of the Federal Capital of Vienna, Austria.
Keywords: Long COVID, small fiber neuropathy, NK cell, NKG2C

OP-004
Pandemic preparedness and health services research

Unlocking the full potential of wastewater-based epidemiology for pandemic preparedness: realistic quality assurance measures for the reliable detection of circulating and emerging viruses in wastewater

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AIM: The aim of this study was to evaluate measures for ensuring quality assurance in wastewater-based epidemiology (WBE) for the effective implementation of comprehensive surveillance of human pathogenic viruses in wastewater. Building on the experience from SARS-CoV-2 monitoring (COVIDready), this subsequent study (WBEready) seeks to ensure reliable WBE data through comparative quality control.
METHODS: To assess PCR- and NGS-based assays and interlaboratory variability, authentic inactivated SARS-CoV-2 was spiked into wastewater samples collected during low-incidence periods. An interlaboratory comparison was conducted, allowing each participating laboratory to perform routine in-house workflows. Sample sets, derived from frozen reserved wastewater samples from a low COVID-19 incidence period, were spiked with inactivated SARS-CoV-2 variants.
RESULTS: During comparative assay evaluations, reduced specificity and sensitivity were observed for several published and commercially available SARS-CoV-2 spike mutation assays, such as for the Δ69/70 or the K417N substitution, underscoring the need for interlaboratory comparisons. Despite heterogeneous methods in the participating laboratories, genome copy equivalents and proportions of individual SARS-CoV-2 variants were largely reproduced by PCR-based analysis (RT-qPCR and RT-d(d)PCR). Reliable identification of variants using NGS required more than 100 copies/ml of wastewater and varied significantly between analysis workflows.
CONCLUSIONS: To maximize the potential of WBE for pandemic preparedness, it is essential to implement reliable WBE for a wide range of pathogens. We recommend realistic and regular evaluations of PCR- and NGS-based WBE analysis and interlaboratory variability using wastewater spiked with inactivated authentic viruses.
SOURCES OF FUNDING: German Federal Ministry of Health
Keywords: Wastewater; Epidemiology; Pandemic preparedness

OP-005
Pandemic preparedness and health services research

AI-Optimized Vaccination Strategies to Mitigate Smallpox Outbreaks in Urban Settings: A Case Study of Frankfurt

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AIM: Vaccination regimens played a crucial role in eradicating smallpox but haven't been studied in modern contexts where smallpox remains a security threat in times of political tension. This study assessed different vaccination strategies for controlling a smallpox outbreak in the Frankfurt area using modern in-silico methods.
METHODS: A modified SEIRHD (Susceptible, Exposed, Infectious, Recovered, Hospitalized, Deceased) model simulated smallpox spread in Frankfurt via an AI mobility-probability matrix, produced using German Federal Employment Agency, census, and spatial data of Frankfurt's 15 districts and main commuter towns. Force of infection was determined as a factor of workplace, housing/population densities, and residual immunity (8.8%). The AI-modified model adjusted historical infection peaks based on current anthropological factors, with peak infection occurring between 70-100 days. Varied single-dose and dual-dose regimes with different efficacy rates were evaluated on the R-program model.
RESULTS: Vaccination regimes with efficacy rates >75% effectively controlled the smallpox outbreak when assumed at day seven without exceeding the intensive care unit capacity of the Bundesstadt (338 beds). A dual-dose regime with 50-75% efficacy exceeded capacity (563 hospitalised), even with assumed 95% compliance of the second dose after 28 days. Single-dose regimens had statistically superior EIHRD parameters vs. equivalent dual-dose vaccinations (chi-squared P<0.0001). A single-dose, 95% effective vaccine resulted in 372 deaths and an infection peak of 43, with 212 hospitalized patients.
CONCLUSIONS: Single-dose regimes with high efficacy rates proved effective in controlling smallpox outbreaks without overwhelming healthcare resources when processed through the novel AI-simulator.
SOURCES OF FUNDING: Tristan Learoyd, Kevin Yeo and Quang Thai are employees of Emergent BioSolutions.
Keywords: RIng vaccination, Smallpox, Preparedness, SEIRHD Model, AI, Vaccine Efficacy

OP-006
Pandemic preparedness and health services research

Wastewater Surveillance Aids Real-time Tracking of Community Transmission of SARS-CoV-2 and Influenza
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AIM: This study aimed to evaluate the effectiveness of wastewater-based surveillance for early detection of SARS¯CoV¯2 and Influenza viruses, in strengthening pandemic preparedness efforts. We investigated whether viral detection in wastewater correlates temporally with community case surges and compared viral variants and subtypes between wastewater and human samples.
METHODS: Fortnightly samples were collected from all 11 sewage treatment plants in the central Indian city of Bhopal from March 2022 through June 2023. Samples were processed using pasteurization, centrifugation, and filtration. Viral concentration was achieved using the PEG-NaCl method, followed by RNA extraction and real-time RT-PCR for viral detection. Positive samples were further analyzed for subtyping and whole-genome sequencing.
RESULTS: Analysis of 649 samples (548 influent, 101 effluent) identified SARS-CoV-2 in 10 and Influenza A in 32 influent samples. Sequencing revealed two distinct Omicron lineages (XBB.1.16 and XBB.1) in April 2023, which matched with variants circulating among COVID-19 cases (GISAID: EPI_ISL_18705562 to EPI_ISL_18705571). Influenza A subtyping identified H1N1pdm09 and H3N2 strains, aligning with predominant human cases circulating regionally during the study period. Viral detection in wastewater coincided temporally with community surges for either virus. Effluent samples tested negative for both viruses, indicating effective virucidal treatment.
CONCLUSIONS: Alignment with community case surges and viral variants suggest that wastewater detection can serve as an effective surveillance tool. Given the high proportion of asymptomatic or mildly symptomatic cases in both these diseases, this method offers a cost-effective and comprehensive alternative for generating timely warnings, crucial for pandemic preparedness and response.
SOURCES OF FUNDING: DST SERB and DHR ICMR, Govt. of India
Keywords: Wastewater Surveillance, SARS-CoV-2, Omicron Variant, Influenza A, Ion Torrent

OP-007
Respiratory viruses

Characterization of RSV full-length viral genomes after Nirsevimab breakthrough infections in a large national observational real-world study conducted in France

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AIM: Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection (LRTI) in children worldwide. Nirsevimab, a long-acting monoclonal antibody has recently been approved for the prevention of RSV LRTI in infants. In France, more than 200,000 single doses were administered during 2023-24 season to <1year infants. In this context, emergence and spread of escape variants is a concern. Here we characterize RSV viral breakthrough in a large observational real-life study.
METHODS: Multicentre national observational study during RSV 2023-24 season was conducted in RSV-infected infants <1y: (i) RSV(+) nirsevimab-treated breakthrough and (ii) RSV(+) nirsevimab-untreated infection. Full-length RSV RNA sequencing was performed. Changes of F (site Ø) relative to reference sequences were analysed.

RESULTS: Of the 685 RSV infected infants, 635 RSV full-length genome sequences were analysed: 312 from nirsevimab-treated breakthrough infections (284 RSV-A; 28 RSV-B) and 323 from untreated RSV-infected infants (270 RSV-A; 53 RSV-B). Analysis of RSV-A revealed no selection for mutations in site Ø known to be associated with resistance to nirsevimab. However, F:S63N, a known polymorphism, was found in 8/554 samples. One of the 28 RSV-B breakthrough infections evidenced F:N208D, which is known to confer high resistance to nirsevimab. In addition, so far undescribed F:I64V was found in 4/28 treated and 4/53 untreated patients.
CONCLUSIONS: This is the largest viral molecular surveillance study of nirsevimab breakthrough infections conducted in France. Nirsevimab escape variants remain very rare despite its widespread use. Detection of several mutations in RSV-B F highlights the importance of active molecular surveillance.
SOURCES OF FUNDING: ANRS MIE INSERM
Keywords: respiratory syncytial virus - monoclonal antibody - viral escape

OP-008
Respiratory viruses

Age- and cell-type-specific metabolic programming shapes respiratory and systemic immune responses against the Influenza A virus

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AIM: Identifying molecular determinants explaining the age-dependency of severe influenza infections.
METHODS: Human airway epithelial cells (AECs) and blood monocytes from newborn infants and healthy adults were infected with IAV and subjected to functional, transcriptional and metabolic profiling. Moreover, we explored the impact of ketogenic diet (KD) in adults and S100-alarmins as regulator of neonatal immunity on the overall anti-IAV phenotype.
RESULTS: Adult AECs showed a significantly restricted but adult monocytes an enhanced immune response to IAV infection compared to neonatal AECs respective monocytes. This opposite and age-dependent IAV responsivity was linked to the metabolic programming of the individual cell types. Neonatal AECs and adult monocytes were characterized by a high glycolytic activity fueling their strong inflammatory response towards IAV, respectively. We identified the ubiquitously and physiologically high exposure of neonates to S100A8/A9 as factor enhancing glycolysis in AECs but restricting it in myeloid cells. In contrast, KD could not constrain the strong glycolytic and consequently inflammatory activation of adult monocytes upon IAV infection.
CONCLUSIONS: The inflammatory response to IAV primarily depends on the cellular glycolytic activity, which is age- and cell-type-specific. Immunometabolic reprogramming constrains the airway epithelial responsivity with increasing age but fuels systemic inflammatory responses against IAV, which together might increase the risk of adults to severe influenza disease. S100A8/A9 priming might protect against severe influenza, while KD did not promise to be a successful treatment strategy.
SOURCES OF FUNDING: DFG (EXC 2155)
Keywords: Influenza A virus, immune response, neonate, airway epithelium, monocytes, immunometabolism

OP-009
Respiratory viruses

Characterization of antigenic relations of SARS-CoV-2 variants and population immunity using antigenic cartography

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AIM: Antigenic characterization of newly emerging SARS-CoV-2 variants is important to assess their immune escape and judge the need for future vaccine updates. Here, the combination of describing the basic antigenic relations of new variants via single variant exposure antigenic maps and antibody landscapes to identify holes in population immunity should be most helpful.
METHODS: We analyzed titers of neutralizing antibodies against SARS-CoV-2 variants via a focus reduction neutralization assay using authentic virus isolates and performed antigenic cartography. As human single variant exposure sera for newer variants are increasingly difficult to collect, animal sera may be a substitute, which we evaluated. To visualize more complex exposure histories, e.g. after XBB.1.5 boost, we constructed antibody landscapes based on the single exposure map.
RESULTS: To bridge data obtained from animal sera with human sera, we analyzed neutralizing antibody titers in human and hamster first infection sera in the same assay. Using a Bayesian framework, we found that titer fold changes in hamster sera corresponded well to human sera, especially after adjusting for the generally higher reactivity in hamster sera. Using antibody landscapes, we modeled different types of population immunity in human sera and found that number and type of exposures influenced the shape of the antibody landscapes.
CONCLUSIONS: Our results indicate that sera from infected hamsters are a good surrogate for the antigenic characterization of new variants.
SOURCES OF FUNDING: NIH NIAID Centers of Excellence for Influenza Research and Response (CEIRR) contract 75N93021C00014 as part of the SAVE program, Gates Cambridge Trust
Keywords: SARS-CoV-2, neutralizing antibodies, cross-neutralization, antigenic cartography

OP-010
Respiratory viruses

The role of RSV viral load and co-infection on disease severity in a very large cohort of paediatric patients

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AIM: This study aims to explore the frequency of RSV in mono- and co-infection and the clinical outcomes associated with RSV infection in paediatric population.
METHODS: A retrospective analysis of paediatric patients admitted at Bambino Gesù Children's Hospital in Rome who had a RSV positive respiratory sample between January, 2022, and December, 2023 was performed.
RESULTS: Among 13,952 samples collected during the study period, 492(3.5%) were RSV-positive. Of these, 466 belonged to paediatric patients (Median[IQR] age: 0.4[0.1-2.1] years) with ARI diagnosis at hospital admission. 405(86.9%) involved the lower-respiratory tract, while only 61(13.1%) involved the upper-respiratory tract. Viral co-infections were mainly observed in the upper-respiratory tract infections (71.7%) with respect to the lower (68.6%),(P=0.071). However, considering clinical manifestation, in the lower-respiratory tract the RSV co-infection was mainly observed in patients with pneumonia (21.0%) while RSV mono-infection mainly characterized bronchiolitis (89.4%),(P=0.021). Notably, RSV cycle-threshold (CT) value was significantly lower in mono-infection (median[IQR]: 21.3[17.7-25.0]) than in co-infection (23.1[19.1-29.0]),(P=0.003). Logistic regression showed that the highest CT value was positively associated with RSV co-infection (AOR[95%CI]: 1.07[1.00-1.13],P=0.008), while a negative association was observed with bronchiolitis (AOR[95%CI]: 0.32[0.17-0.57],P<0.001).
CONCLUSIONS: These results showed that RSV mono-infection with high viral load mainly characterized bronchiolitis. Conversely, a lower viral-load and pneumonia were observed when RSV was in co-infection, thus suggesting that the presence of another virus can potentially reduce the replicative capacity (and perhaps pathogenicity) of this virus. RSV co-infection is associated with low viral-load, and a specific clinical outcome. Therefore, the quantitative study of RSV is important in combination with virus-virus interactions.
SOURCES OF FUNDING: none
Keywords: RSV, co-infection, disease severity, pediatric population, acute respiratory infection

OP-011
Respiratory viruses

Coronaviruses impair resolution phase in monocytes via aberrant interferon signalling

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AIM: Monocytes play an essential role in the clearance of viral infections and maintenance of tissue homeostasis. In this study, we investigate their re-programming during infection with various coronaviruses.
METHODS: Primary monocytes were subjected to infection with seasonal coronavirus HCoV-229E and pandemic strains such as SARS-CoV, MERS-CoV, and SARS-CoV-2 variants (ranging from B.1 to Omicron BA.5). Our analysis encompassed viral replication, innate immune response, and cellular functions.
RESULTS: While all coronaviruses were internalized by monocytes, only HCoV-229E displayed productive replication within these cells. Noteworthy differences emerged in innate immune signalling across the coronaviruses. HCoV-229E, MERS-CoV, and SARS-CoV-2 variants, Omicron BA.1 and BA.5 induced secretion of type I interferons (IFNs) and subsequent activation of interferon-stimulated genes (ISGs). In contrast, SARS-CoV and SARS-CoV-2 variants, B.1- Delta failed to activate IFN signalling. Time-kinetic experiment revealed that both Omicron- and HCoV-229E-infected monocytes displayed caspase 3/7 activation and pronounced cell death starting at day 3 post infection. Additionally, we observed prolonged increase in IFNß mRNA levels. Intriguingly, early IFN signalling following coronavirus infection could prevent super-infection with Influenza A virus, although no protective antiviral state was evident in later time-points, suggesting aberrant IFN response. We further detected downregulation in the gene expression of pro-resolving mediators (IL1RN, EGR3), concomitant with a decline in monocyte phagocytic activity. Crucially, the administration of an IFN signalling inhibitor, B18R, rescued the observed phenotype.
CONCLUSIONS: Our finding demonstrates functional interplay between the resolution phase and IFN signalling in monocytes exposed to coronaviruses.
SOURCES OF FUNDING: German Research Association, GRK 2336:AVE - Resolution of Inflammation
Keywords: SARS-CoV-2, Monocytes

OP-012
Viromics and virus discovery

Multicenter benchmarking of short and long read wet lab protocols for clinical viral metagenomics

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AIM: Metagenomics is gradually being implemented for diagnosing infectious diseases. However, in-depth protocol comparisons for viral detection have been limited to individual sets of experimental workflows. Here, we present a benchmark of metagenomics protocols used in clinical diagnostic laboratories initiated by the European Society for Clinical Virology (ESCV) Network on NGS (ENNGS).
METHODS: A mock viral reference panel was designed to mimic low biomass clinical specimens. The panel was used to assess the performance of twelve metagenomic wet lab protocols currently in use in the diagnostic laboratories of participating ENNGS member institutions. Both Illumina and Nanopore, shotgun and targeted capture probe protocols were included. Performance metrics sensitivity, specificity, and quantitative potential were assessed using a central bioinformatics pipeline.
RESULTS: Overall, viral pathogens with loads down to 10E4 copies/ml (corresponding to CT values of 31 in our PCR assays) were detected by all the evaluated metagenomic wet lab protocols. In contrast, lower abundant mixed viruses of CT values of 35 and higher were detected only by a minority of the protocols. Considering the reference panel as the gold standard, optimal thresholds to define a positive result were determined per protocol, based on the horizontal genome coverage. Implementing these thresholds, sensitivity and specificity of the protocols ranged from 67 to 100% and 87 to 100%, respectively.
CONCLUSIONS: A variety of metagenomic protocols are currently in use in clinical diagnostic laboratories. Detection of low abundant viral pathogens and mixed infections remains a challenge, implying the need for standardization of metagenomic analysis for use in clinical settings.
SOURCES OF FUNDING: ESCV
Keywords: Clinical viral metagenomics, benchmarking

OP-013
Viromics and virus discovery

Tracing the origin, spread and molecular evolution of Dengue type 1 cases occurred in Northern Italy in 2023
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AIM: Dengue virus (DENV) is a mosquito-borne Flavivirus endemic in many tropical countries. We present data on DENV-1, isolated from 13 patients during an autochthonous outbreak in Northern Italy (August 2023). Adult mosquitoes of the neighboring area were also analyzed and sequenced. We also included in the analysis three cases of imported DENV-1 from Italian people living in Lombardy who traveled between July and August 2023 in Asian countries.
METHODS: Metagenomic approach was used to recover DENV-1 whole genome. INSaFLU pipeline was used to construct consensus sequences. A dataset of DENV-1 sequences from other countries was collected. Genotype analysis and phylogenetic analysis by Bayesian methods were performed.
RESULTS: The dataset included 540 DENV-1 sequences from Europe,Asia and America. Time of sequencing spread from 1977 to 2023 with most abundant samples in 2005-2009 and 2020-2023. Italian autochthonous strains clustered with American strains collected in the period 2020-2023 belonging to genotype V subtype D.The imported Italian strains clustered with Asian strains collected in the period 2015-2019 and 2020-2023 belonging to genotype I subtype K and E. We produced a non-redundant representative dataset (n = 252) preserving genomes of interest and minimizing the loss of genetic variation.The Bayesian analysis estimated a mean evolutionary substitution rate of 7.861x10-4 subs/site/years.The time to the most recent common ancestor (tMRCA) estimate was 1943.
CONCLUSIONS: The results aim to provide better insights in the spread dynamics of DENV-1 Lombardy outbreak and to advocate for the need for molecular surveillance to guide public health interventions.
SOURCES OF FUNDING: Ministry of Health, CCM 2022 Project Surveid, Italy
Keywords: Dengue virus, Phylogenetic analysis, Metagenomic

OP-014
Viromics and virus discovery

Metagenomic and transcriptomic investigation of paediatric acute liver failure cases

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AIM: In 2022, a cluster of severe childhood hepatitis was detected leading to a global alert by the world health organization. Five percent of the cases progressed to acute liver failure, necessitating transplantation. The aim of this study was to use an in-depth molecular analyses into the mechanism of pediatric acute liver failure, using targeted transcriptomics and metagenomic analysis for identifying any virus present in cases, immune profile haplotypes and groups of genes differentially expressed.
METHODS: An upsurge of in total five cases of acute liver failure presented to the Dutch national referral center for pediatric liver transplantation in spring 2022. Explanted liver tissue and plasma samples were subjected to viral metagenomic and human transcriptomic profiling. Signatures of transplanted cases were compared with those of pediatric controls.
RESULTS: AAV2, adenoviruses, and herpesviruses were detected in liver and plasma samples of the cases. In one case, Epstein-Barr virus, and in one case, varicella zoster virus infection with pathognomonic clinical symptomatology preceded the liver failure. AAV2 was detected in 1/3 of control livers. Excessive activation of monocyte pathways was detected in liver explants from cases compared with controls. Remarkably, this signature was comparable for AAV2, adenoviruses and/or herpesviruses positive transplant cases.
CONCLUSIONS: Our multi-omic findings suggest a common pathway predominated by monocytes in the presented transplanted cases, with similar severe clinical outcome. In the cohort presented, AAV2 was not uniquely associated with acute liver failure, suggesting other processes may have contributed to a uniform cascade of irreversible pathology.
SOURCES OF FUNDING: No external funding was obtained for this study.
Keywords: metagenomics, transcriptomics, adeno-associated virus 2, childhood hepatitis

OP-015
Advancements in diagnostics & clinical cases

Evaluating metagenomics and targeted approaches for diagnosis and surveillance of viruses

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AIM: Provide a pragmatic assessment of utility for routine diagnostic viral metagenomics in samples expected to have low microbial abundance and high host content.
METHODS: We evaluated detection of viruses with readily available untargeted metagenomic workflows using Illumina and Oxford Nanopore Technologies (ONT), and an Illumina-based enrichment approach using the Twist Bioscience Comprehensive Viral Research Panel (VRP). We tested samples consisting of a dilution series of a six-virus mock community in a human DNA/RNA background, designed to resemble clinical specimens with low microbial abundance and high host content. Protocols were designed to retain the host transcriptome, since this could help confirm the absence of infectious agents. We further compared the performance of commonly used taxonomic classifiers.
RESULTS: Capture with the Twist VRP increased sensitivity by at least 10-100-fold over untargeted sequencing. While untargeted ONT had good sensitivity at high viral loads, at lower viral loads, longer runs would be required to achieve sensitivities comparable to the untargeted Illumina workflow. Untargeted ONT provided better specificity than untargeted Illumina sequencing. However, the application of robust thresholds standardized results between taxonomic classifiers. Host gene expression analysis is optimal with untargeted Illumina sequencing but possible with both the VRP and ONT.
CONCLUSIONS: Metagenomics has the potential to become standard-of-care in diagnostics and is a powerful tool for the discovery of emerging pathogens. Untargeted Illumina and ONT metagenomics and capture with the VRP have different advantages with respect to sensitivity, specificity, turnaround time and cost, and the optimal method will depend on the clinical context.
SOURCES OF FUNDING: NIHR
Keywords: Clinical metagenomics, diagnostics, surveillance, viromics

OP-016
Advancements in diagnostics & clinical cases

Detection of clinically relevant viral pathogens using antibody capture and metagenomic sequencing
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AIM: Recent technological advances in sequencing techniques enable the high throughput detection of multiple viruses within the same assay. However, linking a detected virus with certain disease remains challenging. Autologous antibody capture can be utilized to selectively enrich immunogenic pathogens directly from clinical samples, followed by metagenomic sequencing to comprehensively characterize the pathogen and investigate transmission and epidemiology.
METHODS: Convalescent serum, collected at different time-points after initial virus detection, was assessed for its efficacy in capturing immunogenic viruses in patients. Antibody capture was performed on eight fecal and six respiratory samples containing known viral pathogens. Samples were sequenced with and without antibody capture using Nanopore sequencing to evaluate the performance of the antibody capture.
RESULTS: Minor enrichment was observed using convalescent serum collected 1-2 weeks after initial viral detection while the most efficient capture was observed using convalescent serum collected between 5 and 10 weeks. If later time points were used, the capture efficiency using convalescent serum decreased. Immunogenic viruses were captured in 13 of the 14 samples, with enrichment factors between 1.01x and 560.80x demonstrating the effectiveness of this approach.
CONCLUSIONS: Antibody kinetics were determined, revealing higher antibody concentrations between 5-10 weeks post infection. The combination of antibody capture and Nanopore metagenomic sequencing resulted in the enrichment of immunogenic viruses and provided more comprehensive genome information compared to metagenomic sequencing alone.
SOURCES OF FUNDING: Department of Viroscience
Keywords: metagenomics, viral discovery, antibody capture, high throughput sequencing

OP-017
Viruses, tumors and immunocompromised hosts

HCMV-specific T-cell response as a tool for predicting late HCMV reactivation in hematopoietic stem cell transplant recipients after letermovir prophylaxis

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AIM: Letermovir (LTV) prophylaxis has changed HCMV management in hematopoietic stem cell transplant recipients (HSCTRs); however, late HCMV reactivations remain a crucial issue. We aimed to analyse the role of HCMV-specific T-cell response as prognostic marker for predicting risk of HCMV reactivation after LTV.
METHODS: We enrolled 52 adult HCMV-seropositive HSCTRs receiving LTV prophylaxis for 100 days. HCMV DNAemia will be prospectively monitored for one year. At 100 and 180 days, lymphocyte T-cell count was quantified and HCMV-specific T-cell response against pp65 and IE1 peptide pools using a commercial ELISpot assay was measured. Response was then normalized on CD3 T cell count and expressed as number of specific cells/µl.
RESULTS: At time of writing, 20/52 (38.6%) patients completed the 180-days follow-up. After LTV, 7/20 (35%) patients showed HCMV reactivation and three of them received pre-emptive therapy. A significant increase in CD3 and CD8 T cells (p=0.0315 and p=0.0142) but not CD4 T cells (p= 0.1359) was observed at 180 days. Pp65 and IE1-specific T-cell response at 180 days was significantly higher than that at 100 days (p=0.003 and p=0.0160, respectively). HCMV-specific T-cell response at 100 days was compared in patients with undetectable and detectable HCMV DNAemia after LTV discontinuation. Pp65 but not IE1 T-cell response was higher in those patients who did not experience late HCMV reactivation (p=0.0568).
CONCLUSIONS: Pp65-specific T-cell response might be used as marker for predicting late HCMV reactivation after LTV. The study is still ongoing and results will be confirmed in a larger sample setting.
SOURCES OF FUNDING: Ministry of Health-Italy
Keywords: Human Cytomegalovirus, Transplant, Letermovir, HCMV-specific T-cell response, prognostic marker, late HCMV reactivation

OP-018
Viruses, tumors and immunocompromised hosts

Characterization of the Blood Virome in Liver Transplant Patients

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AIM: To assess the changes in the blood virome in liver transplant patients by unbiased metagenomics (mNGS).
METHODS: Prospective study on candidates for Orthotopic Liver Transplantation (OLT) at a tertiary-care center. Blood samples collected before transplantation and at months 1, 3, 6, and 12 after transplantation. Pathogen genetic material was extracted from plasma samples and separate DNA and RNA libraries were sequenced using the NovaSeq 6000 platform, (40 million reads per library.) Bioinformatic analysis, including pathogen detection and taxonomic classification, was performed using Kaiju.
RESULTS: This study is ongoing, with 70 patients included to date, divided into two groups: 49 transplanted because viral hepatopathy (HCV, or HBV with/without HDV) and 21 becasue other reasons (alcohol or Non-Alcoholic Steatohepatitis). Complete follow-up is available for 60 of them. When comparing the abundance of viral taxa before and post-transplantation, significant differences between pre-tOLT and month 12 RNA libraries pointed to a higher abundance of Torquetenovirus 13 and Torque teno-like mini virus transcripts at month 12. For DNA libraries, significant differences appeared at each post-OLT time point as compared to pre-OLT. There were significant increases CMV as early as month 1, and in CMV, the Torquetenovirus genus, and the Anelloviridae family at months 3 and 6 post-OLT. At month 12, there was an increase only in Torquetenovirus, like returning to a pre-OLT condition.
CONCLUSIONS: Preliminary analysis suggests that mNGS captures the blood virome composition, viral reactivation, and the viral dynamics in immunocompromised liver transplant recipients.
SOURCES OF FUNDING: Instituto de Salud Carlos III, projects PI2018/01759 and PI2018/01824
Keywords: virome, blood, solid-organ transplantation, cell-free nucleic acid metagenomics sequencing

OP-019
Viruses, tumors and immunocompromised hosts

Intraindividual evolution of noroviral sequences during protracted infections
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AIM: To study noroviral evolution in 19 samples of 4 patients with protracted infections.
METHODS: The 2–7 sequential specimens covered 49–776 days. The capsid gene was amplified by PCR and sequenced on the Oxford Nanopore platform. Sequences were trimmed, filtered by quality, and mapped before variant calling.
RESULTS: All samples except one yielded >3000 reads of which 94%–100% mapped to the reference norovirus genome. The mean length was between 1546 and 1677 nt, encompassing the entire capsid gene. As expected, dN/dS analysis showed negative selection over the entire sequences. Nevertheless, previously described epitopes amino acid sequences varied substantially between and within patients. The patients accumulated 69 to 99 variants sequentially. Interestingly, patient 3 with the noroviral RNA present at least over 776 days accumulated only 69 nucleic acid mutations. Noroviral sequences of patients 1, 2 and 4 mutated 84, 80 and 99 nucleic acids over much shorter time periods of 49, 108 and 81 days. Patient 3 has been strongly immune-suppressed with a previous heart transplantation, followed by a kidney transplantation during the sampling period. Patients 1, 2 and 4 can be assumed to be more immune competent with kidney- and stem cell transplantation >4 years before sampling.
CONCLUSIONS: Intraindividual evolution during protracted correlates to the patients´ immune function.
SOURCES OF FUNDING: German Center for Infection Research (DZIF)
Keywords: mutation rate, variant, selection, dN/dS analysis, immunocompromised

OP-020
Viruses, tumors and immunocompromised hosts

Viral infections in Stem Cell Transplant recipients with posttransplant lymphoproliferative disorder: Torque Teno Virus as a marker of immune function

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AIM: Post-transplant viral infections, like Cytomegalovirus (CMV) or Epstein-Barr Virus (EBV), can lead to serious complications including EBV-driven post-transplant lymphoproliferative disorder (PTLD), caused by immunosuppressive therapy driving the abnormal proliferation of EBV-infected B-cells. Torque Teno virus (TTV) recently gained interest as marker of immune function. Here, we studied kinetics of post-transplant viral infections and their association with clinical parameters in hematopoietic stem cell transplant (HSCT) recipients who developed PTLD.
METHODS: Plasma was collected from 23 post-HSCT patients with PTLD (PTLD-HSCT) and 25 post-HSCT patients without PTLD (CONTR-HSCT) at three timepoints: time of transplantation (T0), 3 months (T1) and 6 months (T2) post-HSCT. Plasma from 25 Healthy Donors (HD) was used as control. We investigated the presence and kinetics of post-transplant viral infections (TTV, EBV, CMV, Human herpesvirus 6 [HHV-6] and Adenovirus [AdV]) and correlated results with clinical parameters.
RESULTS: EBV, CMV, HHV-6 or AdV infections were found in some, while TTV was found in all samples. TTV levels were significantly higher in PTLD-HSCT compared to CONTR-HSCT and HD. Patients with viral co-infections had higher TTV levels compared to patients with only TTV. TTV levels were significantly higher in surviving compared to deceased patients.
CONCLUSIONS: The highest levels of TTV were detected in PTLD-HSCT, suggesting that TTV viral load increases with the fall of the host immunocompetence. A steady rise in TTV levels in the PTLD-HSCT but not in CONTR-HSCT cohort suggests that a continuous rise in TTV viral load could be a predictive factor for developing PTLD after HSCT.
SOURCES OF FUNDING: None.
Keywords: post-transplant lymphoproliferative disorder, transplantation, viral infections, immunocompromised, Anellovirus, herpesvirus

OP-021
Viruses, tumors and immunocompromised hosts

Investigating nosocomial BK Polyomavirus infections in paediatric hematopoietic stem cell transplantation recipients: challenges and prospects

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AIM: BK Polyomavirus (BKPyV) is responsible for hemorrhagic cystitis in hematopoietic stem cell transplantation (HSCT) recipients. The modes of transmission of this virus remain incompletely understood. As BKPyV seroprevalence rises in children, reaching nearly 100% by late adolescence, hospitalization in a hematology unit could represent the first exposure to this virus in pediatric HSCT recipients, given prevalent viral shedding in hematology patients. This study explores the possibility of nosocomial transmission of BKPyV within a hematology department.
METHODS: For 34 HSCT paediatric recipients with BKPyV replication during hematology stay, overlapping stays were analyzed, whole genome sequencing of the virus was conducted and phylogeny was studied.
RESULTS: An unusually high detection of genotype Ia BKPyV in 2021 compared to other periods indicates a potential clinical cross-transmission. However, while closely related, sequences cluster with several Genbank entries, complicating phylogenetic linkage determination. On the other hand, genetic signature investigation did not associate sequences with patient clinical status.
CONCLUSIONS: Despite the small sample size and lack of available serological data, this study suggests potential cross-transmission of BK polyomavirus (BKPyV) within hematology departments, while highlighting the technical challenge of confirming such transmission. This observation underlines the importance of monitoring medical environments, and encourages the consideration of preventive strategies to mitigate the viral impact that would result from such potential transmissions. Further analysis of environmental data and a larger cohort study could improve our understanding of the transmission of this non-enveloped virus.
SOURCES OF FUNDING: This study was conducted without any external funding.
Keywords: BK Polyomavirus, Hematopoietic Stem Cell Transplantation, Pediatrics, Sequencing, Cross infection.

OP-022
Advancements in diagnostics & clinical cases

Transient biopsy-proven progressive multifocal leukoencephalopathy-immune reconstitution inflammatory syndrome in an elderly woman without known immunodeficiency

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AIM: Progressive multifocal leukoencephalopathy (PML) is a severe opportunistic infection caused by lytic replication of JC polyomavirus (JCPyV) in oligodendrocytes. JCPyV infection is common but PML almost exclusively occurs in immunocompromised patients. Immune reconstitution is necessary for recovery. Paradoxically, initiation of antiretroviral treatment of HIV/AIDS or various treatments aiming at restoring anti-JCPyV immunity, may cause severe immune reconstitution inflammatory syndrome (IRIS), exacerbating symptoms. Here we report an unusual case of spontaneous IRIS unmasking PML in a 76-year-old immunocompetent woman.
METHODS: MRI, Flow-cytometry, immunohistochemistry, PCR, serology
RESULTS: The previously healthy patient was admitted with psychosis, speech impairment and behavioural changes over three months. MRI revealed multiple contrast-enhancing lesions and metastases were suspected. Due to suspicion of oedema, dexamethasone was initiated. Eight days later, tonic seizures occurred and dexamethasone was unintentionally discontinued. Ten days later a brain biopsy revealed demyelination, numerous perivascular CD8+ T cells, abundant macrophages, and scattered JCPyV-positive oligodendrocytes, supporting the diagnosis PML-IRIS. The CSF contained low amounts of JCPyV DNA and the plasma contained high levels anti-JCPyV IgG. Extensive immunological testing did not show evidence of cellular immunodeficiency. The patient gradually recovered clinically and radiologically. More than one year after diagnosis, only slight language and behavioural abnormalities persist.
CONCLUSIONS: An apparently immunocompetent elderly person can develop clinically symptomatic, biopsy-proven PML which spontaneously resolves with symptoms and signs of IRIS. The absence of immunological risk factors for PML and atypical MRI lesions with contrast enhancement delayed diagnosis.
SOURCES OF FUNDING: No
Keywords: Polyomavirus, JCPyV, PML, IRIS

OP-023
Advancements in diagnostics & clinical cases

National-scale environmental and clinical surveillance of Poliovirus strains using novel quantitative PCR assays

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AIM: Circulation of vaccine-derived poliovirus (VDPV) becomes an increasing concern, jeopardizing global polio eradication efforts, in spite of continuous vaccination campaigns. In order to enable rapid, high-throughput poliovirus detection, we developed and implemented novel quantitative PCR assays that specifically detect Polio-2 and VDPV-3.
METHODS: Both multiplex assays can detect <10 target copies per sample, and are highly specific. The VDPV-3 assay detects the presence of VDPV-3 even in Sabin-like 3 vaccine RNA background, demonstrating exceptional specificity. National-scale wastewater surveillance using these assays showed substantial circulation of both strains across Israel, during 2021-2023, in different sites.
RESULTS: We screened approximately 3,000 environmental samples from ~30 wastewater sites across Israel, and ~1700 fecal samples from suspected Polio-2 and VDPV-3-circulating regions. We identified ~200 positive samples in different regions, and ~15 infected individuals, with varying viral loads. Comparing the direct qPCR assays results to standard WHO protocol showed 96%-100% positive and negative agreement. The quantitative assays enabled monitoring viral load dynamics in wastewater sites and infected patients over time, providing unique, important information on virus circulation on a national level, leading to public health counter-steps by healthcare authorities. Such direct, quantitative detection of VDPV from clinical and environmental samples cannot be accomplished using currently-used standard protocols that require culturing and obligatory sequencing, and are therefore resource-, time- and labour-consuming, and are not quantitative.
CONCLUSIONS: These assays may therefore improve laboratory diagnostic performance significantly, in terms of speed, high-throughput capacity, and data quantification, compared with current protocols, thereby providing valuable help in the global Polio eradication efforts.
SOURCES OF FUNDING: None
Keywords: Poliovirus, PV2, VDPV3, real-time PCR, direct detection, wastewater surveillance

OP-024
Advancements in diagnostics & clinical cases

Genomic studies of adeno-associated virus hepatitis

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AIM: Adeno-associated viruses (AAVs) are important vectors in gene therapy, yet the occurrence of hepatitis remains a significant complication associated with this treatment. The identification of adeno-associated virus 2 (AAV2) as a potential culprit in the 2022 outbreak of unexplained hepatitis in children raises intriguing parallels, hinting at a shared mechanism in these forms of AAV-related hepatitis. We used genomic methods to compare the two types of hepatitis.
METHODS: We conducted long and short read metagenomic sequencing, transcriptomics and proteomics on a liver sample from a patient who experienced hepatitis following treatment with an AAV gene therapy and 2022 outbreak cases.
RESULTS: Metagenomic sequencing revealed evidence of multiple gene therapy plasmids, characterized by elevated levels of complex structures and concatemerization. We also identified human betaherpesvirus 6B in the liver sample, a finding associated with the 2022 paediatric hepatitis outbreak. Transcriptomic analyses of host responses unveiled significant similarities between the two forms of hepatitis. Additionally, we employed proteomic techniques to investigate the presence of viral and plasmid-related proteins.
CONCLUSIONS: There are many similarities between 2022 paediatric hepatitis outbreak, where AAV2 was implicated, and hepatitis following AAV gene therapy. This investigation provides insights into the mechanisms underpinning AAV-related hepatitis, paving the way for improved therapeutic strategies and mitigation of adverse effects.
SOURCES OF FUNDING: SB, OETM, and SM are funded by NIHR BTRU-GEMS. This work was partly funded by the NIHR GOSH-BRC. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.
Keywords: Genomics, adeno-associated viruses, hepatitis, gene therapy

OP-025
Advancements in diagnostics & clinical cases

Impact of inherited human herpesvirus 6 in transplantation

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AIM: To investigate the impact of inherited chromosomally integrated human herpesvirus 6 A/B (iciHHV6) in two pediatric hematopoietic stem cell or solid organ transplant recipients.
METHODS: We used qPCR and hybrid capture sequencing for analysis of the viral genomes. Moreover, we substantiate viral replication by RISH (U38-U94 genes), RT-PCR, immunohistochemistry, and immunofluorescence. We performed whole transcriptome sequencing to profile the host immune response.
RESULTS: We introduce two graft recipients positive for iciHHV-6 A or B in whom we demonstrate reactivation of this inherited virus and subsequent infection of the graft. We integrate qPCR and next generation sequencing for analysis of the viral genomes and demonstrate active replication via the emergence of viral minor variants across time points. Furthermore we demonstrate positive viral mRNAs and antigen stainings in tissue sections coupled to significant upregulation of cell surface molecules, transcription factors, and cytokines associated with antiviral immune responses.
CONCLUSIONS: Our analysis underscores the potential pathological impact of iciHHV-6, emphasizing the need for close monitoring of reactivation in transplant recipients. Most crucially, it highlights the critical role that the host's virome can play in shaping the outcome of transplantation, urging further investigations.
SOURCES OF FUNDING: Finnish Medical Foundation, the Life and Health Medical Foundation, the Helsinki University Hospital Research and Education Fund.
Keywords: Virome, Inherited chromosomally integrated human herpesvirus 6, Transplantation.

OP-026
Advancements in diagnostics & clinical cases

A Novel Next Generation Sequencing Approach for Detecting Antiviral Resistance Mutations in Cytomegalovirus

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AIM: Genetic characterization of Cytomegalovirus (CMV) has become increasingly important due to new antiviral drugs. This study aims to validate an in-house next-generation sequencing (NGS) protocol to determine genotype/s (UL55) and detect antiviral resistance mutations (UL27, UL54, UL56, UL89 and UL97).
METHODS: The technique involves amplifying these regions using overlapping amplicons of 400-800 base pairs, followed by Illumina-based sequencing and a custom data processing pipeline to generate consensus sequences. The method was validated with serial 1/2 dilution of an American-Type-Culture-Collection strain (ATCC VR-538DQ) with replicates. Moreover, a comparison was made with 37 previously Sanger-sequenced samples, considered the gold-standard method. This included 7 with resistance-mutations in UL54 and 10 without, and 10 with resistance-mutations in UL97 and 10 without.
RESULTS: The ATCC strain validation showed 100% coverage and sequence identity across all dilutions and replicates. The Sanger comparison yielded 99.97% concordance for UL54 and 99.91% for UL97 in non-resistant samples. In resistant samples, concordance was 99.91% for UL54 and 100% for UL97. Despite some nucleotide divergence, this not resulted in mutations associated neither polymorphisms nor resistance.
CONCLUSIONS: The in-house NGS protocol demonstrated robust validation with the ATCC-VR-538DQ strain, achieving 100% coverage and identity. Comparison with Sanger-sequenced samples for UL54 and UL97 confirmed protocol's accuracy and reliability for CMV genetic characterization. NGS has some advantages over Sanger, including the ability to sequence multiple regions simultaneously and detect minority variants and mixed viral genotypes. This makes the protocol well-suited for genotypic characterization and drug resistance detection supporting its use in clinical practice in CMV infection.
SOURCES OF FUNDING: No funding
Keywords: Cytomegalovirus, Next Generation Sequencing, Antiviral Resistance, Human Herpesvirus, Immunocompromised Patients, Genotyping

OP-027
Diagnostic stewardship & Diagnostic test accuracy

Performance of a novel dual marker RT-qPCR- based whole-blood assay for the detection of SARS-CoV-2-reactive T cells

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AIM: The COVID-19 pandemic has now officially come to an end. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become endemic and continues to pose a risk to a significant proportion of the population. T-cell immunity against SARS-CoV-2 plays a central role in controlling the virus and disease severity. The objective of this study was to analyse T-cell responses using a new molecular whole blood assay in participants with and without a history of SARS-CoV-2 exposure (by infection or vaccination).
METHODS: Evaluation of SARS-CoV-2 reactive T cells applying a novel quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay, which is based on the combined assessment of *IFNG* and *CXCL10* mRNA levels in response to the S1 and NP SARS-CoV2-antigens in a cohort of 335 participants with or without a history of SARS-CoV-2 infection or vaccination.
RESULTS: Of the 62 convalescent donors, 100% responded to S1 and 88.7% to NP antigens. In comparison 68 naive donors, 4.4% were reactive to S1 and 19.1% to NP. Convalescent donors aged <50 and ≥50 years showed 100% S1 reactivity and 89.1% and 87.5% NP reactivity, respectively. Cellular and humoral responses were consistent for at least 36 weeks post-immunization in vaccinated and convalescent donors.
CONCLUSIONS: Our results demonstrate the very good performance of this new molecular assay and suggest that it may be suitable for monitoring SARS CoV-2 specific T cell responses in COVID-19 vaccination trials and cross-reactivity studies.
SOURCES OF FUNDING: This research was funded by Mikrogen and received no external funding.
Keywords: SARS-CoV-2, T-cell immunity, RT-qPCR, CXCL10, IFNG, COVID-19

OP-028
Diagnostic stewardship & Diagnostic test accuracy

Are we using syndromic meningitis testing rationally? five-year experience of a university hospital laboratory

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AIM: Molecular syndromic panels offer the benefit of rapid diagnosis with high sensitivity/specificity, particularly in cases of central nervous system infections. However, the overabundance of these tests combined with their irrational use may mislead physicians into erroneous diagnosis and inappropriate therapy decisions leading to detrimental effects on patient management and raising hospital expenses. This study aimed to assess the clinical impact of syndromic meningitis panel (SMP) testing.
METHODS: Retrospective analysis was done on the outcomes of SMP testing carried out on cerebrospinal fluid samples between 1.1.2019 and 31.12.2023. Clinical and demographic information was gathered from the hospital's information system. Clinical follow-up and medication modifications after laboratory diagnosis were also assessed for patients with positive SMP results.
RESULTS: Of the 1491 patient samples evaluated during the study period, 122 (8.18%) were positive for at least one etiological agent. The most frequently detected viruses were HHV-6 (27.86%), enterovirus (11.47%), HSV-1 (8.19%), and CMV (8.19%). S.pneumoniae (10.65%) was the most common bacteria. Multiple agents were detected in 13 positive samples (10.65%), with HHV-6+Parvovirus-B19 being the most prevalent (61,53%). The most frequent indications for SMP requests were fever, altered consciousness, and headache. Surprisingly, only 40.98% of positive results were considered clinically significant and nearly one-third of the test results were not even followed up on.
CONCLUSIONS: SMP testing is over-requested.
Approximately two-thirds of the positive results are overlooked or not clinically significance by the clinicians. When not performed on correct patients SMP do not provide clinically relevant results and increase hospital costs.
SOURCES OF FUNDING: Funding not used
Keywords: Syndromic meningitis panel, rapid diagnosis, rational use of molecular testing

OP-029
Diagnostic stewardship & Diagnostic test accuracy

Prospective comparison of cytomegalovirus quantification in whole blood and plasma samples among hematopoietic stem cell transplant and kidney transplant recipients

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AIM: Cytomegalovirus (CMV) induces multi-organ pathogenesis in hematopoietic stem cell transplant (HSCT) and kidney transplant (KT) recipients. Effective management involves systematic monitoring for CMV reactivation by quantitative real-time PCR, allowing timely preemptive intervention. However, the optimal blood compartment for CMV surveillance remains undetermined. The aim of the study was to compare the quantification of CMV DNA in paired plasma and whole blood samples.
METHODS: From June and October 2022, we conducted a prospective study with 390 sets of paired plasma and whole blood specimens collected from 60 HSCT and 24 KT recipients. CMV DNA levels were compared between the cobas® CMV assay on the automated cobas® 6800 system for plasma and the reference assay, Abbott RealTime CMV assay on the m2000 RealTime platform for whole blood.
RESULTS: The sensitivity and specificity of CMV quantification in plasma using the cobas® CMV assay were 90.0% and 94.8%, respectively, compared to whole blood quantification with the Abbott assay. The overall agreement between these two strategies was 0.89 (95%CI: 0.86–0.91). In samples with quantifiable results, a correlation was observed between the two methods (R2=0.62, 95%CI: 0.65–0.87, p<0.0001). CMV loads were significantly higher in whole blood, with a mean bias of 0.41 log10 IU/mL (95%CI: -0.32–1.15).
CONCLUSIONS: The cobas® CMV assay in plasma showed significant concordance with the Abbott RealTime CMV assay in whole blood, confirming the relevance of plasma samples for CMV monitoring in HSCT and KT recipients.
SOURCES OF FUNDING:
Keywords: cytomegalovirus, CMV load, HSCT, letermovir, Plasma

OP-030
Diagnostic stewardship & Diagnostic test accuracy

Dengue virus shedding kinetics in body fluids of infected patients, Rome, Italy, 2018-2023
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AIM: Dengue is a mosquito-borne viral disease caused by dengue virus (DENV), whose geographic distribution has been expanding. A proper diagnostic algorithm is essential to control dengue burden. We investigated the dynamics of DENV-RNA in samples longitudinally collected from 42 patients (median age 38 years, 57% female).
METHODS: We collected: 118 serum, 110 plasma, 90 whole blood (WB), 118 urine, 68 oral-swabs, 42 saliva, and 41 ocular-swabs.
RESULTS: During the first week, the highest rate of DENV-RNA detection was observed in WB (87%), followed by serum (84%), saliva (76%), plasma (63%), oral-swabs (59%), urine (42%), and ocular-swabs (2%). At week 2, DENV-RNA was detected in 84% WB, 62% serum, 55% urine, 38% saliva, 29% oral-swabs, and 19% plasma. Viral persistence was observed for 9 days from symptom onset (DSO) in saliva, 14 DSO in oral-swabs, 16 DSO in serum and plasma, 32 DSO in WB, and 39DSO in urine. The viral load in all the body fluids tended to decrease with time from symptom onset. Regarding the genital compartment, DENV-RNA was revealed in 20% cervicovaginal (n=49, released until 11DSO) and 30% seminal (n=23, 35 DSO) fluids.
CONCLUSIONS: WB should be the preferred sample for DENV molecular diagnostics; urine may be helpful at longer DSO. In the early phase, saliva is of advantage for testing infants and children, a population at higher risk for severe dengue. Understanding DENV dynamics in different anatomical districts is crucial to correctly apply diagnostic tools and shed light on viral tropism, alternative routes of transmission, or unusual clinical presentation.
SOURCES OF FUNDING: not-applicable
Keywords: dengue, viral shedding, molecular diagnostics, viral persistence

OP-031
Diagnostic stewardship & Diagnostic test accuracy

Diagnosing acute Epstein-Barr Virus infections: do all roads lead to Rome?

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AIM: Serology has long been the established diagnostic tool for diagnosing acute Epstein-Barr Virus (EBV) infections. In April 2021 during an EBV external quality control samples dispatch by the Belgian health authority (Sciensano), a survey on EBV test strategies was included to gain better insight into the approach for EBV diagnostics in Belgium.
METHODS: A survey was issued to 121 Belgian laboratories, querying the test strategy in use to diagnose an EBV infection based on the analysis of Epstein-Barr Nuclear Antigen 1 (EBNA-1) and Viral Capsid Antigen (VCA) antibodies. Besides five standard response options, the respondents could provide their own response option.
RESULTS: Of the 121 participants, 115 (95%) participants completed the survey. Eighty-two (71.3%) used the standard response options, whereas 33 (28.7%) laboratories responded differently. After analysing the results, two answers were reclassified to the standard response options. The majority of laboratories (n=104; 90.4%) use a predefined test strategy, while the other 11 (9.6%) laboratories leave the choice to the requesting physician. Almost half (n=57; 49.6%) of the laboratories perform all tests immediately, while the other half (n=58; 50,4%) use a reflex testing strategy. In 28 out of 115 (24%) laboratories EBNA-1 IgG is not performed.
CONCLUSIONS: Although most laboratories have a defined EBV test strategy, there is a high variability in the initial approach for EBV testing. Based on these results, we should critically evaluate these different algorithms for their ability to provide a correct interpretation of the patient's EBV status.
SOURCES OF FUNDING: None
Keywords: Epstein-Barr Virus, serology, diagnostic algorithms

OP-032
Development of new antiviral strategies

Safety, tolerability and immunogenicity of PRV-101, a multivalent vaccine targeting coxsackie B viruses associated with type 1 diabetes: a double-blind randomised placebo-controlled Phase 1 trial

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AIM: Coxsackie B viruses (CVBs) cause diseases ranging from mild respiratory illness to severe life-threatening conditions. The significant disease burden together with their association with chronic diseases such as cardiomyopathies and type 1 diabetes has prompted the development of human CVB vaccines. The current study evaluated the safety and immunogenicity of the first human vaccine designed against CVBs in a double-blind randomised placebo-controlled Phase 1 trial.
METHODS: In total, 32 participants (17 men) aged 18–44 years were randomised to receive a low (n=12) or high (n=12) dose of a multivalent, formalin-inactivated vaccine including CVB serotypes 1–5 (PRV-101), or placebo (n=8), given by intramuscular injections at weeks 0, 4 and 8. The participants were followed for another 24 weeks. Safety and tolerability were the primary endpoints. Anti-CVB IgG and virus-neutralising titres were analysed using an ELISA and neutralising plaque reduction assays, respectively.
RESULTS: No serious adverse events or adverse events leading to study treatment discontinuation were observed. Treatment-emergent adverse events considered to be related to the study drug occurred in 37.5% of the participants in the placebo group and 62.5% in the PRV-101 group (headache, injection site pain, injection site discomfort and injection site pruritus being most common). PRV-101 induced dose-dependent neutralising antibody responses against all five CVB serotypes included in the vaccine. Titres ≥8 against all five serotypes were seen in >90% of participants over the entire follow-up period.
CONCLUSIONS: The tested multivalent CVB vaccine is well tolerated and immunogenic, supporting its further clinical development.
SOURCES OF FUNDING: Provention Bio, a Sanofi company
Keywords: Enterovirus, Coxsackie B virus, vaccine, phase 1 trial, first-in-human trial, type 1 diabetes

OP-033
Development of new antiviral strategies

Development and characterisation of a novel SARS-CoV-2 macrodomain (Mac1) targeting inhibitor
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AIM: The SARS-CoV-2 replicase protein nsp3 encodes a macroD-like macrodomain (Mac1) with de-ADP-ribosylating (ADPR) activity [1]. Structurally similar domains are found in other Coronaviridae, but also in Togaviridae (e.g. ChikV) and Orthohepeviruses (e.g. HEV) [2], thus representing an interesting target for antiviral therapy.
METHODS: In-vitro testing of potential Mac1 inhibitors was performed by HPLC and/or fluorescence derivatisation based, and biophysical ligand binding assays were established. Candidate compounds were synthesised. Mac1 mutant viruses encoding a luciferase (orf7a) were generated using a reverse genetics approach. SARS-CoV-2 inhibitory potential of compounds was assessed in-vivo by infection experiments (Calu-3, Vero E6 cells). SARS-CoV-2 replication was assessed by RT-qPCR, luciferase assay and virus titration.
RESULTS: Testing a number of ADPR analogues resulted in an initial structure-activity-relationship for ADPR. A set of novel Mac1 inhibitor candidates were synthesised and evaluated regarding inhibition of the enzyme. Candidate inhibitors proved to specifically impair Mac1 activity in-vitro. For these compounds, membrane permeability and cellular uptake proved to be effective. Infection experiments revealed SARS-CoV-2 inhibition in the nanomolar range with complete virus growth inhibition at >0.5 µM without affecting cell viability (>50% viability at 10 µM (≙ >100x IC50)). Moreover, in mutant viruses with inactive Mac1 the IC50 was higher compared to wildtype viruses.
CONCLUSIONS: We developed and characterised a new effective inhibitor of the catalytically active macrodomain of SARS-CoV-2 bearing the potential of novel broad-spectrum treatment options.
SOURCES OF FUNDING: This study was supported by the Deutsche Forschungsgemeinschaft (DFG) (Project number: 335447717 –SFB 1328 Project A18 granted to Chris Meier, Susanne Pfefferle and Ralf Fliegert)
Keywords: SARS-CoV-2, MAC-1,MAC-1 inhibitor

OP-034
Development of new antiviral strategies

Prevention of BK virus DNAemia by IVIG administration in at-risk kidney transplant recipients

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AIM: BK virus (BKV) replication is a major problem in kidney transplant recipients (KTR) as it may lead to BKV nephropathy and graft loss. BKV is mainly transmitted through the donor kidney, and KTR with low levels of neutralizing antibodies (NAbs<4 log10IC50) against the donor strain are particularly at risk. In this study, we investigated whether early IVIg administration prevents BKV replication in at-risk KTR.
METHODS: In a prospective randomized clinical trial, twenty KTR were included (IVIG group n=9, Control group n=11). IVIG were administered at D10±4 days, D31±4 days and D52±10 days post-transplant. The incidence of BKV viruria and DNAemia was assessed at M6 and M12 post-transplant.
RESULTS: During follow-up, 5/11 KTR in the control group and 1/9 in the IVIG group showed BKV replication. There was a 100% match between donor neutralizing profile and replicating strain. At M6, viruria incidence was 11.1% in the IVIG group vs 55.6% in the control group (p=0.045) and DNAemia incidence was 0% vs 22.2% (ns). At M12, viruria incidence was 0% in the IVIG group vs 60.0% in the control group (p=0.009) and DNAemia incidence was 0% vs 20.0% (ns).
CONCLUSIONS: IVIG administration decreased the incidence of BKV viruria and DNAemia in at-risk KTR at any timepoint post-transplant. These preliminary results suggest that IVIG may be a valuable strategy for preventing BKV replication.
SOURCES OF FUNDING:
Keywords: BK virus, nephropathy, kidney transplantation, neutralizing antibodies, IVIG

OP-035
Development of new antiviral strategies

Brincidofovir sensitivity testing of vaccinia virus containing potential resistance associated mutations
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AIM: Brincidofovir (BCV) is one of the two antivirals approved for treatment of smallpox virus infections. The current study aimed to evaluate the sensitivity of six vaccinia virus (VACV) mutants encoding potential resistance-associated substitution mutations to BCV. These were identified by next-generation sequencing (NGS) of DNA samples collected during in vivo efficacy studies of BCV against mousepox.
METHODS: Mutant viruses were generated using CRISPR/Cas9 and virus reactivation methods. Plaque reduction assays were used to test the sensitivity of each mutant to BCV. VACV strains WR and GD1.3 were used as wild-type (no resistance) and resistant controls, respectively. Mean EC50 and 95% confidence intervals of BCV for each mutant were determined by ANOVA modeling. BCV resistance was defined as a mutant virus exhibiting a >2.5□ mean fold change in EC50 compared to VACV WR.
RESULTS: VACV clones containing six different substitutions (E581V, E581K, F964L, H389N, F419L, R1019G) in three different genes (E9L, J6R, and A24R) were successfully constructed as confirmed by NGS. Each mutant was evaluated for sensitivity to BCV in plaque reduction assays. The EC50 of the wild-type virus was 0.58μM whereas, all six mutants had mean EC50 values below 0.5μM (range 0.29-0.47μM) with EC50 ratios <1.0 (range 0.50-0.81) compared to the wild-type, confirming sensitivity of VACV mutants to BCV. By comparison VACV GD1.3, bearing A314T and A684V BCVR mutations in the E9L (DNA polymerase) gene, exhibited an EC50=5.7μM.
CONCLUSIONS: None of the mutants tested showed resistance to BCV compared to VACV WR wild-type control.
SOURCES OF FUNDING: DHHS/ASPR/BARDA (contract 75A50122C00047)
Keywords: Brincidofovir, Vaccinia virus, resistance, mutations, sensitivity

OP-036
Development of new antiviral strategies

Emerging host-targets for anti-mpox therapy

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AIM: Mpox virus (MPXV) is an emerging zoonotic pathogen which can cause severe disease in susceptible individuals. The rapid emergence of resistance to Tecovirimat, the only approved drug for MPXV treatment, urges for novel antiviral therapeutics.
METHODS: We performed a comparative screening using well-characterized tool compounds with known targets (drug-repurposing/kinase inhibitors sets). The antiviral activity of these drug-like molecules against MPXV, HSV-1 and AdV was assessed in primary human fibroblasts (HFF) using high-content imaging.
RESULTS: Two compounds currently in clinical use, nitroxoline (an antibiotic) and tirbanibulin (a Src/tubulin inhibitor), were identified as effective inhibitors against both MPXV and HSV-1. These compounds hindered the replication of a wide range of MPXV isolates in HFFs and primary keratinocytes at concentrations achievable in plasma. Additionally, both nitroxoline and tirbanibulin maintained their efficacy against a Tecovirimat-resistant strain of MPXV. Importantly, in our recently developed ex vivo human skin explant model for MPXV and HSV-1 infection, both compounds significantly reduced the number of virus-infected cells, highlighting their therapeutic potential. Importantly, we discovered that antiviral mechanism of nitroxoline against MPXV differs from its antibacterial action - the disruption of biofilms via metal ion chelation. Instead, nitroxoline interfered with cellular signalling pathways crucial for MPXV replication, specifically the PI3K/AKT/mTOR and Raf/MEK/ERK pathways. We have also demonstrated that antiviral effect of tirbanibulin was mediated by its anti-Tubulin activity, which disrupted post-entry steps of MPXV infection.
CONCLUSIONS: Our findings indicate that both nitroxoline and tirbanibulin hold promise for repurposing as antiviral therapeutics against MPXV.
SOURCES OF FUNDING: No project-specific funding
Keywords: mpox, antivirals, host-target, nitroxoline, tirbanibulin

OP-037
Immune response and vaccines

T-cell immunity to a conserved coronavirus epitope correlates with improved outcome of SARS-CoV-2 infection

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AIM: T-cell immunity is a major determinant of the outcome of SARS-CoV-2 infection and vaccination. How pre-exposure cross-reactive T cells to conserved epitopes between common cold Coronaviruses influence disease outcome is poorly understood. We determined T-cells to SARS-CoV-2-specific and cross-reactive peptides in HLA-matched patients with mild or severe COVID-19 and in individuals pre- and post-BNT162b2-mRNA vaccination.
METHODS: The study involves 142 individuals (73 patients, 51 vaccinated individuals, 18 pre-pandemic donors). T-cell responses were determined with ELISPOT assays, using a set of naturally processed peptides and peptide pools covering the entire spike protein. Peptide:MHCII binding strength was predicted for conserved peptides in question with all 222 different MHC class II molecules of the study cohort using the Immune Epitope database.
RESULTS: We observed an immunodominant response against a highly conserved peptide (S813-829), located in the fusion domain of the spike, with frequencies of 19%, 51% and 55% in pre-pandemic donors, patients, and BNT162b2-vaccinated individuals, respectively. Patients with S813-829-specific T-cell responses were significantly less likely to develop severe COVID-19, including necessity for ICU admission. High frequencies of S813-829-specific T cells relative to the total spike response were observed in patients with mild disease and in individuals post-vaccination. In patients with critical illness, these S813-829-specific cell frequencies were significantly reduced (p=0.0006).
CONCLUSIONS: The results suggest that SARS-CoV-2 infection and vaccination frequently induces responses against cross-reactive T-cell epitopes presented by highly prevalent HLA-alleles that enhance T-cell immunity and correlate with a favorable disease outcome.
SOURCES OF FUNDING: Medical-Scientific fund of the Mayor of the federal capital Vienna (grant 23096)
Keywords: SARS-CoV-2, T-cell, Immunity

OP-038
Immune response and vaccines

IL-12 is a key responsive element to corticosteroid treatment in COVID-19

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AIM: Treatment with corticosteroids is standard of care for patients with severe COVID-19. Yet, little is known about the specific effect of steroid treatment during the acute infection. Here, we investigated the impact of steroid treatment on the immune responses during acute COVID-19.
METHODS: We took use of COVID-19 patient samples collected before steroid treatment was implemented as standard of care and applied propensity score matching to assess the impact of corticosteroids on the antiviral immune response. To this end, we studied the soluble proteome as well as immune cell numbers in relation to steroid treatment. Additionally, we combined single-cell RNA sequencing of in vitro stimulated cells with publicly available data from patients with severe COVID-19.
RESULTS: Of note, the key cytokine reduced by steroid treatment was IL-12, displaying a close correlation to the total dose of steroids administered before sampling. Furthermore, via publicly available and newly generated single-cell RNA sequencing data we show that IL-12 signaling is enhanced in patients with severe COVID-19 and that corticosteroids predominantly target the IL-12 pathway via myeloid cells. Downstream, NK cell function is altered by the loss of this key cytokine.
CONCLUSIONS: Collectively, we show that corticosteroids have a surprisingly narrow effect on the inflammatory immune mediators during acute infection with SARS-CoV-2 affecting predominantly innate immune cells.
SOURCES OF FUNDING: European Research Council, Swedish Research Council, Swedish Cancer Society, Swedish Society for Medical Research, Swedish Foundation for Strategic Research, Wallenberg Foundation, Novo Nordisk Foundation, CIMED at Karolinska Institutet, Norwegian Health Authorities, Oslo University Hospital, Nordstjernan, Region Stockholm, and Karolinska Institutet.
Keywords: Corticosteroids, COVID-19, IL-12, antiviral immune response

OP-039
Immune response and vaccines

Specific long-term changes in anti-SARS-CoV-2 IgG modifications and antibody functions in mRNA, adenovector, and protein subunit vaccines

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AIM: Various vaccine platforms were developed and deployed against the COVID-19 disease. The Fc-mediated functions of IgG antibodies are essential in the adaptive immune response elicited by vaccines. However, the long-term changes of protein subunit vaccines and their combinations with mRNA vaccines are less well known. Therefore, we investigated the IgG profile of the Taiwanese protein subunit vaccine (Medogen) in comparison to adenovector and mRNA vaccines.
METHODS: A total of 272 serum and plasma samples were collected from individuals who received first to third doses of the protein subunit Medigen, the mRNA (BNT, Moderna), or the adenovector AstraZeneca vaccines. The IgG subclass level was measured using enzyme-linked immunosorbent assay, and Fc-N glycosylation was measured using LC-MS/MS. Antibody-dependent-cellular-phagocytosis (ADCP) and complement deposition (ADCD) of anti-spike (S) IgG antibodies were measured by flow cytometry.
RESULTS: IgG1 and 3 reached the highest anti-S IgG subclass level. IgG1, 2, and 4 subclass levels significantly increased in mRNA- and Medigen-vaccinated individuals. Fc-glycosylation was stable, except in female BNT vaccinees, who showed increased bisection and decreased galactosylation. Female BNT vaccinees had a higher anti-S IgG titer than that of males. ADCP declined in all groups. ADCD was significant lower in Astrazeneca vaccinated individuals.
CONCLUSIONS: Each vaccine produced specific long-term changes in Fc structure and function. This finding is critical when selecting a vaccine platform or combination to achieve the desired immune response.
SOURCES OF FUNDING: Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan (grant number: EMRPD1N0071) and “National Institutes of Health, USA (grant number: U01 AI151698)
Keywords: IgG, COVID-19, protein subunit vaccine, Fc-function, adenovector vaccine, mRNA vaccine

OP-040
Immune response and vaccines

Individual T-cell Cytokine patterns and b-cell reactions after HPV vaccination elucidated by multi-colour EliSpot

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AIM: Human Papillomaviruses (HPV) cause most anogenital warts and anogenital and oropharyngeal cancers. Vaccination with virus-like-particle-based (VLP) HPV vaccines is recommended for persons age 9-18 years. We characterized vaccine-induced T- and B-cell-mediated immunity and investigated cellular cross-reactivity against L1-VLPs of genotypes 6,11,16,18 by EliSpot.
METHODS: T-cell cytokine secretion was monitored multiparametric to elucidate T-cell pattern changes. IFN-γ (active), IL-2 (memory) and IL-5 (transient response during vaccination-induced T-cell activation) were detected simultaneously in T-cells in one well. HPV-specific B-cells were detected using fluorescently labelled VLPs from vaccine HPV types to investigate potential antibody cross-reactivity.
RESULTS: HPV-reactive T-cells were clearly distinguishable between vaccinated and naïve blood donors. In vaccinated IL-2 expression was highest (mean SI 91,58 [CI 34,77-148,4] versus 4,347 [CI 1,992-6,701]). IL-5 expression (mean SI 7,217 [CI-1,16-15,6] versus 0,27 [CI 0,04-0,5]) was exclusively detectable in freshly vaccinated donors, indicating functional T-helper 2 cells against HPV 6,11,16,18 VLPs. Functional B-cells and cross-reactive antibody responses were seen between VLPs from HPV 6&11 (low-risk-mix) and 16&18 (high-risk-mix) that were labeled with fluorescent dyes. B-cell numbers for both mixes were evaluated in one well simultaneously. The vaccine dose-dependent spot number was higher for all HPV types in vaccinated than in naïve donors. B-cell reactions to the low-risk-mix were observed in vaccinees of the bivalent vaccine containing exclusively high-risk HPV16/18 VLPs. This might indicate a cross-reactive, potentially protective cellular immune reaction after vaccination.
CONCLUSIONS: We show detection of differences between HPV-vaccinated and naïve blood donors which offers possibilities to correlate clinical effectiveness with cellular immune responses.
SOURCES OF FUNDING: not available
Keywords: T-cells, EliSpot, HPV, vaccination, B-cells, multi-color EliSpot

OP-041
Immune response and vaccines

A Combined Cross-Sectional Analysis and Case-Control Study Evaluating Tick-Borne Encephalitis Vaccination Coverage, Disease and Vaccine Effectiveness in Children 0-17 in Switzerland, 2005-2022

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AIM: Tick-borne encephalitis (TBE) is a severe, vaccine-preventable viral infection of the central nervous system. Symptoms are generally milder in children and adolescents than in adults, though severe disease does occur. A better understanding of the disease burden and duration of vaccine-mediated protection is important for vaccination recommendations. Here we aimed to estimate TBE vaccination coverage, disease severity and vaccine effectiveness (VE) among individuals aged 0–17 years in Switzerland.
METHODS: Vaccination coverage between 2005 and 2022 was estimated using the Swiss National Vaccination Coverage Survey (SNVCS), a nationwide, repeated cross-sectional study assessing vaccine uptake. Incidence and severity of TBE between 2005 and 2022 were determined using data from the Swiss disease surveillance system and VE was calculated using a case–control analysis, matching TBE cases with SNVCS controls.
RESULTS: Over the study period, vaccination coverage increased substantially, from 4.8% (95% confidence interval (CI): 4.1–5.5%) to 50.1% (95% CI: 48.3–52.0%). Reported clinical symptoms in TBE cases were similar irrespective of age. Neurological involvement was less likely in incompletely (1–2 doses) and completely (≥ 3 doses) vaccinated cases compared with unvaccinated ones. For incomplete vaccination, VE was 66.2% (95% CI: 42.3–80.2), whereas VE for complete vaccination was 90.8% (95% CI: 87.7–96.4). Vaccine effectiveness remained high, 83.9% (95% CI: 69.0–91.7) up to 10 years since last vaccination.
CONCLUSIONS: Even children younger than 5 years can experience severe TBE. Incomplete and complete vaccination protect against neurological manifestations of the disease. Complete vaccination offers durable protection up to 10 years against TBE.
SOURCES OF FUNDING: Swiss Federal Office of Public Health, EMDO Stiftung, University of Zurich.
Keywords: Tick-borne Encephalitis, Vaccine Effectiveness, Infectious Disease, Epidemiology, Pediatric Vaccination, Immunity

OP-042
Viral infections in pregnancy

Sequelae of congenital cytomegalovirus following maternal primary infections are limited to those acquired before 12 weeks

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AIM: To refine the period at risk of sequelae of congenital cytomegalovirus (cCMV) infection, using a logarithm model to date maternal primary infection (MPI)
METHODS: Among the children with sequelae following MPI during pregnancy we calculated the date of MPI using CMV serology. If positive IgM was associated with negative IgG (IgM+/IgG-), we estimated the date of MPI 10 days earlier. In case of seroconversion, MPI was estimated at the mean date between IgG-negative and IgG-positive. In other cases, we dated the MPI using a logarithmic model and IgG avidity Vidas. This model was trained on 208 cases of MPI for which the date of MPI was known.
RESULTS: 69 children with cCMV related sequelae were identified. Precise dating was obtained for 61 (88%) of cases using IgM+/IgG- results (26%), seroconversion (15%) or the logarithmic model (59%). Unilateral hearing loss (HL), bilateral HL and motor/intellectual retardation were identified in 43 (64%), 14 (21%) and 15 (22%) respectively. 14 (23%), 30 (49%), 17 (28%) and 0 (0%) of the MPIs were dated before 2, 3 to 7, 8 to 12, and after 12 weeks respectively. No neurological sequelae or bilateral HL were associated with MPI after 10 weeks. No unilateral HL was associated with MPI after 12 weeks.
CONCLUSIONS: In our study, the MPI occurred before 12 weeks in all and before 10 weeks in 90% of cases. No severe sequelae were observed following a MPI occurring after 10 weeks.
SOURCES OF FUNDING: The study was funded by a grant from PHRC (Ministère de la Santé N° 2013-A00213-42)
Keywords: Congenital, cytomegalovirus, CMV, maternal primary infection, hearing loss, sequelae

OP-043
Viral infections in pregnancy

Universal neonatal screening for congenital Cytomegalovirus, nine years after implementation and still going strong

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AIM: We demonstrate the impact on patient care of universal neonatal screening for congenital Cytomegalovirus (cCMV) in our hospital.
METHODS: From February 2nd, 2015 onwards, a universal neonatal CMV screening program was implemented in our Belgian secondary care hospital. Within 48 hours postpartum, saliva of the newborn is collected by the pediatrician using Eswab (Copan, Italy) and analyzed for CMV DNA by a lab developed RT-PCR. Positive saliva tests are confirmed by demonstrating CMV presence on a urine sample of the newborn and/or by comparison with CMV serology of the newborn's mother. Results are analyzed until February 1st 2024.
RESULTS: Almost all newborns were tested (19653/19842, 99.0%). 221 newborns (1.1%) showed a positive screening result. Only in 84 (0.4%) cCMV infection was confirmed, of which 32.1% (n=27) were symptomatic. Without the screening program, 64 (76,2%) cCMV cases, of which 29.7% (n=19) symptomatic, would not have been detected immediately after birth. 28 (33.3%) newborns with cCMV were infected following a non-primary maternal infection. Approximately ⅓ (n=9) of these newborns were also symptomatic. Prevalence fluctuated from only 4 cCMV cases in 2017 to maximum 14 cases in 2015.
CONCLUSIONS: Universal neonatal cCMV screening was easily adopted and is here to stay at our hospital as we were able to detect many prima facie unrecognised (symptomatic) cCMV cases. As confirmation of positive saliva CMV screening results is necessary by analysis of an independent urine sample or serology of the mother, a decentralized approach of universal cCMV screening seems more efficient.
SOURCES OF FUNDING: None
Keywords: congenital Cytomegalovirus, newborn screening, PCR, saliva

OP-044
Viral infections in pregnancy

Exploring the urinary metabolomic fingerprint of human cytomegalovirus: a 1H-NMR study on congenitally infected newborns

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AIM: Human cytomegalovirus (HCMV) is the leading cause of congenital infections resulting in severe morbidity and mortality among newborns worldwide. Nevertheless, little is known about the metabolic response triggered by HCMV in congenitally infected newborns. As such, urinary metabolic profiling by 1H-nuclear magnetic resonance (NMR) might represent a promising tool to be exploited in the context of cHCMV. This study aims to investigate the impact of HCMV infection on the urine metabolome in a population of newborns by 1H-NMR spectroscopy combined with multivariate statistical analysis.
METHODS: Thirty-five newborns diagnosed with cHCMV and fifteen uninfected controls were recruited. The 1H-NMR spectra of patients and controls allowed the identification of 55 metabolites.
RESULTS: Principal Component Analysis (PCA) and clustering correctly assigned 48 out of 50 newborns into the infected and control group. Partial least squares-discriminant analysis (PLS-DA) revealed that newborns with cHCMV resulted to have increased betaine, citrate, succinate, acetate, urea, galactose, glycolate, and formiate levels in the urine. On the other hand, healthy controls showed increased 1-methylnicotinamide, myoinositol, ethanolamine, glycine, taurine, fumarate, creatinin, and creatinin-phosphate levels. Specifically, succinate emerged as the discriminating metabolite in cHCMV newborns, whereas glycine and taurine were characteristic of healthy controls.
CONCLUSIONS: These results showed a clear difference in terms of metabolomic fingerprint between newborns with cHCMV infection and healthy controls. Thus, metabolomics can be considered a new promising diagnostic and prognostic tool in the clinical management of cHCMV patients.
SOURCES OF FUNDING: Italian Ministry of University and Research (PRIN 2022S3AZCC; Project CH4.0 under the MUR program “Dipartimenti di Eccellenza 2023-2027”)
Keywords: metabolomics, proton nuclear magnetic resonance spectroscopy, human cytomegalovirus, urine, congenital infections.

OP-045
Viral infections in pregnancy

Pichinde virus infection of pregnant guinea pigs as a comparative model of human Lassa virus infection during pregnancy

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AIM: Arenaviruses, including Lassa virus (LASV) and Junin virus (JUNV), can cause severe and lethal arenaviral hemorrhagic fevers (AHF) in West/South Africa and South America. LASV can cause spontaneous abortion and maternal demise in nearly 90% of the cases. Unlike LASV and JUNV that must be handled in A/BSL4 laboratory, Pichinde virus (PICV) is a nonpathogenic arenavirus that was in vivo adapted to cause AHF-like disease in guinea pigs and is therefore a good surrogate AHF model. We sought to determine whether PICV can infect placental tissues in vitro and in vivo and to assess the degrees of maternal and fetal disease severity.
METHODS: Explanted term human placenta, trophoblast stem cells (TSCs), and TSC-derived trophoblasts were used. Virus production in pregnant and non-pregnant guinea pigs was assessed. Infected tissues were stained for pathologic analysis, and RNA-scope was used to assess the type and location of the infected cells. RNA-seq was used to determine the effect of infection on host gene expressions.
RESULTS: PICV was found to efficiently replicate in term human placental explants and caused dysregulated gene expressions. Viremia were evidenced and stillbirth or fetal resorption was also observed. Acute tissue inflammation and necrosis of placenta were noted. PICV appeared to initially infect the parietal yolk sac epithelium, decidual endothelium, and monocytes before spreading into the labyrinth. The amnion and yolk sacs were also found to be infected.
CONCLUSIONS: These findings demonstrate the utility of PICV-infected guinea pigs as a convenient surrogate model for studying disease pathogenesis caused by AHF infection during pregnancy.
SOURCES OF FUNDING: None.
Keywords: Arenavirus, Lassa virus, Junin virus, Pichinde virus, pregnancy, guinea pigs

OP-046
Viral infections in pregnancy

Valacyclovir in Pregnancy To Prevent and Treat Congenital Cytomegalovirus Infection: Results from a Large Tertiary Care Hospital in Rome

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AIM: Clinical studies showed that high dose (8 g/day) valacyclovir (HD-VCV) is a good option for the prevention and treatment of congenital cytomegalovirus (CMV) infection. The study aims to evaluate the effects of HD-VCV on fetal infection and sequelae in a large tertiary care hospital in Rome.
METHODS: 48 pregnant women with CMV infection and admitted to the Fondazione Policlinico A. Gemelli IRCCS, Rome, were divided into two groups: the first group (group 1, N=19) was treated with HD-VCV from the diagnosis of CMV maternal infection to the amniocentesis. In particular, 14/19 women withdrawn from HD-VCV due to the negative amniocentesis, whereas 5/19 continued the drug because of CMV detection in the amniotic fluid. The second group (Group 2, N=29) started HD-VCV as early as the diagnosis of CMV fetal infection was made and continued treatment until delivery.
RESULTS: In group 1, the vertical CMV transmission rates at birth were 21% and 100% in those women treated with HD-VCV until amniocentesis and delivery, respectively. Importantly, only 1/19 (5%) newborns was symptomatic. Regarding group 2, the vertical CMV transmission rate at birth was 90%. Interestingly, in this group, 8/29 (28%) fetuses resulted symptomatic at ultrasound but only 4/29 (14%) newborns were symptomatic. Viral load on amniotic fluid was higher among the symptomatic than the asymptomatic infected newborns (P=0.001). No adverse effects in HD-VCV-treated women were reported.
CONCLUSIONS: This study supports the idea that valacyclovir should be started as soon as the maternal diagnosis is performed and continued until delivery.
SOURCES OF FUNDING: Nothing to disclose.
Keywords: Cytomegalovirus, Valacyclovir, Pregnancy

OP-047
(Re-) emerging viral infections and one health

High West Nile virus infection rates in mosquitoes from Berlin, 2023

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AIM: West Nile virus (WNV) is a mosquito-borne flavivirus that is maintained in nature through a transmission cycle involving birds and mosquitoes. WNV infection in humans may lead to West Nile Fever and West Nile neuroinvasive disease. WNV is endemic in eastern Germany including Berlin since 2018. Here, we aimed to get insight into urban areas that favour WNV amplification in Berlin.
METHODS: We collected mosquitoes at five sites with less than 1 km distance to each other: a sponge city site (S), a residential courtyard (RB), a park-like residential area (RA), a park-like cemetery (C) and a natural conservation area (N). Mosquitoes were collected for four consecutive nights once per month from June to September 2023, identified morphologically and tested for WNV infection by qPCR.
RESULTS: In total, 13.627 mosquitoes were collected, with highest densities at N (n=4202) and C (n=3927), and lowest at RB (n=669). For all sites, the main vectors of WNV were the most prominent (up to 92%). WNV was detected in 78 mosquito pools. While mosquito density peaked in July, WNV infection rates peaked in August, with the highest minimum infection rate (MIR) at C (2.08 %) and RA (1.46%). Surprisingly low MIR (0.26% in August) were detected at N. All detected WNV strains contained unique Berlin-specific nucleotide variants and emerged from local amplification.
CONCLUSIONS: An unexpected high risk for acquiring a WNV infection at RA and C was found. Overall, our study provides evidence for extensive endemic circulation of WNV in Berlin in 2023.
SOURCES OF FUNDING: Berlin University Alliance
Keywords: West Nile virus, mosquito-borne virus, virus ecology

OP-048
(Re-) emerging viral infections and one health

A 10 year long-lived cellular and humoral MERS-CoV immunity cross-recognizing the Wild-type and Variants of SARS-CoV-2: a potential one-way MERS-CoV cross-protection towards a pan-coronavirus vaccine

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AIM: The Middle East respiratory syndrome (MERS) is a respiratory disease caused by the highly pathogenic Middle East Respiratory Syndrome coronavirus (MERS-CoV). It is unknown how long the immune responses last beyond 6 years, and the effectiveness of MERS-CoV memory humoral and cellular immunity in protecting against MERS-CoV re-infection and its potential cross-recognition of SARS-CoV-2 and its variants.
METHODS: This study investigates the long-lasting cellular and humoral immunity against MERS-CoV and its potential cross-recognition of SARS-CoV-2 and its variants. We conducted a comprehensive study involving two co-horts of individuals with a history of MERS-CoV infection (with or without COVID-19 vaccination) spanning 10 years. We assessed the persistence of IgG antibodies, memory CD4+ and CD8+ T cells specific to MERS-CoV, and their cross-reactivity against Wild-type and Variants of SARS-CoV-2. Additionally, we examined the neutralizing antibodies against HCoV-EMC/2012 and Nigeria/NV1657 MERS-CoV strains.
RESULTS: Our findings reveal a remarkable longevity of cellular and humoral immunity against MERS-CoV. MERS-CoV-specific cellular and humoral memory immunity were detected a decade after infection. Importantly, this immunity exhibited cross-recognition of SARS-CoV-2 and its variants, suggesting a potential one-way cross-protection from MERS-CoV towards SARS-CoV-2. Furthermore, we found that MERS-CoV survivors vaccinated against SARS-CoV-2 had significantly higher anti-MERS IgG, cellular immune response and neutralization than unvaccinated survivors.
CONCLUSIONS: These findings indicate that the COVID-19 vaccination boosted and reshaped the long-term humoral and cellular immune memory established from the MERS-CoV infection. Our findings may have significant im-plications for the development of a pan-coronavirus vaccine.
SOURCES OF FUNDING: King Fahad Medical City funded this study (Grant No IRF 019-058).
Keywords: MERS-CoV, SARS-CoV-2, cross-reactivity, cellular immunity, humoral immunity, short-term T cell line.

OP-049
(Re-) emerging viral infections and one health

Seroprevalence and vaccination coverage of tick-borne encephalitis in Sweden
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AIM: In Sweden, little is known about the tick-borne encephalitis virus (TBEV) seroprevalence in the population, including vaccine coverage and infection. This is due to the absence of a national TBEV vaccination register and few previous studies. Furthermore, it is believed that a majority of TBEV infections are undiagnosed. The aim was to use a method that can differentiate vaccination antibodies from infection antibodies to estimate the TBEV seroprevalence in Sweden and compare the results with reported cases of TBE.
METHODS: 2,700 serum samples from blood donors in nine Swedish regions, collected in 2018 and 2019, were analyzed using a serological method that can distinguish antibodies induced by vaccination from antibodies elicited by infection. The regions were chosen to reflect differences in notified TBE incidence.
RESULTS: The overall TBEV seroprevalence varied from 9.7% to 64% between the counties. The proportion of vaccinated and infected individuals ranged from 8.7% to 57.0% and from 1.0% to 7.0%, respectively, between the counties. According to our estimation, more than 160,000 and 1,600,000 individuals could have been infected by TBEV and vaccinated against TBE, respectively. The mean manifestation index was 3.1%
CONCLUSIONS: As expected, a difference was observed between low- and high-incidence TBE regions, on the overall TBEV seroprevalence and when separated into vaccinated and infected individuals. The estimated incidence and manifestation index argue that a large proportion, probably >90% of TBEV infections are not diagnosed and consequently unreported.
SOURCES OF FUNDING: Sources of funding: Region Uppsala, Swedish Research Council, European Union Horizon 2020 Research innovation programme, SciLifeLab Pandemic Laboratory Preparedness projects.
Keywords: Tick-borne encephalitis, seroprevalence, vaccination

OP-050
(Re-) emerging viral infections and one health

Identifying gaps in population immunity in light of large measles outbreaks in Austria in 2023 and 2024
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AIM: Measles is a highly contagious viral infection with potentially severe complications. Despite efforts to eradicate measles, 598 cases have been reported in Austria since 2023. Therefore, we characterized the current outbreak by comprehensive genotyping (over 80% of all cases). Further, we analyzed a potential decrease of population immunity to measles as the facilitating factor of the high incidence.
METHODS: Since 2023, 492 (2023: n=156; 2024: n=336) measles cases were genotyped at the National Reference Laboratory using sanger sequencing. In parallel, we estimated the anti-measles IgG seroprevalence in the Austrian population stratified by the year of birth (sample cohort from diagnostic testing, n = 53,119). The ELISA cut-off for seropositivity was validated using a measles neutralization test as a reference.
RESULTS: Genotyping revealed a large number (n=16) of measles subtypes of the genotypes B3 and D8 in Austria since 2023, indicating a high frequency of independent importations. The most commonly detected subtypes were D8-8350, D8-5963 and B3-6418. Of note, the seroprevalence data revealed that while over 95% of individuals born before 1970 were immune to measles, more than 20% of individuals born since 1990 were seronegative. However, there was no large effect of antibody waning over time.
CONCLUSIONS: Here, we show genetic evidence for multiple independent importations of measles to Austria, together with comprehensive data on immunity gaps specifically in the younger population. These factors likely facilitated the recent measles outbreaks in Austria, emphazising that the vaccination coverage has to be specifically increased in children and young adults.
SOURCES OF FUNDING: Medical University of Vienna
Keywords: measles, genotypes, outbreak, seroprevalence, vaccination, immunity

OP-051
(Re-) emerging viral infections and one health

Comparison of neutralizing and binding antibodies levels against different orthopoxviruses in mpox patients and healthy vaccinated individuals

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AIM: Since May 2022, mpox affected to at least 104 non-endemic countries. Traditional serological tests present diagnostic accuracy limitations due to cross-reactivity among human-pathogenic-orthopoxviruses (OPXVs). Understanding OPXVs cross-reactivity can aid diagnosis and enhance cross-protection comprehension. Our study aimed to assess both specific-OPXVs IgA, IgM, and IgG responses and OPXVs neutralizing-antibodies (NAbs) cross-reactivity in mpox patients and MVA-BN-vaccinated HCWs.
METHODS: Sequential serum samples were collected up to three months from mpox convalescent subjects (n=15) and MVA-BN-vaccinated HCWs (n=6). Sera were tested for specific OPXVs IgG, IgA, and IgM through IFA, and for specific-MPXV, -VACV, and -CPXV NAbs through PRNT50. Moreover, FRNT50 was assessed employing MVA-BN/GFP recombinant virus with an external Guinea-pig-complement source.
RESULTS: In mpox patients OPXV-IgG levels steadily increased following the symptoms onset; higher IgA levels were observed earlier compared to IgM. In same patients, PRNT50 did not allow to clearly distinguish among OPXVs showing in some cases higher VACV-NAbs titers than MPXV-NAbs. FRNT50 and PRNT50 titers show similar trends. Conversely, differential NAbs titers effectively allowed to distinguish MVA-BN-vaccinated individuals, showing significantly higher VACV-NAbs compared to other OPXVs.
CONCLUSIONS: In our study, both mpox patients and MVA-BN-vaccinated individuals exhibited cross-reactive NAbs against OPXVs, affirming the efficacy of vaccination as well as the natural infection in inducing NAbs against other OPXVs. Assessment of differential neutralizing titers could provide a diagnostic tool suitable for retrospective analyses OPXVs outbreaks.
SOURCES OF FUNDING: EU fundings within MUR-PNRR Extended Partnership Initiative on Emerging Infectious Diseases (No. PE00000007, INF-ACT); Italian Ministry of Health fundings "Fondi Ricerca Corrente".
Keywords: mpox, cross-neutralizing antibodies, orthopoxviruses, MVA-BN vaccine

OP-052
Hepatitis, HIV and other blood borne viruses

HDV infection in the UK

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AIM: HBV/HDV co-infection is the most severe form of chronic viral hepatitis. We aimed to define the epidemiology and clinical burden of HDV infection in the UK
METHODS: Laboratories undertaking either/both anti-HDV/HDV RNA testing in the UK were identified through a questionnaire administered through the UK Clinical Virological Network and asked to provide testing data. Data were deduplicated at UKHSA. A national HDV register was established. Clinicians were sent lists of HDV RNA positive patients thought to be resident within their catchment area and asked to fill in the register data. Further data linkage was performed using Hospital Episode Statistics to identify in-hospital stays for end-stage liver disease and/or a diagnosis of hepatocellular carcinoma (HCC), and with the National Transplant database.
RESULTS: Testing data returned from 11 laboratories revealed 55,392 individuals were tested for hepatitis D (either antibody and/or antigen) between 2011-2021. 97,009 individuals were newly diagnosed with HBV, of whom 42% were linked to a HDV test. 2626 were anti-HDV positive, 56% of whom subsequently underwent HDV RNA testing. 927 individuals were HDV RNA positive. Where data was returned to the register, 94% were born outside the UK, 45% were cirrhotic, 21% had at least one episode of decompensation, 13% had undergone liver transplantation, and 9% had a diagnosis of HCC.
CONCLUSIONS: Testing for HDV infection in the UK has been sub-optimal over the past 10 years. There is a need to improve this. HDV RNA positivity was associated with severe life-threatening liver disease.
SOURCES OF FUNDING: Grant from Gilead Sciences Limited
Keywords: Hepatitis delta; HDV; anti-HDV; HDV RNA; UK

OP-053
Hepatitis, HIV and other blood borne viruses

HIV-2 infections in Southern France and genotypic assessment though genome next generation sequencing

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AIM: HIV-2 virological management requires specific molecular biology approaches including for RNA and DNA quantification and drug resistance genotypic testing to guide therapy. We aimed to describe HIV-2 genotypes over the last 2 years, particularly by genomic analysis.
METHODS: The study covered years 2022-2023. Genotyping of the HIV-2 protease, reverse transcriptase and integrase genes used in-house protocols and Sanger technology. For two patients, genotyping was performed after next generation sequencing (NGS) using Illumina technology on a MiSeq instrument post-enrichment with the Illumina Virus Surveillance kit. Genomic obtaining and analysis used bioinformatic tools CLCGenomics, BLAST, NCBI genotyping, HIV grade, geno2pheno, Stanford HIV database, MEGAX; and the ANRS-MIE resistance algorithm.
RESULTS: 33 patients underwent virological management in our institution laboratory, among whom five newly underwent a genotypic drug resistance testing. There were 2 HIV-2 subtype A and 3 non-A subtype (B: 1; AB: 2). For two patients whose HIV-2 RNA loads were 5.0 and 5.7 log10copies/mL, quasi-complete genomes were obtained by NGS post-enrichment from plasma. These genomes were a recombinant CRF01_AB and of subtype A, and their closest relative were genomes obtained in 1989 from Ivory Coast and in 2001 from Guinea-Bissau, respectively. They did not show antiretroviral resistance-associated mutations and showed a R5 tropism.
CONCLUSIONS: HIV-2 remains of non-exceptional prevalence in our region. It is important to optimize its genotypic characterization, which is possible using NGS. HIV-2 genomes' number worldwide is very low (c.a.100), although HIV-2 is of tremendous interest given both its similarities and differences with HIV-1.
SOURCES OF FUNDING: National Agency for Research (ANR) (Méditerranée-Infection 10-IAHU-03).
Keywords: HIV-2, genome, next-generation sequencing, mutation, genotype, resistance

OP-054
Hepatitis, HIV and other blood borne viruses

Heterogeneity in the diagnostic performances of HDV RNA quantification assays used in clinical practice in Italy: data from the first national quality control multicenter study

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AIM: This quality-control study compares the diagnostic performances of quantitative HDV-RNA assays, used in clinical-practice.
METHODS: Two HDV-RNA sample panels were quantified at 30 laboratories by 6 commercial assays: RoboGene(N=9 laboratories), Eurobio/EliTech-platform(N=7), Altona(N=5), Anatolia(N=3), DiaPro(N=2), Nuclear-Laser-Medicine(N=1) and 3 in-house assays. Panel-A and -B comprised 8 serial dilutions of WHO/HDV standard(range:5-0.5logIU/ml) and 20 clinical samples(range:6-0.5logIU/ml), respectively. Laboratories quantified dilutions of Panel-A and -B 9 and 5 times, respectively, in order to define for each assay: i)sensitivity by 95%LOD(limit of detection), ii)precision by intra- and inter-run CV(coefficient of variation), iii)accuracy by the differences between expected-observed HDV-RNA loads, iv)linearity by linear-regression analysis.
RESULTS: In Panel-A, 95%LOD varied across the assays underlining heterogeneous sensitivities: Altona had the lowest median 95%LOD (10[min-max:3-316]IU/ml), followed by Robogene (31[3-316] IU/ml), Nuclear-Laser-Medicine (31IU/ml) and EliTech (100[100-316]IU/ml). Moreover, 5 assays (Robogene/EliTech/Altona/Nuclear-Laser-Medicine/In-house) showed <0.5logIU/ml differences between expected and observed HDV-RNA for all dilutions while the remaining assays had HDV-RNA underestimations>1logIU/ml. In Panel-B, Altona and EliTech had the highest precision (mean intra-run CV<20%), followed by Robogene and Nuclear-Laser-Medicine (<30%).

Inter-run CV was higher for all assays with only Altona, Nuclear-Laser-Medicine and EliTech maintaining this parameter<25%. Five assays (Robogene/Altona/EliTech/Nuclear-Laser-Medicine/In-house) showed a good linearity(R2>0.9) between LLOQ and 6.7logIU/ml. Conversely, a linearity drop emerged for HDV-RNA<1000IU/ml, with only Altona and Robogene retaining R2>0.85.
CONCLUSIONS: This study underlines heterogeneous sensitivities (inter- and intra-assays), that could hamper the proper HDV-RNA quantification, particularly at low viral-loads. This raises the need to improve the diagnostic performance of most assays for properly identifying virological response to anti-HDV drugs.
SOURCES OF FUNDING: Gilead Research Grant
Keywords: hepatitis Delta; HDV-RNA assays, HDV-RNA sensitivity, diagnostic performance

OP-055
Hepatitis, HIV and other blood borne viruses

Large Hepatitis E virus Genotype 3 outbreak in New Caledonia Island

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AIM: Between August and December 2023, 127 symptomatic cases of hepatitis E virus (HEV) infections were reported to the Public Health Service of New Caledonia island. This prompted epidemiologic and virological investigations to identify the source of infection.
METHODS: HEV RNA was assessed in symptomatic patients, several food items and pig farms of the island. HEV strains were characterized by sequencing. A seroprevalence study in asymptomatic blood donors was also performed before and after the outbreak.
RESULTS: 127 symptomatic cases were reported. An hospitalization was required in 29/127 patients (22,8%). Hospitalized patients had more frequently comorbidities (80.7% versus 27%, p< 0.01). Three persons died. Among the 100 HEV RNA positive patients received at the French National refence Centre for HEV, sequencing was possible in 76 patients. All strains belonged to HEV genotype 3 and 74/76 strains grouped together (nucleotide identity of 98-100%). Full length sequencing indicated a new HEV-3 subtype within HEV-3 subclade abjk. In pig farms of the island, only genotype 3f strains were detected. No food item tested was HEV RNA positive. The seroprevalence of HEV IgG and IgM in blood donors was 9.2% (9/98) and 0% respectively in 2020 and raised to 17.9% (26/312) and 2.5% (8/312) in blood donors collected in 2024.
CONCLUSIONS: Although large epidemics were previously associated to genotype 1 or 2 in Asia and Africa, this outbreak in New Caledonia was associated with HEV-3. Among symptomatic cases there was a high number of hospitalizations and a case fatality of 2.3%.
SOURCES OF FUNDING: Santé Publique France
Keywords: Hepatitis E, Outbreak, epidemiological investigation, sequencing

OP-056
Hepatitis, HIV and other blood borne viruses

Evaluation of a Sanger sequencing-based kit for genotyping drug resistance mutations in various HIV-1 subtypes using clinical and analytical samples

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AIM: HIV drug resistance (HIVDR) compromises antiretroviral therapy (ART) effectiveness. Accurate detection of resistance-associated mutations (RAMs) in reverse transcriptase (RT), protease (PR) and integrase (IN) regions across various HIV-subtypes and viral-load (VL) ranges is essential. We evaluated the performance of Applied Biosystems™ TaqPath™ Seq HIV-1 Genotyping Kit (TaqPath™ Kit) compared to ViroSeq™ HIV-1 Genotyping Assays (ViroSeq™ Assays).
METHODS: Study included 20 HIV-1 subtype C positive patient plasma samples (median VL 3.79 log10 copies/ml) and 23 HIV-positive Virology Quality Assurance (VQA) samples including subtypes A, B, C, D, F, G, CRFO2_AG, and URF (median VL 6.68 log10 copies/ml). Extracted RNAs were tested with TaqPath™ and ViroSeq™ Assays. Sequencing success rates (SSR), mutation profiles and nucleotide similarity scores (NSS) were compared.
RESULTS: SSR with TaqPath™ Kit were 83.7% (n=36/43) for PR-RT and 95.3% (n=41/43) for IN regions vs. 37.2% (n=16/43) and 62.8% (n=27/43) with ViroSeq™ assays, respectively. TaqPath™ Kit showed high SSR in non-B subtype (81.6% for PR-RT and 94.7% for IN region) and lower VL samples. Mean NSS were 98.8±1.3% for PR-RT and 99.2±0.7% for IN region in samples genotyped by both kits. ViroSeq® Assays detected 34 RAMs in 31 samples with 97% (n=33/34) agreement to TaqPath™ Kit. In samples that failed sequencing with ViroSeq® Assays, the TaqPath™ Kit detected 83 mutations including 39 major RAMs.
CONCLUSIONS: TaqPath™ Seq HIV-1 Genotyping Kit demonstrated high sequencing performance for detection of RAMs in PR, RT and IN regions of various HIV-1 subtypes, offering a reliable tool to identify and monitor HIVDR to recommended ART.
SOURCES OF FUNDING: ThermoFisher Scientific
Keywords: HIV drug resistance, HIV-1, genotyping assays

OP-057
Viral infections in pregnancy

Genomic diversity of cytomegalovirus in congenital infections in France

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AIM: To study the inter-host and within-host genomic diversity of the cytomegalovirus (CMV) according to the type of maternal infections and samples.
METHODS: We identified maternal and neonatal samples for CMV whole genome sequencing using an Illumina platform. A Neighbor-net split phylogenetic network analysis was undertaken to assess the distance. Multiple viral populations were investigated with HaROLD, a tool for reconstructing haplotypes. Multidimensional scaling was used to analyze the conserved portion of the genome along with 13 European, African, and Asian GenBank sequences to identify the geographic origin.
RESULTS: 18 amniotic fluids (AF), 9 saliva, 3 chorionic villus samples (CVS), and 1 maternal blood were sequenced from 28 patients with maternal primary infection (PI, 74%) or non-primary infection (NPI, 26%). Most samples (n=29) showed low within-host diversity. Two samples (one maternal blood and one CVS from 2 patients) displayed higher variability; however, the reconstructed haplotypes clustered very closely together in the phylogenetic network, suggesting these were not mixed infections, but multiple viral populations derived from the same strain. Of the 26 different genomes, 15 clustered with European GenBank strains, 8 with African strains, and 3 were possibly hybrid strains.,Samples were paired in 5 cases: CVS associated with AF (n=3) and AF associated with saliva at birth (n=2). All the CMV genomes of the paired samples clustered together.
CONCLUSIONS: Low within-host genetic diversity was observed in AFs and saliva in both maternal PI or NPI. Most genomes clustered either with European or African GenBank sequences, except for 3 hybrid sequences.
SOURCES OF FUNDING: AP-HP
Keywords: cytomegalovirus, CMV, whole genome sequencing, haplotypes, genomic

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POSTER PRESENTATION ABSTRACTS

PP-001
(Re-) emerging viral infections and one health

Correlation of circulating levels of free secreted dengue nonstructural 1 (sNS1) antigen with severity of disease in adult patients admitted with dengue

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AIM: To look at the levels of circulating sNS1 Antigen and correlate it to the severity of disease in adult patients admitted with Dengue.
METHODS: This was a prospective descriptive study done among adult in-patients. Baseline data was summarized using descriptive statistics. Qualitative DEN NS1 ELISA and IgM ELISA were performed as the part of routine care (sample 1). Quantitative sNS1 ELISA titres were measured for patients with IgM and/or NS1 Antigen positive (sample 2).
RESULTS: Among 93 patients, M:F ratio was 1.58:1. 90% patients had Dengue Fever (DF) with a median age of 29 years. 69% of the patients had thrombocytopenia, 24.4% had persistent thrombocytopenia. Median Optical Density (O.D.) values and titres for sample 1 and sample 2 were 0.464 & 0.437 and 1400 & 380 respectively. The correlation between day of fever and O.D. 1 was found to be weak and negative with a p value of 0.78. The association between presence of quantitative sNS1 and the change in platelet count (p = 0.065), in first four days, trending towards significance. Quantitative NS1 and persistent thrombocytopenia (p=0.698) had no association. No significant association was found between quantitative titres and severity of the patients (p = 0.77).
CONCLUSIONS: Rising severe dengue cases in tropical regions (75% global burden) are concerning. Dengue, a resurging viral illness, surged 30-fold globally in 50 years. This study concluded that sNS1 titres were significant in early phase of the disease, qsNS1 is not associated with severity. sNS1 is therefore not useful in predicting the course of disease.
SOURCES OF FUNDING: Self-funded
Keywords: Dengue Fever, sNS1 titres, Dengue Haemorrhagic Fever, Dengue Shock Syndrome, DEN IgM, DEN NS1

PP-002
(Re-) emerging viral infections and one health

The 2024 Parvovirus B19 epidemic analyzed through blood donors in the Netherlands
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AIM: Parvovirus B19 (B19V), a small nonenveloped DNA virus, is the causal agent of erythema infectiosum or fifth disease, mostly affecting children. It spreads via the respiratory route, mostly between spring and early summer. The estimated seroprevalence is 70% in the general Dutch population. We monitor B19V outbreaks through donor screening.
METHODS: B19V infections in the Netherlands are monitored by screening >800,000 blood and plasma donations for presence of high loads (>1E6 IU/ml) of B19V DNA annually.
RESULTS: During the COVID pandemic (2020-2022) B19V was virtually absent in both clinical samples and blood donations in the Netherlands. On average 0.42 per 10.000 donations were detected before 2020 and in 2023. In January 2024, the start of an exceptionally large B19V outbreak was first noticed, characterized by a ~10-fold increase in the number of viremic donations, peaking in April (6.5 per 10,000) and subsiding in June. Analysis of basic donor characteristics indicates that viremic donors are relatively young (18-50). The observed B19V epidemic in blood donors coincided with an increase in fifth disease cases in Dutch day care centers and primary schools.
CONCLUSIONS: Most likely, the lower infection rate in older donors is related to less contact (as parent or caregiver) with infected children. The cause of the epidemic is likely an increased reservoir of suscepible children that were not previously B19V-exposed as a result of social distancing measures during COVID years. Whole genome sequencing is currently being performed to investigate the alternative explanation of circulation of a novel B19V (immune escape) variant.
SOURCES OF FUNDING: Internal funding
Keywords: Parvovirus B19

PP-003
(Re-) emerging viral infections and one health

Re-emergence of measles - adults outbreak: experience of a Romanian tertiary hospital

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AIM: Describe clinically and epidemiological the present measles outbreak in adults from the perspective of a Romanian tertiary hospital.
METHODS: A retrospective, descriptive study was conducted on a group of patients admitted to "Dr. Victor Babes" Clinical Hospital for Infectious and Tropical Diseases in Bucharest, Romania, between 8th of December 2023 and 29th of April 2024 with the diagnosis of measles. Data was collected from the informatics system of the hospital and a consent for data processing was obtained upon patient admission. The diagnosis of measles was made epidemiological, clinical and by serology tests.
RESULTS: A total of 219 patients were enrolled, median age (IQR) 34 years (23;42). The majority were originated from urban areas (164;71,3%). 111 were males and 108 were females. All patients associated hepatic cytolysis, median (IQR) AST 115.5 U/L (60;211), ALT 158 U/L (78;303), the most frequent complication was viral pneumonia and in third of cases respiratory failure but irrespective of age, sex or associated risk factors. Mean hospitalization period was 5 (4;6) days. There were 4 immunocompromised patients (living with HIV) that associated inclusion body encephalitis and had unfavorable outcome. The overall cost was 54.910,11 euro with a mean 743,82 euro per patient and 107 patients had no medical assurance.
CONCLUSIONS: Hepatic, pulmonary and neurological impairment were the most frequent complications of measles. Even in an era with easy and free of charge access to vaccination, measles continues to be a burden, both medically and financially, thus measures to support vaccination should be enforced.
SOURCES OF FUNDING: No funding.
Keywords: measles, hepatic cytolysis, pulmonary failure, encephalitis

PP-004
(Re-) emerging viral infections and one health

Dengue circulation in early 2024: are Italian autochthonous cases and Latin America epidemiology having an affect?

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AIM: In 2023, 82 Dengue virus autochthons infections were detected in Italy; moreover, an unprecedented virus circulation is occurring in Latina America. As a consequence, stronger efforts were put in place in surveillance. We describe how local epidemiological scenario changed accordingly.
METHODS: The study included results of molecular and serological tests performed for DENV infection diagnosis from January to April 2024: data were compared with those from the same period of previous years (2016-2023). Subjects tested were symptomatic individuals presenting at Emergency Room or referred to Infectious Disease specialist by General Practitioner. RealTime PCR was performed with Dengue Serotyping Virus kit (CLONIT Srl, Italy), while IgG and IgM detection was run on VIRCLIA® LOTUS (VIRCELL S.L., Spain).
RESULTS: A total of 76 subjects were RT-PCR screened in January-April 2024, whit 18 (23.68) positive: half patients harboured a DENV-2 virus, while no DENV-4 was detected. Molecular screening doubled compared to 2023 (N=33), as well as a 3-fold increase in positive rate was found; tests ranged 10-25 in 2016-2019, while reduced to 3-4 in 2021 and 2022 due to pandemic. IgG and IgM were performed for 89 subjects in 2024: IgM were positive in 12 cases, all but one supported by RT-PCR, with 21 positive IgG. As for molecular screening, the serological tests doubled compared to 2023, following a similar trend in years 2016-2022. No autochthonous case was detected.
CONCLUSIONS: The greater attention on DENV led to growth in screening and positive rate, suggesting a higher burden in next months.
SOURCES OF FUNDING: No funding source to be declared.
Keywords: Dengue virus, epidemiology

PP-005
(Re-) emerging viral infections and one health

Seroprevalence of Crimean-Congo hemorrhagic fever virus in small ruminants, northern Greece
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AIM: Crimean-Congo hemorrhagic fever virus (CCHFV, genus Orthonairovirus, family Nairoviridae) is a highly pathogenic tick-borne virus circulating in nature in an enzootic cycle between ticks and vertebrates. It presents a wide distribution in three continents (Africa, Asia and Europe) with a potential to cause outbreaks. Up to now one CCHF human case has been reported in Greece, while seroprepavalence studies in humans showed a positivity of approx. 4%. Aim of the present study was to screen small ruminants from farms in northern Greece for CCHFV IgG antibodies.
METHODS: A number of 364 serum samples from clinically healthy young (<5 years old) sheep and goats were tested for the presence of CCHFV IgG antibodies. The samples were collected during 2021-2023 from animals residing in 12 regional units of northern Greece using the commercial ID Screen® CCHF Double Antigen Multi-species ELISA kit (Innovative Diagnostics, Grabels, France). Testing of the positive samples with additional serological methods is in progress.
RESULTS: A total of 15.4% (range 0-59.1%) of the tested animals had anti-CCHFV IgG antibodies. CCHFV-positive animals were detected in three regional units of Greece. The highest seroprevalence was seen in goats from a farm in northeastern part of the country.
CONCLUSIONS: Seroprevalence studies in animals enable the mapping of CCHFV distribution in a region, and are the basis for further studies in humans, animals, and ticks, following the One Health approach.
SOURCES OF FUNDING: The Study was supported by EU Durable (Research Network against Epidemics)
Keywords: CCHF, Hemorrhagic fever, tick-borne virus, seroprevalence study

PP-006
(Re-) emerging viral infections and one health

Six pediatric cases of entervirus EV-C105: experience of a hospital located in northeast Italy
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AIM: EV-C105 is an emerging genotype with reported neurological implications. The present work is aimed to report for the first time EV-C105 circulating in patients in Northeast Italy.
METHODS: EV-C105 cases were found by respiratory panel FilmArray RP2.1 and genotyped by Sanger sequencing (VP1 region) in patients attending the emergency room and/or admitted in our hospital. Multiple sequences alignments were carried out by AliView software using EV-105 sequences deposited in NCBI Virus. Phylogenetic trees were reconstructed by IQ-TREE, based on maximum likelihood approach 1000 bootstrap.
RESULTS: Between March 2023 and May 2023, EV-C105 was identified from upper respiratory tract in 6 children (4 males and 2 females, median age 4 years). All patients were Italian from Verona province, presented respiratory symptoms, fever and viral co-infection (n=3 adenovirus, n=1 astrovirus, n=1 human coronavirus HKU1, n=1 adenovirus/rotavirus). No severe complications were reported. Partial sequence of VP1 was phylogenetically compared with all those publicly annotated. They place together in a unique cluster sharing 99% of nucleotides, and belong to the European lineage. Compared to the reference prototype (EV-C105 strain 34S, JX514943), 2/6 sequences show 90.62% identity whilst 4/6 89.73%.
CONCLUSIONS: These data provide first insights into the circulation of human EV-C105 in Veneto Region (Northeast Italy), showing the presence of this potentially neurotropic genotype and suggesting the need to continue the monitoring analysis.
SOURCES OF FUNDING: EU funding within the MUR PNRR Extended Partnership Initiative on Emerging Infectious Diseases (Project No. PE00000007, INF-ACT) and Italian Ministry of Health “Fondi Ricerca Corrente”.
Keywords: enterovirus, EV-C105, Italy

PP-007
(Re-) emerging viral infections and one health

First two Oropouche fever cases diagnosed in Italy in epidemiologically non-related travellers from Cuba

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AIM: A neglected arbovirus, Oropouche virus (OROV), an Orthobunyavirus with negative sense, segmented RNA genome, is spreading in the Americas where several countries reported an ongoing outbreak. Vector competence for OROV is still largely unexplored. Culicoides are considered the preferred vectors for humans and animals. Here we report OROV detection and virological results in two epidemiologically distinct travellers who returned to Italy from Cuba in late May-early June.
METHODS: Both patients (Case1 and Case2) tested negative for dengue, chikungunya and Zika viruses. Samples were then analyzed for OROV infection using a specific end-point RT-PCR targeting a partial fragment of segment S. Whole genome sequences and viral isolation were also tried to perform.
RESULTS: Results from Case1 showed both whole blood and serum samples positive; whole blood and urine from Case2 tested positive, while serum sample was negative. Both amplicons sequences clustered with OROV sequences in GenBank. Successful viral isolate was obtained. OROV whole genome sequence was achieved from viral isolate. Serology tests were also performed by both indirect-immunofluorescence and microneutralization tests.
CONCLUSIONS: The two cases we report here, detected in epidemiologically non-related travellers from Cuba indicate that, in the shadow of a massive dengue outbreak in the Americas, the Oropouche outbreak might be more widespread than previously estimated. In order to stem the spread it is crucial to increase the laboratories capability, place OROV in the diagnostic landscape of imported fevers, and determine potential vector competence outside the tropical region.
SOURCES OF FUNDING: EU-fundings MUR-PNRR-Extended-Partnership-Initiative Emerging Infectious Diseases (No.PE00000007, INF-ACT); Italian Ministry of Health-fundings “Fondi Ricerca Corrente”.
Keywords: Oropouche fever, Oropouche virus, emerging pathogen, arbovirus, diagnosis, neglected diseases.

PP-008
(Re-) emerging viral infections and one health

Detection of measles virus genotype D8 in wastewater of the Brussels capital region, Belgium, March 2024

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AIM: Following the ECDC threat assessment brief regarding increased measles case numbers and outbreaks in Europe, we investigated sewage samples from different wastewater treatment plants (WWTP) in Belgium for the presence of measles virus (MV).
METHODS: We initiated measles testing on 24 hours influent wastewater samples from WWTP of Louvain, Brussels and Antwerp. Molecular detection and quantification of MV RNA was performed by real-time RT-PCR. A nested PCR method was used to sequence the 450 nucleotides encoding the carboxyterminal 150 amino acids of the MV nucleoprotein, the minimum sequence data required for MV genotyping according to WHO guidelines.
RESULTS: Wastewater samples from the Brussels North WWTP, covering 1.1 million population equivalents, taken between February 28th and March 19th 2024 contained between 2 and 22 MV genome copies/ml wastewater. There were 12 confirmed measles cases reported in the Brussels Capital Region from the beginning of 2024 up to mid-March. Detectable presence of MV in the wastewater samples indicates that additional cases are likely occurring under the radar. No MV was detected in wastewater samples from Louvain, Antwerp and Brussels South. The partial MV sequence retrieved from the Brussel North wastewater sample showed highest similarity to genotype D8, which is currently circulating in Europe. It was most closely related to recent strains from Romania, where an outbreak has been ongoing since mid-February 2023.
CONCLUSIONS: The methodology we have developed enables highly sensitive genotyping of MV from wastewater. Wastewater testing for measles can offer a valuable surveillance tool to trace hot spots of virus circulation.
SOURCES OF FUNDING: None
Keywords: measles, sewage, wastewater based surveillance, Belgium

PP-009
(Re-) emerging viral infections and one health

(Re)Emerging respiratory infections for patients in a regional university hospital: towards combined molecular virological diagnosis with wastewater-based epidemiology

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AIM: SARS-CoV-2 and other respiratory pathogens can cause severe illness in vulnerable or initially-healthy people. Rapid viral molecular diagnosis is needed, combined with population epidemiology. The aims were (i) to increase data on the spread of non-COVID-19 respiratory viruses during SARS-CoV-2 circulation, (ii) to focus on severe respiratory infections in a partner intensive care unit (ICU), (iii) to justify adequate viral markers for wastewater-based epidemiology such as done in a border country.
METHODS: From a retrospective study during the high circulation of SARS-CoV-2 in France (Jan-2021_June-2022), on a population of 806 patients, respiratory samples were explored by FilmArray-Pneumonia panel. For 18 patients (partner ICU), clinical features were analyzed by descriptive statistics. Moreover, a wastewater epidemiology approach was selected in the literature, yet used in a country showing a viral co-circulation similar to ours.
RESULTS: The Pneumonia panel on 806 patients detected at least one virus and/or one bacteria for 412 patients, including 58 patients from intensive care. For 18 patients (partner ICU), viruses were detected showing 7/18 patients with influenza virus or adenovirus, then treated by direct antiviral molecules. Moreover, in a Spanish reference-(1), influenza A(H3/N2) viruses co-circulated with SARS-CoV-2 in wastewaters, matching the chronological incidence of infections from the Catalan Government clinical database closed to our regional epidemiology.
CONCLUSIONS: Our work showed (i) the co-circulation of respiratory viruses during COVID-19 spreading, (ii) the usefulness of molecular diagnosis of respiratory infections on clinical intensive care practices, (iii) a justified combination of molecular assays and wastewater viral markers for human respiratory infection epidemiology.
SOURCES OF FUNDING: Hospital standard-of-care
Keywords: Severe respiratory infections, syndromic panel-based assay, non-COVID-19 viruses, SARS-CoV-2, clinical management, wastewater-based epidemiology

PP-010
(Re-) emerging viral infections and one health

Analysis of the monophyletic lineage of avian Influenza H5N1 which circulated in Venezuelan birds during the 2022-2023 outbreak

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AIM: Avian influenza subtype H5N1 has caused outbreaks worldwide since 1996, with the emergence of the Guandong lineage in China. The current clade 2.3.4.4b has evolved from this lineage, with increased virulence and mass mortality events in birds and mammals. The objective of this study was the analysis of 17 viral genomes of H5N1 avian influenza isolated in Venezuela during the 2022-2023 outbreak.
METHODS: The 8 segments of the viral genome were amplified using universal primers, and sequenced by Next Generation Sequencing. The sequences were analyzed to confirm the H5 hemagglutinin clade, identify possible genetic rearrangements, and perform a phylogenetic and docking analysis of the viral isolates.
RESULTS: The results showed that the viruses found in Venezuela belonged as expected to clade 2.3.4.4b, and formed a monophyletic clade with North American influenza viruses, with no evidence of further rearrangements. The introduction of the virus in South America is associated with bird migration through the Atlantic (Venezuela) and Pacific migratory flyways (two independent lineages), with the emergence of three viral lineages. Several mutations were found in all segments of the genome, although none of the key mutations involved in mammal´s adaptation. Moreover, in silico structural analysis suggests that the virus maintained a predilection for avian α2,3-linked sialic acid.
CONCLUSIONS: The unprecedented pathogenic outbreak of avian Influenza in South America was associated with the circulation of 3 different lineages, which maintains a lower affinity for the mammalian receptor.
SOURCES OF FUNDING: MinCyt, Venezuela
Keywords: Avian Influenza, South America, clade 2.3.4.4b, mortality, avian receptor

PP-011
(Re-) emerging viral infections and one health

The Origin of SARS-CoV-2 and its Furin Cleavage Site, a Parsimonious 5-Stage Evolutionary Model
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AIM: SARS-CoV-2 differs from all its near wild relatives by having a 12 nucleotide insertion at the S1/S2 junction of the viral Spike protein. This insertion of four amino acids (PRRA) created a protein motif (RRAR) which is a furin cleavage site (FCS) that likely was a decisive factor in SARS-CoV-2's switch from a faecal/oral life-history strategy in bats to respiratory transmission in humans. Can this insertion be reconciled with an evolutionary origin that is both a) parsimonious, and b) consistent with the complex chronological and biological constraints which biochemical, structural, and other studies infer limit how SARS-CoV-2 Spike could have acquired its lung cell infection capabilities, in particular its cleavability by both furin and TMPRSS2 (Zhang et al., 2021; Jackson et al., 2022; Vu et al., 2022)?
METHODS: Literature review, sequence comparison, genome analysis using GenBank, BLAST, and other phylogenetic and sequence comparison tools.
RESULTS: It can if SARS-CoV-2 initially acquired its FCS through recombination with the human mRNA encoding the lung epithelial sodium channel subunit (ENAC-α). ENAC-α represents an ideal donor since it combines a furin site with 5 additional amino acids identical to SARS-CoV-2 downstream from the insertion. Subsequently, just one additional non-synonymous change (A to P) at the upstream inserted codon achieves the final SARS-CoV-2 sequence.
CONCLUSIONS: This model precisely fulfills every constraint imposed by the above-mentioned chronological, structural, biochemical, and genetic predictions.
SOURCES OF FUNDING: The Bioscience Resource Project
Keywords: furin, SARS-CoV-2, coronavirus, protease, entry

PP-012
(Re-) emerging viral infections and one health

Post-Pandemic Measles Re-Emergence in a High Vaccinated Country – Portugal 2024
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AIM: Laboratory investigation of possible measles cases in Portugal from January to April 2024.
METHODS: The National Institute of Health received oral fluids (OF), urine and serum from 171 possible measles cases for confirmation, 131 (76.8%) of which were in adults (≥18yo). Viral RNA detection was performed in urines and OF by real time RT-PCR and IgG and IgM in serum by ELISA. Measles genotype was determined by sequencing of 450 nucleotides of the N protein.
RESULTS: Measles were confirmed in 25(14.6%) cases.
Nineteen (76%) were foreigners and of which 15(79%) lived in Portugal. Two cases belonging to genotype B3 and 23 to D8.
Seventeen (68%) cases were adults.
Eleven (44%) were vaccinated and in 3(12%) the vaccinated status is unknown.
Five cases were imported, 3 of which originated secondary cases.
In 14(56%) cases, all with identical genotype D8, no epi-link was identified and 11 of these cases (79%) were foreigners residing in Portugal.
CONCLUSIONS: During the first 4 months of 2024 Portugal confirmed 25 measles cases, 14(56%) in vaccinated patients which could happen in sustainable measles elimination settings due to a secondary vaccine failure. Three chains of transmission were identified, one associated to genotype B3 and the others to D8. However, the 11 cases with unknown epi-link, are a cause of major concern, despite the high-vaccinated coverture in the country, due to the possibility of virus circulation in the community.
References: Iwamoto M, Hickman CJ, Colley H et al. Measles infection in persons with secondary vaccine failure, New York City, 2018–19. Vaccine 39(2021)5346–5350.
SOURCES OF FUNDING:
Keywords: Measles, Re-Emergence, High Vaccinated Country

PP-013
Advancements in diagnostics & clinical cases

Whole genome sequencing of RNA viruses on clinical samples using RNA-seq technology

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AIM: Viral genome sequencing is becoming more important in research, epidemiology and disease management. But, due to their high genome variation, Whole Genome Sequencing (WGS) of RNA viruses remains a challenge especially for PCR success and priming design.In this work, we experienced a high sensitivity RNA-seq sequencing technology with non-specific amplification to sequence whole genome of various RNA viruses directly from clinical sample.
METHODS: This technology has been optimized to provide high sensitivity for degraded or low copy number viral RNA in human samples.
Four plasma samples of patients infected by HIV (n=1), HCV (n=2) and HDV (n=1), two feces samples of patients infected by HEV and one nasopharyngeal swab of patient infected by SARS-CoV-2 were selected for WGS using RNA-seq kit, followed by short read sequencing. The raw data were analyzed in order to generate consensus sequence.
RESULTS: Two to 4.5 million reads were obtained by sample. 0.2% to 50% of the reads were mapped on the interest genomes. For each sample, complete viral genomes were successfully sequenced with a mean depth of 300X to 25 000X and a coverage up to 100% at 10X.
CONCLUSIONS: This RNA-seq technology enables with success Whole-Genome-Sequencing without specific genome amplification. Thus, it can be of great interest to give a rapid response for sequencing emerging RNA viruses like Influenza, Dengue or Respiratory Syncytial viruses. Sequence evolution of viral variants can thus be followed as well as the emergence of new resistance mutations all along the genome.
SOURCES OF FUNDING: none to declare
Keywords: RNA-seq, Whole-genome sequencing, RNA viruses

PP-014
Advancements in diagnostics & clinical cases

A case of non conventional clinical presentation of Parvovirus B19 infection in a healthy baby

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AIM: We present a case of Parvovirus B19 (B19V) infection in lymph nodes and lung. At onset, persistent neck pain due to compression of enlarged cervical lymph nodes was reported. Biopsy was performed to differentiate diagnosis between malignancy and infection. Several herpes viruses were excluded, while high viral load positivity of 5 million-IU was detected for B19V. Presence of B19V was confirmed in BAL and whole blood samples ≥ 900,000 and 350,000 copies/mL, respectively. International literature regarding lymphadenopathy and respiratory distress during B19V infection in childhood has been reviewed reviewed.
METHODS: According to the PRISMA Extension guidelines for Scoping Review.
Parvovirus B19, Human/pathogenicity" [Mesh] Filters: Humans, English, Child: 6-12 years, Preschool Child: 2-5 years, Adolescent: 13-18 years, from 2000 – 2024 Pubmed
Parvovirus B19, Human" [Mesh]) AND "Lung" [Mesh] Filters: Humans, English, Preschool Child: 2-5 years, Child: 6-12 years, Adolescent: 13-18 years, from 2000 – 2024 Pubmed; Parvovirus B19 AND ('complication'/dm OR 'lymphadenopathy'/dm OR 'respiratory distress'/dm); limits 7-12 + 13-17 years; English, from 2000 – 2024-Embase.
RESULTS: Of 94 studies, 6 records met the inclusion criteria. No data are available in the literature on lymphnode and lung involvement during B19V infection in the pediatric population, with the exception of one pediatric case after heart-transplantation.
CONCLUSIONS: Parvoviruses have the ability to activate apoptosis causing tissue damage and disease progression. It is still uncertain whether lung or other tissues (different from bone marrow) injury results from B19V direct damage. Lymph nodes and lung involvement should be investigated even if no respiratory symptoms are present but only fever.
SOURCES OF FUNDING: Not applicable
Keywords: Parvovirus B19, Children, lymphadenopathy infection

PP-015
Advancements in diagnostics & clinical cases

Comparison of Cytomegalovirus (CMV) Polymerase Chain Reaction (PCR) Test Results Run on Fully Automated Systems: Qiagen NeuMoDx Experience with Roche Cobas 6800

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AIM: In this study, it was aimed to investigate the quantitative compatibility between measurements made with Cobas6800 (Roche Diagnostics, Mannheim, Germany) and NeuMoDx (Qiagen, Ann Arbor, USA) Cytomegalovirus (CMV) polymerase chain reaction (PCR) tests, which are fully automated systems calibrated with the WHO CMV Standart.
METHODS: The results of 214 plasma samples, which were studied simultaneously with Cobas6800 and NeuMoDx CMV PCR tests, were analyzed. In the tests, the extraction, amplification and detection stages were carried out fully automatically.
RESULTS: CMV DNA was detected in 144(67.28%) samples in both tests and was not detected in 53(24.76%) samples. Incompatible results were obtained in a total of 17(7.94%) samples. Good agreement was found between the qualitative results of both tests (kappa=0.80, p<0.001). According to Bland-Altman analysis; the average difference between the results of the NeuMoDx CMV PCR test and the Cobas6800 CMV PCR test was found to be -0.14 log10 IU/mL, and it was determined that the Cobas6800 CMV PCR test had lower measurements than the NeuMoDx CMV PCR test. In 120 of 129 samples (93%) whose results were within the dynamic measurement range of both tests, the measurement difference was within ±0.5 log10 IU/mL.
CONCLUSIONS: Quantitative agreement was found in the measurements made in plasma samples with the fully automated tests calibrated with the CMV Standart. To the best of our knowledge, a study comparing viral load measurements made with Cobas6800 and NeuMoDx fully automated systems in the detection of CMV DNA has not yet been conducted, and this is the first study on this subject.
SOURCES OF FUNDING: No fund
Keywords: CMV; WHO; PCR; quantitative agreement; NeuMoDx, Cobas 6800

PP-016
Advancements in diagnostics & clinical cases

Optimizing Diagnostic Workflows: Machine Learning-Aided Result Interpretation
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AIM: The aim of our study is to introduce a machine learning-aided system designed to optimize an existing diagnostic workflow by facilitating fast and accurate result interpretation, thereby reducing hands-on time without a loss of performance.
METHODS: We implemented a machine learning software tailored to enhance the interpretation of real-time PCR results within an established diagnostic workflow. This software integrates seamlessly into existing in vitro diagnostic processes with minimal modifications required for implementation. Our evaluation utilized data from a variety of pathogen-detecting commercial products, encompassing over 28,000 PCR curves for iterative learning and error optimization.
RESULTS: Our automated result interpretation system demonstrated comparable performance to manual analysis across various diagnostic assays. Verification data including e.g. limit of detection and linearity studies from pathogen-detecting products were analysed in parallel with a fully automated analysis and a manual analysis of PCR curves. The obtained data confirmed very good agreement not only for qualitative (100% concordance) but also quantitative results (max. 0.1 Δlog IU/ml).
CONCLUSIONS: Our findings underscore the reliability of employing machine learning for result interpretation within a diagnostic workflow. Additionally, the sample to result time can be decreased considering the high throughput capability of machine learning aided software.
SOURCES OF FUNDING: Altona Diagnostics GmbH
Keywords: Diagnostic, Workflow, machine learning, real-time PCR, automated analysis

PP-017
Advancements in diagnostics & clinical cases

TTVGuideIT: validation of and quantitative use of a PCR assay across 13 different sites

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AIM: Torque teno virus (TTV) is being evaluated in the TTVGuideIT trial adjusting tacrolimus dosing in kidney transplant recipients when participants are outside of a predetermined TTV range. Thirteen centers in six countries are participating in the trial.
METHODS: Internal Quality Control (IQC) containing only TTV22 was diluted using TTV negative donor plasma to a concentration of 4 log copies/ml. IQC is performed with each sample run.
RESULTS: Of 1014 IQC TTV runs, 27 were rejected (2.7%); four times as the value was 3SD over target, 23 times as the result was outside 2SD in two of three consecutive samples. Analysis of the remaining 987 results showed a mean of 4.00 log copies/mL (Min=3.43, Max=4.58), an inter-laboratory SD of 0.19 log copies/mL and an intra-laboratory SD 0.07 – 0.18 log copies/mL. Seven labs had an average below the target and two above the target.
CONCLUSIONS: IQC assessment shows that the bioMérieux R-Gene® PCR has excellent accuracy and reproducibility. However, a patient enrolled in a center with an IQC mean which is lower than the inter-laboratory mean may be more likely to have dose adjustments upward, potentially leading to net increased tacrolimus dosing. Given the structural bias exhibited by all laboratories, we suggest that it would be beneficial to use a correction based on the IQC- variation individually calculated for each laboratory using the mean +/- the SD for each laboratory.
SOURCES OF FUNDING: This study is partly funded by a Horizon-2020 EU grant programme under grant agreement number 896932. TTV kits were provided by bioMérieux.
Keywords: TTV, transplantation, PCR, clinical trial, TTVGuideIT

PP-018
Advancements in diagnostics & clinical cases

Identification of the new herpes simplex virus 2 variant in three patients suffering from recurrent infections unresponsive to antiviral therapy

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AIM: Herpesviruses are ubiquitous pathogens that cause lifelong infections by establishing latency. Herpes simplex virus 2 (HSV-2) mainly causes genital herpes, characterized by recurrent genital ulcers. Like all herpesviruses, HSV-2 exhibits low genetic diversity (mean pairwise distance of 0.1%). Here, we characterized five clinical HSV-2 isolates showing high genetic variability in the viral DNA polymerase (DP).
METHODS: Under our translational research platform RegaVir, five HSV-2 isolates from three Belgian patients were genotyped by Sanger sequencing of the thymidine kinase (TK) and DP. Two isolates were additionally phenotyped by CPE reduction assays. Clustal Omega was used for multiple sequence alignments.
RESULTS: Three isolates [RV-938 (Patient #1); RV-2760 and RV-2765 (Patient #2)] were recovered from 2 non-immunocompromised women suffering from recurrent genital herpes under long-term valacyclovir therapy. Two isolates (RV-1472 and RV-1484) were collected from a man with persistent HSV-2 ocular infections. The isolates harbored no known drug-resistance mutations but >20 novel changes in the DP, primarily in the thumb domain, when aligned to HSV-2 laboratory strains. Additionally, two novel TK changes (P273L and E276K) were found in isolates from patient #2. None of these changes affected the susceptibility to anti-HSV-2 drugs, indicating their association with inter-strain variability. Alignment of the DP sequences to different clinical HSV-2 isolates showed high similarity to the new HSV-2 variant (HSV-2v) identified in sub-Saharan African patients, characterized by unexpectedly high DP variability (2.4% divergence).
CONCLUSIONS: To our knowledge, these are the first HSV-2v strains isolated in Belgium warranting further studies evaluating their replication capacity and pathogenicity.
SOURCES OF FUNDING: Sciensano.
Keywords: Herpesviruses, case series, sequencing, genetic diversity, herpes simplex virus 2

PP-019
Advancements in diagnostics & clinical cases

Integrating Hepatitis C virus whole-genome sequencing into clinical diagnostics using Oxford Nanopore Technology

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AIM: Hepatitis C virus (HCV) represents a significant public health challenge, impacting over 70 million individuals with chronic infections worldwide. Current diagnostic methods involve genotyping (NS5 gene) through Sanger sequencing, followed by separate assessments for antiviral resistance in NS3, NS5a and NS5b genes. Here, we introduce a promising HCV whole-genome approach, enabling both genotyping and identification of antiviral resistance patterns.
METHODS: Plasma samples were processed using the MagNaPure followed by cDNA synthesis with Superscript-IV. A nested PCR approach was applied to generate two fragments (8.4kb and 1.2kb) using LongAmp polymerase. Amplified products were sequenced on a GridION using the Native Barcoding Kit-v14 (ONT). Consensus sequences were derived by mapping and de novo assembly (Flye). Genome Detective and Geno2pheno were used for genotype determination and antiviral resistance profiling.
RESULTS: Sequencing success (defined as >100-fold read coverage) was achieved for commonly reported genotypes (1a,1b,2b,3a), with viral loads >3log10IU/mL. Whole-genome sequencing (WGS) demonstrated a 98% nucleotide concordance with Sanger, identifying a rare HCV genotype (6a) previously undetectable by conventional methods. Primer analysis revealed significant mismatches, potentially explaining previous Sanger challenges. This finding has critical implications for patient management, determining susceptibility to first-line Direct-Acting Antiviral agents.
CONCLUSIONS: Integrating a single HCV workflow into diagnostics, along with currently applied WGS targets, could help streamline NGS within the clinic. Additionally, as current antiviral resistance testing is typically limited to genotypes 1-4, WGS eliminates the need for further selection or optimization of specific primers following genotyping. We anticipate this approach could improve therapeutic decision-making, leading to more efficient treatment outcomes.
SOURCES OF FUNDING: ErasmusMC
Keywords: Hepatitis C virus; Whole-genome sequencing; Nanopore sequencing

PP-020
Advancements in diagnostics & clinical cases

Enhancing mutation analysis with minMutFinder: a versatile solution for accurate viral population characterisation

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AIM: minMutFinder aims to accurately identify mutations and assess their frequencies, accounting for multiple nucleotide mutations occurring within a single codon. Additionally, it can annotate mutations associated to phenotypical changes in viral populations based on user-supplied datasets.
METHODS: minMutFinder uses genome mapping algorithms and variant calling techniques to analyse Fastq files. This software, implemented in nextflow, follows a bioinformatics pipeline of quality control, read-mapping, variant calling, and post-processing steps to identify mutations and characterise them. The program was tested on serial SARS-CoV-2 positive samples from immunocompromised patients undergoing monoclonal antibodies treatment.
RESULTS: This tool identified low-frequency mutations, with special attention to codons containing multiple changes. Among others, as an example, nucleotide mutations T1111C and C1112T in Spike codon position 371 were identified within a unique sample. These mutations were previously characterised as two independent nucleotide changes resulting in mutations S371P and S371F. After using minMutFinder, these changes were classified as C1112T and T1111C+C1112T, giving the following codon variations S371F and S371L, respectively. Performance at this codon was confirmed on translated-read sequence alignments.
CONCLUSIONS: minMutFinder is a reliable and user-friendly option for mutation detection and characterisation directly from Fastq files, identifying variants even at low frequency in viral populations. Furthermore, it generates comprehensive reports, including several quality control metrics and the identified variants. Additionally, when a reference dataset of phenotypic profiles is given, matching mutations are highlighted in the report. With its consistent and robust performance, minMutFinder serves as a versatile tool applicable across diverse domains, including clinical genetic studies and epidemiological research.
SOURCES OF FUNDING: No Funding
Keywords: bioinformatics, viral populations, minority mutation detection, variant calling, next-generation sequencing

PP-021
Advancements in diagnostics & clinical cases

High-throughput DNA sequencing of human cytomegalovirus genomes in formalin-fixed paraffin-embedded tissues

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AIM: Congenital cytomegalovirus disease (cCMV) is an uncommon but sometimes severe condition that affects 1 in 150 live births. Studies investigating possible associations between severe cCMV and infection with either multiple human cytomegalovirus (HCMV) strains or particular HCMV genotypes have to date involved only small sample sizes derived from fresh tissues. In this context, extensive formalin-fixed, paraffin-embedded (FFPE) cCMV tissue repositories established worldwide would provide an invaluable resource for strengthening future investigations. However, due to the difficulty of recovering intact DNA of sufficient quality, there are no published reports involving sequencing whole HCMV genomes from such material. In this study, we have assessed the feasibility of sequencing whole HCMV genomes from cCMV FFPE material.
METHODS: A total of 16 FFPE samples of foetal kidney or placenta tissue from ten cCMV cases (2008-2018) were processed. Two commercial kits for DNA extraction were evaluated, HCMV DNA was enriched from the resulting DNA preparations, and sequencing libraries were prepared and analysed on the Illumina platform. The sequence data were processed using the GRACy computing toolkit.
RESULTS: Five complete HCMV genomes were determined from nine unique cCMV cases using either DNA extraction kit.
CONCLUSIONS: Determining complete HCMV genomes from cCMV FFPE material is feasible. This advance will facilitate comprehensive studies of the effects of multiple-strain infection or particular HCMV genotypes on clinical outcomes in cCMV.
SOURCES OF FUNDING: Medical Research Council (grant numbers MC_UU_12014/12 and MC_UU_12014/3) and the Wellcome Trust (grant number 204870/Z/16/Z)
Keywords: human cytomegalovirus (HCMV), congenital cytomegalovirus infection (cCMV), formalin-fixed, paraffin-embedded (FFPE) tissue, DNA sequencing

PP-022
Advancements in diagnostics & clinical cases

Enterovirus RNA testing in cerebrospinal fluid and respiratory samples: consideration of feasibility across different age groups

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AIM: Enteroviruses (EVs) are known to primarily infect children and cause a significant burden of disease in newborns, yet EV testing is frequently used in older patients. The aim of this study was to investigate the feasibility of EV testing in different ages.
METHODS: A total of 3,733 cerebrospinal fluid (CSF) and 13,844 respiratory samples collected in Slovenia between January 2014 and September 2023 were analyzed for EV RNA. A ROC curve was constructed using the patient's age as a predictor of an EV-positive test result.
RESULTS: A total of 238 (6.4%) CSF and 740 (5.3%) respiratory samples tested positive for EV RNA. A lower age to predict an EV-positive result resulted in an area under the curve of 0.776 (95% confidence interval (CI): 0.754-0.797) and 0.707 (95% CI: 0.694-0.719) for the CSF and respiratory datasets, respectively. With the age cut-off of 45.3 and 25.3 years for CSF and respiratory samples, respectively, the sensitivity of this diagnostic procedure for the detection of EV corresponds to >98% and would prevent unnecessary EV testing in CSF in 1,736 (47%) cases and in respiratory samples in 5,184 (37%) cases. A sensitivity of 95% results in a cut-off of <39.2 and <12.7 years for the CSF and respiratory dataset, respectively.
CONCLUSIONS: Although syndromic multiplex testing is commonly used, these results suggest that EV testing is not feasible in older age.
SOURCES OF FUNDING: Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana and the Slovenian Research and Innovation Agency (grant number P3-0083).
Keywords: enteroviruses, cerebrospinal fluid, respiratory samples, testing, sensitivity

PP-023
Advancements in diagnostics & clinical cases

Is the quantification of CMV DNA in patients on letermovir prophylaxis affected by the presence of lysed cellular material in plasma samples?

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AIM: Letermovir differs from other antiviral drugs, as it does not affect the replication of the viral genome itself, but acts at the level of viral DNA cleavage. A previous study has shown that the determination of cytomegalovirus (CMV) DNA concentration is suitable for measuring the efficacy of letermovir prophylaxis when testing plasma or other cell-free samples. However, contamination of plasma samples with lysed cellular material due to delayed sample preparation could hinder diagnostic monitoring, which we wanted to investigate.
METHODS: Parallel blood samples were collected from patients receiving letermovir prophylaxis, one being processed immediately and the other centrifuged and plasma collected after one- or two-day storage at 4oC. CMV DNA was quantified in all samples using Alinity m CMV assay (Abbott Molecular, Des Plaines, IL, USA).
RESULTS: Parallel testing was performed for 126 samples. Only 9/126 (7.1%) baseline samples had a CMV DNA result of >30 IU/ml (all below 200 IU/ml), with similar CMV DNA results for immediately processed samples and samples with delayed sample preparation.
CONCLUSIONS: Cellular CMV DNA does not significantly affect the determination of CMV DNA in plasma. Since all samples analysed had a relatively low viral load, we cannot exclude a greater influence of cellular CMV DNA in samples with a higher viral load.
References: Weinberger S, Steininger C. Reliable quantification of cytomegalovirus DNAemia in letermovir treated patients. Antiviral Res. 2022;201:105299.
SOURCES OF FUNDING: Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana and the Slovenian Research and Innovation Agency [grant number P3-0083].
Keywords: letermovir, cytomegalovirus, quantification, DNA

PP-024
Advancements in diagnostics & clinical cases

Detection of Acyclovir-Resistant Herpes Simplex Virus-1 Without Prior Long-term Acyclovir Treatment
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AIM: Detection of acyclovir-resistant HSV is unusual in patients without prior long-term acyclovir prophylaxis or suppression.
METHODS: We describe here a patient who had acyclovir-resistant HSV-1 detected in multiple broncho-alveolar lavage samples without any prior long-term acyclovir. Multiplex PCR detected HSV-1 in multiple successive broncho-alveolar lavage samples. Sample was sent to the reference laboratory for genotypic resistance testing.
RESULTS: A 49-female was admitted for severe necrotising Streptococcus pneumoniae pneumonia requiring veno-venous extra-corporeal membrane oxygenation and acyclovir prophylaxis was commenced. Subsequently, Herpes Simplex Virus-1 was detected in multiple bronchoalveolar lavage samples, with decreasing cycle threshold values. Acyclovir was switched to foscarnet, and genotypic resistance testing revealed mutations in the viral thymidine kinase associated with high-level resistance to acyclovir.
CONCLUSIONS: ACV-resistant HSV-1 infection should be considered even if the patient has not been on long-term ACV prophylaxis, as the patient may have acquired resistant HSV-1 infection at primary infection. In addition, the clinical presentation of acyclovir-resistant HSV-1 infection is not limited to recurrent oro-genital infections or infections of immune-privileged sites, and consideration for other sites of infection should be made when diagnosing ACV-resistant HSV-1 infections.
SOURCES OF FUNDING: Nil
Keywords: Herpes Simplex Virus, Acyclovir, Drug Resistance, Immunocompetent, Thymidine kinase

PP-025
Development of new antiviral strategies

Evaluation of Antiviral Efficacy and Validation of EN 14476 Standard Test for Virucidal Activity
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AIM: The European standard EN 14476 outlines a quantitative suspension test for assessing the virucidal activity of disinfectants, however, existing ISO standard method have been found to contain ambiguities that may affect the accuracy and comparability of results. This study aims to evaluate the antiviral efficacy of various agents using the EN 14476 test, validate its application in clinical settings, address ambiguities in the method, and investigate the impact of cell line on antiviral efficacy results.
METHODS: A series of experiments was conducted using the EN 14476 test to evaluate the virucidal activity of several antiviral agents against a panel of viruses, including enveloped and non-enveloped viruses. A key part of this study involved comparing the performance of two cell lines, Vero cells and BHK-21 cells, infected with Vaccinia Virus, Ankara strain, to assess potential discrepancies in antiviral efficacy results. The test involved exposing viral suspensions to the disinfectants for specified contact times, followed by neutralisation and titration to determine the reduction in viral infectivity.
RESULTS: The findings demonstrate that the test reliably measures the virucidal efficacy of antiviral agents, with significant reductions in viral titers observed for effective disinfectants. However, discrepancies were noted when comparing results from Vero cells and BHK-21 cells infected with the Vaccinia Virus (Ankara strain), indicating that cell line selection can impact the perceived efficacy of antiviral agents.
CONCLUSIONS: Adoption of the test, with consideration of cell line implications, can enhance the standardisation and comparability of antiviral efficacy studies, contributing to better infection prevention and control strategies.
SOURCES OF FUNDING: UKRI
Keywords: Antiviral agents, Virucidal activity, EN 14476, ISO standards, Clinical virology, Disinfectants, Validation, Cell lines, Infection control

PP-026
Development of new antiviral strategies

New antiviral therapies against paramyxoviruses: Boosting innate immunity by delivery of defective interfering RNA and targeting of adenosine deaminase acting on RNA 1

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AIM: The family *Paramyxoviridae* includes medically relevant viruses like measles (MeV), Nipah, and parainfluenza viruses. Direct therapeutic interventions are limited for all. Here, we present novel approaches to suppress paramyxovirus replication by inducing the double-stranded RNA-mediated host innate immune response.
METHODS: We have previously shown that MeV and canine distemper virus (CDV) replication are severely diminished *in vitro* and *in vivo* when the viral C protein is knocked out, leading to accumulation of immunostimulatory viral defective interfering RNAs (DI RNAs). We have also shown that MeV replication highly depends on the RNA editing activity of adenosine deaminase acting on RNA 1 (ADAR1), which interferes with DI RNA detection by innate immune receptors. Based on these findings, we have developed a system for *in trans*-delivery of *in vitro*-transcribed DI RNAs. Additionally, we have investigated the molecular requirements for the pro-viral activity of ADAR1. using reverse genetics and pharmacological approaches targeting its functional domains.
RESULTS: DI RNA delivery efficiently reduced replication of MeV and CDV in cell lines and primary cells by a dual mechanism: first, by activating antiviral innate immune responses, and second, by directly interfering with viral genome replication. Blocking the cytoplasmic localization of ADAR1 was essential for pro-viral activity. In addition, mutations affecting Z-RNA binding reduced the ability of ADAR1 to suppress infection-induced innate immune activation.
CONCLUSIONS: Our data indicate that DI RNA therapeutics may present a novel approach to treat paramyxoviral infections and that ADAR1 is a promising host factor for therapeutic targeting.
SOURCES OF FUNDING: Deutsche Forschungsgemeinschaft, SFB 1021, Project B12
Keywords: RNA editing, dsRNA, interferon, protein kinase R, inosine, A-to-I editing

PP-027
Development of new antiviral strategies

Shedding light on the mechanism of action of an antiviral compound against Zika virus
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AIM: Zika virus (ZIKV), transmitted by Aedes aegypti mosquitoes and from mother to fetus, can cause severe birth defects. A series of molecules synthesized at the University of Sassari, active against Bovine viral diarrhea virus, were tested for antiviral activity against ZIKV, focusing on their mechanism of action. The study aimed to assess these compounds' antiviral potential against ZIKV and understand their mechanism of action METHODS: HuH7 cells infected with ZIKV were treated with varying compound concentrations to determine their effectiveness (EC50) and cytotoxicity. Western blot analysis was used to examine viral protein synthesis and cellular thermal shift assay to evaluate compound interaction with RNA-dependent RNA polymerase (RdRp) RESULTS: Results showed that PS1097 exhibited the highest selectivity index (SI) and demonstrated activity against various viruses, indicating a broad spectrum. This probably indicates that it didn't directly bind to ZIKV RdRp. Western blot analysis revealed a reduction in viral protein synthesis in HuH7 cells but not in other cell lines, suggesting a cell-specific effect. Further investigations will explore PS1097's indirect mechanism of action, possibly by interfering with viral protein binding to cellular proteins crucial for replication. Co-immunoprecipitation studies will examine its impact on viral protein NS4A's binding to endoplasmic reticulum proteins
CONCLUSIONS: In summary, PS1097 shows promise as a broad-spectrum antiviral agent against ZIKV and other viruses. Its mechanism of action likely involves indirect interference with viral replication processes, warranting further research into its synergistic effects with known antiviral compounds
SOURCES OF FUNDING: Research Projects of Major National Interest (PRIN 2020)
Keywords: Zika, virus, compound, antiviral, efficacy, flavivirus

PP-028
Diagnostic stewardship & Diagnostic test accuracy

No evidence for ONNV or MAYV infections in returning travelers diagnosed in Belgium, from 2006-2023: a retrospective study

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AIM: The rising prevalence of arboviruses in tropical regions poses a global health threat, compounded by the challenge of symptom-based differentiation. O'nyong-nyong (ONNV) and Mayaro virus (MAYV), both Alphaviruses in the Togaviridae family, genetically closely related to chikungunya virus (CHIKV) are endemic to Sub-Saharan Africa and Latin America, respectively, and have the potential for global dissemination (Esposito et al., 2017; Pezzi et al., 2019). This study investigated the presence of ONNV and MAYV in samples previously testing PCR positive for CHIKV at the Institute of Tropical Medicine in Antwerp, Belgium.
METHODS: From 2006 to 2023, 54 samples from 52 patients tested positive with the CHIKV PCR adapted from (Panning et al., 2008), known to cross-detect ONNV and MAYV. Of the 49 patients with a known travel history, 16 travelled to Africa and nine to Latin America. In this study, we tested the leftover RNA extracts, mostly from serum samples, utilizing real-time PCRs targeting the ONNV and MAYV 5'UTR-nsP1 genes (Waggoner et al., 2017 and 2018) and re-analyzed them with the CHIKV PCR as a positive control.
RESULTS: All 54 samples reaffirmed positivity for CHIKV. However, none of the samples tested positive for ONNV or MAYV using the specific real-time PCR assays designed for these viruses.
CONCLUSIONS: This retrospective study did not find evidence for of ONNV and MAYV infections in returning travelers in Belgium. From 2024 onwards, ONNV and MAYV PCR will be performed prospectively on all CHIKV PCR-positive samples for further differentiation.
SOURCES OF FUNDING: Institute of Tropical Medicine, Antwerp
Keywords: arboviruses, diagnostics, PCR, retrospective study

PP-029
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of multiplex reference control preparations to support the performance monitoring of rapid meningitis and encephalitis nucleic acid based diagnostic testing

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AIM: Rapid detection and accurate diagnosis of the varied and many common causal agents of Meningitis/ Encephalitis (ME) are critical for positive patient prognosis. Several commercial multiplex, nucleic acid assays are available to aid diagnosis. The ME multiplex (MEX) reference control materials detect 15 causative agents of ME and are representative of human clinical specimens. Multiplex controls provide a cost-effective, reliable method to validate assay performance, as shown in this study.
Composition of MEX controls
Bacteria: Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae
Viruses: Adenovirus 7, Cytomegalovirus AD169, Enterovirus Coxsackie B3, Herpes Simplex Virus 1 and 2, Human Herpes Virus 6A, Human Parechovirus 3
Fungi: Cryptococcus spp.
METHODS: MEX controls were characterised using digital droplet PCR and internal assessment performed using commercially available molecular workflows.
RESULTS: Intra laboratory assessment showed reproducible detection and Cq values for each MEX control target ($P>0.05$).
CONCLUSIONS: There is a continued need for appropriately characterised reference control preparations for the assessment of multiplex assays across a range of clinically appropriate levels. Standardisation across molecular workflows and laboratories is critical for comparison of performance. This study demonstrates the reliable performance of utilising the MEX controls to validate target amplification in multiplex assays.
SOURCES OF FUNDING: Not applicable
Keywords: Meningitis, encephalitis, reference control material, quality assurance, performance monitoring

PP-030
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of Two Fully Automated PCR Assays for the Quantification of CMV and EBV DNA in Whole Blood

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AIM: Standardized cytomegalovirus (CMV) and Epstein-Barr virus (EBV) DNA quantification is important for the patient management in immunosuppressed individuals. The aim of this study was to evaluate the performance of the novel Alinity m CMV and EBV WB PEO assays compared to the corresponding routine real-time quantitative PCR tests.
METHODS: Fresh and frozen EDTA whole blood samples (CMV, n=151; EBV, n=153) were retested with Alinity m, and results were compared to those obtained with the in vitro diagnostics (IVD)/Conformité Européenne (CE)-labeled Artus CMV RG PCR kit and Artus EBV RG PCR kit. Sample concentrations ranged between <2.87 and 5.06 log(10) IU/ml (CMV) and between <2.15 and 6.05 log(10) IU/ml (EBV).
RESULTS: Overall qualitative concordance across all clinical samples was 94.7% (143/151) for CMV and 86.3% (132/153) for EBV and increased to 100% for samples ≥3 Log IU/mL by Artus (CMV, n=58; EBV, n=66). In quantified clinical CMV samples, correlation between the Alinity m CMV WB PEO test and the comparator was high (n=62; r=0.89) with a mean bias of +0,35 log(10) IU/mL. In quantified clinical EBV DNA samples, correlation between the Alinity m EBV WB PEO test and the comparator was high as well (n=79; r=0.91) with a mean bias of -0.28 log(10) IU/mL. Discrepant results (n=8/21) were observed only for low-titer samples ≤3.13 log(10) IU/mL. Alinity m processed each sample in less than two hours.
CONCLUSIONS: Alinity m CMV and EBV WB assays showed concordance >86% and correlation r≥0.89 with on-market routine diagnostic assays.
SOURCES OF FUNDING: Reagent kits were provided by Abbott GmbH.
Keywords: Cytomegalovirus, Epstein-Barr virus, Viral loads, DNA, PCR, Automated PCR assays

PP-031
Diagnostic stewardship & Diagnostic test accuracy

First fully-automated human Adenovirus qPCR reported in IU/ml is accurate and reliable for timely monitoring of HAdV DNAemia in transplant recipients

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AIM: Rapid, accurate and reliable detection and quantification of human Adenovirus (HAdV) DNAemia is essential to guide decision making and patient management. Existing PCR tests are non-standardised, inefficient, and have long turnaround time. Roche Diagnostics recently developed a fully-automated prototype HAdV qPCR (TIB HAdV) that is calibrated to the WHO standard. However, there is limited data on the performance of this assay. The aim of this study is to determine the analytical and diagnostic performance of the TIB assay to detect and quantify HAdV DNAemia in transplant recipients.
METHODS: The limit of detection (LoD), limit of quantification (LoQ), imprecision, linearity, PCR efficiency, bias, total analytical error (TAE), analytical specificity, and cross-contamination rate was determined using plasma samples spiked with WHO standard. The accuracy of the assay was assessed with a WHO-standard-calibrated panel. The diagnostic performance of the assay was evaluated prospectively with a reference laboratory PCR using samples collected from haematology-oncology patients.
RESULTS: The LoD and LoQ was 10.1 and 100.0 IU/ml respectively. The TIB assay was precise (coefficient of variation ≤10%), linear (R2 =0.99) and efficient (1.10) between 100 and 10,000,000 IU/ml. Within these concentrations, the bias and TAE was <0.30 and <0.70 log10 IU/ml respectively. The analytical specificity was 100%. Cross-contamination rate was 0%. The assay detected and quantified HAdV accurately. The overall, positive, and negative percent agreement with reference assay was 98.5%, 100.0%, and 97.2% respectively.
CONCLUSIONS: Excellent analytical and diagnostic performance of the TIB HAdV assay. The assay is suitable for laboratory detection of HAdV DNAemia.
SOURCES OF FUNDING: Roche Diagnostics.
Keywords: Adenovirus, PCR, fully-automated, transplant, diagnostic, accuracy

PP-032
Diagnostic stewardship & Diagnostic test accuracy

Proof of high stability and reproducibility of quantitative PCR results of HBV, HCV and HIV positive samples and the relevance for look back testing in hemovigilance

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AIM: Blood transfusions are generally safe but can carry risks such as transmission of bloodborne viruses like HBV, HCV or HIV. The risk of such transmissions has been greatly reduced by the introduction of NAT techniques. In this context, traceability protocols require blood samples to be kept for at least 3 years for future reference testing. The aim of this work was to determine the stability and reproducibility of longterm frozen samples that are positive for HIV, HBV and HCV in qPCR assays.
METHODS: We retrospectively examined the stability of diluted and aliquoted internal reference controls (IRCs) from single positive patient samples that were used every working day in qPCR assays spanning different time frames (up to 4 years). Descriptive statistics were used to describe the variability.
RESULTS: We found strong robustness of the samples used over a period of 2 years (HBV), 3 years (HIV) and 4 years (HCV) (Figure 1). Only about 1–1.6% of the samples gave a result outside a range of three standard deviations from the mean of all tested samples. Additionally, only 0.6–0.8% of the tested samples did not meet the required Rili-BÄK values (mean of the first 20 samples ± 0.5 log).
CONCLUSIONS: We demonstrated strong robustness of retained HIV, HBV and HCV positive samples stored for up to 4 years. We have also shown that all assays are highly reproducible. This work also highlighted the importance of manufacturer-independent controls and demonstrated the useability of long term stored samples for look back purposes.
SOURCES OF FUNDING: Not applicable
Keywords: hemovigilance, lookback testing, longterm stability, qPCR

PP-033
Diagnostic stewardship & Diagnostic test accuracy

Performance evaluation of a cytomegalovirus and an Epstein-Barr virus DNA real-time PCR assay in whole blood specimens on a fully automated platform

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AIM: Accurate quantitation of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) DNA in clinical specimens from immunocompromised individuals is crucial for effective therapy management. In this study, the performance characteristics of the Alinity m CMV and EBV whole blood (WB) assays were evaluated.
METHODS: Precision, accuracy, and specificity of the Alinity m assays were investigated with high positive (n=48), low positive (n=48), and negative (n=96) CMV and EBV WB panels. Fresh clinical WB samples were tested for CMV (n=115) and EBV (n=135) DNA on both Abbott m2000 (RealTime assays) and Alinity m analyzers. Finally, reproducibility of DNA quantitation after an average frozen storage of 6.2 days at -70°C was examined.
RESULTS: Precision testing across both Alinity m assays yielded a standard deviation ≤0.08 Log IU/mL with a difference to the assigned concentration ≤0.19 Log IU/mL while all negative panel members tested negative. Evaluating 52 CMV and 67 EBV samples that were quantified by both assays demonstrated a correlation of r=0.953 and 0.836 and a mean bias (Alinity-RealTime) of 0.18 and -0.22 Log IU/mL, respectively. Frozen storage showed low impact on quantitation (mean bias -0.04 and -0.15 Log IU/mL). Alinity m median onboard turnaround time for study samples was <3 hours.
CONCLUSIONS: The Alinity m CMV and EBV WB assays demonstrated precision ≤0.08 Log IU/mL, accuracy ≤0.19 Log IU/mL, and a mean bias 0.18 and -0.22 log IU/mL for CMV and EBV, respectively.
SOURCES OF FUNDING: The study was supported by Abbott GmbH.
Keywords: CMV, EBV, DNA load, whole blood, fully automated platform, performance evaluation

PP-034
Diagnostic stewardship & Diagnostic test accuracy

CMV IgG seroconverts two days earlier than CMV IgM on a commercial platform

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AIM: Knowledge of the performance characteristics of Cytomegalovirus (CMV) IgG and IgM analyses is essential when choosing which test method to use in your laboratory. The aim of this study was to investigate the time from infection to seroconversion in patients with primary CMV infection.
METHODS: Oslo University Hospital is the national transplant centre in Norway, and the Department of Microbiology performs CMV DNA PCR analyses weekly in risk groups. We performed a search in our laboratory system and selected patients with primary CMV infection over a three-year period between 2021 and 2024. We included patients that had several samples during the first thirty days after infection confirmed by a positive primary CMV DNA. In addition all selected patients had a previously negative CMV IgG result. CMV IgG and IgM analyses were performed using a commercial platform. We included equivocal results to the positive antibody results. Comparison with two other commercial platforms is planned.
RESULTS: Sixty-tree patients met the inclusion criteria. Seventeen of the 63 patients (27 %) never seroconverted during the 30 day long study period. Seroconversion of either IgM, IgG or both appeared after median 9 days (n=46). IgM was detected after median 13.5 days (n=40) while IgG was detected after median 11.5 days (n=46).
CONCLUSIONS: Twenty-seven % of the patients did not seroconvert within the first 30 days after CMV DNA was detected. Seroconversion was detected after median 9 days. IgG was detected in median two days earlier than IgM.
SOURCES OF FUNDING: None.
Keywords: CMV, Cytomegalovirus, serology, antibodies, seroconversion

PP-035
Diagnostic stewardship & Diagnostic test accuracy

Clinical performance evaluation of a CE-IVD diagnostic test for detection of Parvovirus B19 in patient plasma

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AIM: In immunocompromised hosts, Parvovirus B19 (PVB19) infection has been linked to various complications including hepatitis, myocarditis, chronic pure red cell aplasia, and dysfunction of transplanted organs in both solid and allogeneic hematopoietic stem cell transplantation recipients. Active infection is diagnosed by serology or PCR. As serology has limited use in immunocompromised patients due to delayed antibody response, development of PCR assays has enabled reliable diagnoses of PVB19 infections. Here we evaluated the performance of real-time PCR-based diagnostic test for detection of PVB19 infection in patient plasma - TaqPath Menu I GeneProof Parvovirus B19 PCR Kit.
METHODS: In total 47 plasma samples were tested in parallel using TaqPath Menu I GeneProof Parvovirus B19 PCR Kit and either RealStar® Parvovirus B19 Kit 1.0 or Parvovirus B19 R-GENE® assay. One sample was excluded from analysis due to an invalid result. Diagnostic sensitivity and specificity were evaluated through agreement of results to comparators.
RESULTS: Of 46 samples with valid results, presence of PVB19 was detected in 12, while 34 samples tested negative for the PVB19 virus with both the TaqPath Menu I GeneProof Parvovirus B19 PCR Kit and the comparators. As all results were concordant the diagnostic sensitivity of the TaqPath Menu I GeneProof Parvovirus B19 PCR Kit was 100% (95% CI, 69.87-100.00%) and the diagnostic specificity was also 100% (95% CI, 87.36-100.00%) in this cohort.
CONCLUSIONS: TaqPath Menu I GeneProof Parvovirus B19 PCR Kit is a highly accurate method for detection of PVB19 DNA in patient's plasma with 100% diagnostic sensitivity and specificity.
SOURCES OF FUNDING: Funded by GeneProof.
Keywords: Parvovirus B19, Immunocompromised, transplant

PP-036
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of a multiplex real-time PCR-based CE-IVD test clinical performance for detection and differentiation of JC and BK viruses in urine samples

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AIM: BK and JC viruses can cause significant complications in transplant or immunocompromised patients. Early detection and monitoring of viral load levels can aid timely identification, diagnosis, efficient management and reducing complications risk in these patients leading to improved patient outcomes. Here we evaluated clinical performance of multiplex real-time PCR-based TaqPath Menu I GeneProof BK/JC Virus (BK/JC) PCR Kit designed for qualitative and quantitative detection of BK and JC viruses.
METHODS: Clinical sensitivity and specificity of multiplex TaqPath Menu I GeneProof BK/JC Virus (BK/JC) PCR Kit for BK and JC viruses detection were assessed against the singleplex RealStar® BKV PCR Kit on N=38 and RealStar® JCV PCR Kit on N=48 patients' urine samples. Positive (PPV) and negative predictive values (NPV) were calculated.
RESULTS: BK virus was detected in 28.95% of samples (N=11/38) using both assays, while 71.05% of the samples were negative (N=27/38), with no discordant results. JC virus was detected in 22.92% of samples (N=11/48) with remaining samples negative (N=37/48) and 100% concordant results between 2 tests. PPV and NPV of TaqPath Menu I GeneProof BK/JC Virus (BK/JC) PCR Kit for both BK and JC virus were 100%. The kit demonstrated diagnostic sensitivity of 100% for BK (95%CI, 67.85-100.00%) and JC (95%CI, 95.45-100.00%) virus, with diagnostic specificity of 100% for BK (95%CI, 84.50-100.00%) and JC (95%CI, 98.65-100.00%) virus in this cohort.
CONCLUSIONS: TaqPath Menu I GeneProof BK/JC Virus (BK/JC) PCR Kit is a highly accurate multiplex real-time PCR diagnostic test for detection of BK and JC viruses in patients' urine.
SOURCES OF FUNDING: Funded by GeneProof.
Keywords: BK virus, JC virus, transplant, immunocompromised, real-time PCR, multiplex

PP-037
Diagnostic stewardship & Diagnostic test accuracy

Comparisons of two syndromic multiplex and three miniplex panel tests for the detection of viruses causing upper respiratory tract infections

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AIM: Various pathogens cause respiratory infections. Identifying the specific pathogen is usually not necessary for treatment, but detecting multiple viruses and bacteria simultaneously can be crucial in medical emergencies or outbreaks. Diagnostic options include semi-automated RT-PCR systems and cartridge-based multiplex panels. These systems vary in terms of hands-on time, time to results, cost, throughput, and pathogen coverage.
METHODS: Here, we initially compared the performance of the two cartridge-based systems the cobas eplex RP2 and the BioFire RP2.1 and the four Allplex respiratory panels using up to 100 de-identified clinical respiratory samples or virus bank samples. As data indicated comparable performance of all assays, the non-inferiority of the eplex RP2 panel to the lab based solution by Seegene was verified by testing >700 samples obtained from hospitalized patients with symptoms of an upper respiratory tract infection.
RESULTS: In the initial study, the sensitivity of the three tests was comparable considering the limited samples size per target. Specificity of the two multiplex panels was ranking from 97.6-100%. Testing of additional clinical samples collected during winter season 2023/24 confirmed an equal performance with some discrepancies observed of which resolution testing is ongoing.
CONCLUSIONS: The eplex RP2 panel demonstrated a comparable performance to an established lab-based test while it is faster and easier to be used, allowing a potential placement at Point of Care. Additionally, the study underlined a high positivity rate with a broad variation of respiratory pathogens observed in hospitalized patient with symptoms of an upper respiratory tract infection.
SOURCES OF FUNDING: Roche Diagnostics International AG
Keywords: Multiplex PCR, respiratory infections

PP-038
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of T cell responses detected by the Elecsys IGRA SARS-CoV-2 assay

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AIM: Increasing evidence suggests that monitoring T cell responses may play an important role in assessing protective immunity against symptomatic and severe SARS-CoV-2 infection. This study aims to evaluate the clinical performance of the Elecsys IGRA SARS-CoV-2 assay to measure specific T cell responses, enabling better assessment of immune status. Additionally, the aim is to stratify clinical sensitivity based on positive RT-PCR results and days after vaccination.
METHODS: Whole blood samples were collected prospectively and tested with the Elecsys IGRA SARS-CoV-2 immunoassay. Sensitivity cohorts included individuals who either have had a positive RT-PCR diagnosis for SARS-CoV-2 infection or have been vaccinated against COVID-19. The specificity cohort comprised individuals with no vaccination and no known infection in the past. Anti-SARS-CoV-2 antibody titers (anti-Nucleocapsid, anti-Spike) were determined for each donor.
RESULTS: The Elecsys IGRA SARS-CoV-2 assay demonstrated an overall sensitivity of 99.7% (384/385) in the combined cohorts. The blood draw was performed between 12 and 734 days after a positive RT-PCR result (13 days and 736 days post symptoms onset), and between 5 and 307 days after the last vaccination. No dependencies between days after infection / last vaccination and blood draw were observed. Furthermore, assay performance was stratified based on demographic information.
CONCLUSIONS: This study significantly contributes to the characterization of the Elecsys IGRA SARS-CoV-2 assay by demonstrating its high sensitivity of partly longtime-past SARS-CoV-2 infections and vaccinations. Assessment of T cell responses may enable clinicians to make better informed decisions for patient care.
SOURCES OF FUNDING: This study was funded by Roche Diagnostics GmbH (Mannheim, Germany).
Keywords: cellular immunity, interferon-gamma release assay

PP-039
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of the QIAstatDx meningitisencephalitis syndromic test kit in a university hospital
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AIM: Rapid diagnosis of infectious meningitis and encephalitis (ME) is essential for appropriate treatment. Rapid syndromic tests are important tools to ensure the necessary timely diagnosis. This study evaluated the one-year performance of the QIAstat-Dx ME panel in the routine diagnostic workflow at a university hospital.
METHODS: A total of 107 CSF samples were analyzed with the QiaStat Dx ME (QS-ME) panel between January 2022 and January 2023. Simultaneously, microscopic evaluation, biochemical analyzes and bacterial, fungal and mycobacterial cultures were performed in CSF according to the physician's order.
RESULTS: CSF samples were obtained from patients aged between 0 days and 90 years (mean: 28,9). Nine viruses (Ct >20) and three bacteria (Ct <20) were detected by the panel in 12 of 107 (11.2%) samples. Detected pathogens were: enterovirus (n:5) and parechovirus (n:1) in the infant group; HHV-6 (n:1) in the child group, HSV-1 (n:1), S.pneumoniae (n:1) and N.meningitidis (n:1) in the adult group; VZV (n:1) and S.pneumoniae (n:1) in the elderly group. Sample with N. meningitidis was culture negative due to antibiotic use before the lumber puncture. No culture positive PCR negative samples were detected for the bacteria covered by the panel. The QS-ME panel was operated 24/7, and the average time between sample acceptance and reporting of the result was 122 minutes.
CONCLUSIONS: QS-ME panel enabled the detection of pathogens in 10 samples that could not be diagnosed with routine conventional diagnostic tests in the laboratory and provided results 24/7, within an average of 2 hours.
SOURCES OF FUNDING: None
Keywords: Meningitis, Meningitis and encephalitis panel, Rapid diagnosis

PP-040
Enteric viruses & Neurovirology

Enterovirus in children with acute respiratory infection (2022-2023)
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AIM: This study aimed to evaluate clinical and microbiological characteristics of enterovirus (EV) infections in children with acute respiratory infections in a university hospital in Izmir, Turkey
METHODS: Between March 2022 and November 2023, 640 respiratory samples of patients with acute respiratory infections were tested with syndromic respiratory panels (Bio-Speedy respiratory tract RT-qPCR MX-24, Bio-Speedy Respiratory ID-2 kit, QIAstat-Dx respiratory SARS-CoV-2 panel). Rhinovirus/enterovirus positive samples were re-tested with a specific in-house RT-qPCR assay to confirm the presence of enterovirus. Demographic and clinical characteristics, comorbidities, coinfections were analyzed.
RESULTS: Of the 640 samples, 376 (58%) were positive for one or more respiratory pathogens; 219 of which were positive for rhino/enterovirus. 157 samples were retested with EV-specific RT-qPCR and 29 (18.4%) were confirmed as EV. Among the EV+ patients, 21 (72%) were ≤5 years old (mean: 3.80 years; 1 month-15 years), 10 (34%) had underlying medical condition, 6 of whom had asthma or reactive airway disease. Most EV+ children (86%) required hospitalization, 51% were treated for bronchospasm, 4 received respiratory support. Coinfection was detected in 7 EV+ patients (24%) and the second most common agent was adenovirus. None of the EV+ patients had positive bacterial cultures in respiratory samples. EV sequencing studies are continuing.
CONCLUSIONS: Among pediatric patients admitted to hospital with acute respiratory tract infection, EV positivity was more common in children under 5 years of age, and most of these cases required inpatient care, half of which were treated for bronchospasm.
SOURCES OF FUNDING: None
Keywords: Enterovirus, Pediatric respiratory infection, RT-qPCR

PP-041
Enteric viruses & Neurovirology

Detection of pegivirus and norovirus by viral metagenomics in suspected adult central nervous system infections of unknown etiology, Spain, 2023

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AIM: Virus-induced diseases of the central nervous system (CNS) represent a significant burden to global health, although the etiology remains unknown in about half of cases. Using viral metagenomics (mNGS) we investigated the etiologies of cases with a suspected viral CNS infection that remained undiagnosed despite conventional testing.
METHODS: Retrospective study with cerebrospinal fluid (CSF) samples from hospitalized patients with suspected viral CNS infections (n=60) in Spain, 2023, using capture viral probe-based mNGS. We used as controls positive infection cases (DNA and RNA viruses) and CSF samples from patients sampled for non-infectious indications. Data was analyzed through an in-house NGS analysis workflow and IDSeq, a cloud-based, open-source bioinformatics platform.
RESULTS: In 9 out of 60 (15%) idiopathic samples, a virus was detected by mNGS. Partial or complete genome sequences were obtained for RNA viruses (3 human pegivirus (HPgV) and 4 norovirus) and DNA viruses (1 herpes simplex virus type-1 and 1 cytomegalovirus). All HPgV-positive samples were confirmed by an in-house RT-PCR covering part of the E1/E2 glycoprotein coding sequence. Phylogenetically, all HPgVs belonged to genotype 2, the most prevalent in Europe. Noroviruses belonged to genotypes GII.17 [P17] (n=1), GII.3 [P12] (n=2) and GII.2 [P16] (n=1). No coinfecting pathogens were detected in these samples.
CONCLUSIONS: The study shows the importance of viral mNGS to identify unsolved etiologies in CSF infections. We detected uncommon viruses (pegivirus and norovirus) that should be considered as possible causative agents of idiopathic CNS infections in adults. Further studies are needed to better define their clinical relevance.
SOURCES OF FUNDING: Acción Estratégica en Salud Intramural (AESI), ISCIII.
Keywords: metagenomics, idiopathic meningitis, etiology, cerebrospinal fluid

PP-042
Enteric viruses & Neurovirology

Human Herpesvirus Type 6 (HHV6) as a Causal Agent of Encephalitis in Immunocompetent Patients: Case Study from 2019-2023

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AIM: To investigate the prevalence and clinical significance of Human Herpesvirus Type 6 (HHV6) as a causal agent of encephalitis in immunocompetent patients as well as the positive and negative predictive value of the test based on the level of immunocompetence.
METHODS: A retrospective observational study was conducted on all HHV6 PCR requests in CSF from 2019 to 2023 at a tertiary hospital in various clinical-demographic groups.
RESULTS: A total of 607 HHV6 PCR tests were requested in CSF, with 89 not performed due to lack of samples/ justification. The remaining 519 tests corresponded to 464 patients, 63 of whom were immunocompromised and 402 non-immunocompromised.
There were 5 patients with a positive result in the immunocompromised group, with encephalitis associated with the virus in 4 cases, one case was associated with an episode of ICANS linked to CART therapy. In the non-immunocompromised group, 7 positive HHV6 cases were detected. Only in one case was HHV6 considered the causal agent of meningoencephalitis. In the others, all with a normal electroencephalogram, viral encephalitis was ruled out. One patient had the virus integrated into her chromosome.
The prevalence of HHV6 in CSF as a responsible agent for viral encephalitis was 9.30% in immunocompromised patients, with a positive predictive value (PPV) of 80%, while in immunocompetent patients, the prevalence was 0.33%, with a PPV of 14.3%
CONCLUSIONS: Any positive result in CSF in an immunocompetent adult should be studied with caution as its association with encephalitis in this patient group is minimal.
SOURCES OF FUNDING: This project was undertaken without any funding.
Keywords: HHV6, non-immunocompromised, Encephalitis

PP-043
Enteric viruses & Neurovirology

Epstein-Barr virus can infect rabbits and induce brain pathology similar to what has been observed in patients with multiple sclerosis

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AIM: An accumulating body of data implicates Epstein-Barr virus (EBV) in the pathogenesis of multiple sclerosis (MS). However, how EBV induces the pathology seen in MS has been frustratingly difficult to addressed, primarily due to the lack of a naturally susceptible animal model of EBV infection. We have recently established a novel rabbit model of EBV infection, which recapitulates EBV infection in humans. In this study, we investigate the impact of peripheral EBV infection on virus dissemination and pathology in the CNS of rabbits.
METHODS: Rabbits were infected with EBV via IV route. Animals were sacrificed at 2-4 weeks post infection and all major organs were collected. Histopathological changes and viral dynamics were examined in peripheral blood, spleen and brain, using a range of molecular and histopathology techniques.
RESULTS: Our findings revealed that: (1) IV administration of EBV results in widespread infection with readily detectable virus in the spleen, PBMCs and plasma, (2) circulating infected cells and not free virus correlates with CNS infection, (3) EBV induces CNS pathology similar to that seen in MS, including neuroinflammation and demyelination, (4) the expression EBV latent transcripts, EBER1 and EBNA1, correlate with the levels of expression of proinflammatory cytokines such as IL1β and IL6.
CONCLUSIONS: Our study provides, for the first time, direct evidence that primary peripheral EBV infection can lead to the virus crossing the blood-brain barrier, entering the CNS and inducing pathological features similar to those seen in the brain of MS patients.
SOURCES OF FUNDING: UAE University, Zayed Centre-based grants: 12R001/12R168
Keywords: EBV, multiple sclerosis, neuroinflammation, demyelination

PP-044
Enteric viruses & Neurovirology

Molecular characterization of Enteroviruses in patients with acute respiratory infection in the Central African Republic

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AIM: Enteroviruses (EVs) cause numerous pathologies, notably neurological, respiratory, cutaneous and enteric, leading to morbidity and mortality, especially in young children. These pathologies particularly affect countries in Asia and Sub-Saharan Africa. We set out to evaluate the impact of these viruses on children suffering from Acute Respiratory Infections in the Central African Republic (CAR).
METHODS: A retrospective study was carried out on nasopharyngeal samples collected as part of influenza surveillance at five sentinel sites in CAR in 2019, having a negative result for influenza viruses. A total of 178 samples, grouped into 20 pools, were screened in rRT-PCR 5' UTR for the detection of EVs, and the positives were amplified in RT-PCR and nested PCR and sequenced in NGS. Phylogenetic analyzes were carried out on the VP1 region of the EV genome.
RESULTS: EVs were detected in 17/20 pools of the nasopharyngeal samples, 13 were amplified and sent for sequencing, of which 11 yielded sequences. A total of 42 read5able sequences were generated in NGS, of which 24 cover the VP1 region. Among the EVs found, 14/24 (58.3%) were EV-C (7 PV3, 3 CV-A24, 2 EV-C99, 1 CV-A11 and 1 PV2), 7/24 (25.0%) were EV-B (3 E7, 1 E1, 1 E29,1 CV-B4 and 1 CV-B5) and 3/24 (12.5%) were EV-A (1 CV-A4, 1 CV-A5, and 1 CV-A16).
CONCLUSIONS: Our study showed the presence of EVs in nasopharyngeal samples in CAR, dominated by EV-C. In addition, viruses of vaccine origin were also found in these suspected influenza samples
SOURCES OF FUNDING: PTR-161, Institut Pasteur, Paris, France
Keywords: Molecular Characterization, Enterovirus, ARI, CAR

PP-045
Enteric viruses & Neurovirology

Molecular surveillance of enterovirus A71 in Spain after 2016 outbreak (2017-2023)

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AIM: Enterovirus A71 (EV-A71) belongs to “species A” of “Enterovirus” genus, “Picornaviridae” family. In Spain, it was a low incidence EV, accounting for 0-2% of all typed EV from 2002 to 2015. In 2016, however, EV-A71 detection increased to 26.5%. Outbreak was associated with emergence of a new variant (C1v) which caused many severe encephalitis pediatric cases. This study describes EV-A71 infections in Spain after outbreak (2017-2023) to investigate whether 2016-C1v strain is still circulating.
METHODS: EV-positive samples were genotyped and clinical and epidemiological data of EV-A71 infections were analyzed. Phylogenetic analysis was performed
RESULTS: Between 2017 and 2023, 112 EV-A71 infections were detected, with an annual rate of 1.3% (n=6), 11.9% (n=58), 2.2% (n=10), 3.1% (n=2), 0.6% (n=1), 6% (n=20) and 4.1% (n=15), respectively. Most infections (92.8%) were in children < 6 years-old (mean, 3.25 yr). Clinically, 4.5% patients presented muco-cutaneous symptoms, 21.4% fever-without-source, 22.3% respiratory symptoms, 20.5% meningitis and 31.2% other neurological pathologies such as encephalitis, ataxia or paralysis. Phylogenetic analysis with 95 study sequences showed that most of them (81/95; 85.2%) grouped with 2016-C1v strain. Noteworthy, a separate cluster within C1 genogroup formed by sequences from 2018 and 2023 was observed
CONCLUSIONS: EV-A71C1v continued to circulate after 2016 outbreak, especially in 2018, although with lower incidence. As others infections, EV-A71 was virtually undetected during and after pandemic but it has re-circulated in 2022 and 2023. C1v remains a priority strain but new variants appear to be emerging. Over 30% of EV-A71 infections were associated with more severe neurological diseases
SOURCES OF FUNDING: PI18CIII-00017; PI22CIII-00035
Keywords: Enterovirus, neurological infections, phylogenetic analysis, EV-A71 C1 variants

PP-046
General virology

Host potential genetic risk factors related to Inflammatory and Degenerative Diseases of the CNS in ZIKV and CHIKV infected patients

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AIM: The aim of this study is to delve into the genetic underpinnings of inflammatory and degenerative diseases in patients afflicted by ZIKV and CHIKV infections.
METHODS: We conducted a meticulous screening of 102 patients exhibiting neurological manifestations linked to arbovirus infection, ultimately selecting 28 individuals with neuroinflammatory diseases of the CNS. Employing NGS analysis coupled with a customized SNPs panel, we investigated 200 genetic variants using Ion Torrent technology. For statistical significance, we considered $p<0.05$ in Fisher's exact test.
RESULTS: Our investigation unearthed 14 significant genetic variants. Among these, noteworthy findings include the identification of genetic variants rs7453920, rs2051549, rs2229238, and rs7514452, which are associated with viral susceptibility. Moreover, in patients demonstrating nervous system involvement, we identified variants rs3212227 (of the IL12B gene) and rs3130253 (of the MOG gene), as well as rs73196329 (from the CBLB gene), rs8099917 (from the IFNL4 gene), and SLC30A7 (within the HLA-DRB1*15:01 gene). These SNPs have been previously implicated as risk factors for immune-mediated neurological diseases and likely contributed to nervous system complications following exposure to ZIKV and CHIKV.
CONCLUSIONS: Our study underscores the indispensable role of investigating both viral and host genetic factors in comprehending the pathogenesis of arbovirus infections in patients exhibiting severe neurological phenotypes. These variants may be important biomarkers for further investigation as prognostic tools and therapeutic drugs targets.
SOURCES OF FUNDING: FAPERJ - Fundação Carlos Chagas de Amparo a Pesquisa do Estado do Rio de Janeiro CAPES - Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Keywords: Arbovirus; Central Nervous System Inflammation; Genetic variants; Zika virus; Chikungunya virus

PP-047
General virology

The Dynamic Gain-Loss Network of SARS-CoV-2 Spike Amino Acid Changes During Virus Evolution – United Kingdom, Jan. 2020-Aug. 2023

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AIM: The SARS-CoV-2 spike protein is a multifunctional protein that mediates virus cell entry through its S1 and S2 subunits. We aimed to examine comparatively the gain-loss of amino acids in S1 and S2 during evolution and probe for potential associations with virus control measures (lockdowns and applied vaccinations).
METHODS: We investigated, by code written in R, the temporal gain-loss of amino acids in curated n=1,961,911 GISAID spike protein sequences from the UK from 2020-01-01 to 2023-08-30 and identified the most often changing positions in each of the two subunits. We applied loess and joinpoint regression to study trends of amino acid changes during virus evolution and to examine potential relations to external factors, lockdowns, and vaccinations. Using the 20 residues as nodes and the most frequently altered positions as links, we constructed the evolving gain-loss network of amino acid changes.
RESULTS: In total, 51,790 unique spike sequences were found. Gain-loss trend curves revealed fundamental differences between the S1 and S2 subunits that displayed synchronized multilocational trends of amino acid pairing. Joinpoint analysis revealed critical time points that coincided with the periods of lockdowns and primary scheme and/or booster vaccinations applied in the UK during the pandemic.
CONCLUSIONS: Our results suggest temporal multilocational epistatic amino acid changes in spike variable positions, leading to favored conformational changes and preferred physicochemical traits (e.g., pI, hydropathy, thermostability) potentially crucial for virus functionality, in response to adverse-for-virus-survival conditions.
SOURCES OF FUNDING: None.
Keywords: SARS-CoV-2 spike, amino acid gain-loss network, joinpoint analysis

PP-048
General virology

Frankfurt Virus Platform – acquisition & distribution of newest respiratory virus isolates and tools to enable high quality research

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AIM: Due to their rapid molecular evolution, significant virus research is dependent on the availability of current virus isolates. For this reason, the diagnostics and research departments of the Institute of Medical Virology Frankfurt have jointly established a virus bank, which was built up by the co-operation with clinics at the University Hospital Frankfurt (UKF) enabling access to many patient samples. To make more efficient use of this potential, the aim is to inventory and modernize the virus bank and expand it into a virus platform by adding tools like protocols.
METHODS: As part of a study, nasopharyngeal swabs were collected from UKF patients from December 2023 to April 2024 to refresh the stock of respiratory viruses. The samples were cultivated in cell lines or air-liquid interface human bronchoepithelial (ALI-HBE) cell cultures and then analyzed for cytopathogenic effect (CPE).
RESULTS: The inventory resulted in a number of approximately 320 different virus isolates (from 1975 to 2024) of 17 different virus families. For the digitization of the virus bank, the paper-based sample management system (SMS) is transferred into a cloud. For the renewal of the virus bank, 121 patient samples were cultivated, of which 17 % (21) showed CPE, caused by hMPV (2), PIV3 (7), RSV (4), IAV (5) or IBV (3).
CONCLUSIONS: With the help of continued close cooperation within the UKF and new technologies for sample and document management, the development of the Frankfurt Virus Platform can continue to progress, enabling partners to conduct high-quality research on respiratory and other viruses
SOURCES OF FUNDING: CoroPan (LOEWE)
Keywords: Virus bank, virus platform, sample management system, respiratory viruses, ALI-HBE

PP-049
General virology

A large outbreak of acute hemorrhagic conjunctivitis in Fujian, China in 2023
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AIM: A large outbreak of acute hemorrhagic conjunctivitis (AHC) occurred in Fujian of China between September and October in 2023. This purpose of the study is to determine the causative agent for this outbreak and to analyze virus genetic features.
METHODS: 28 conjunctival swabs were collected from 28 patients with AHC, and viral isolation, molecular typing and phylogenetic analysis were performed.
RESULTS: A total of 5563 AHC cases were reported in Fujian between September and October in 2023. The onset age of AHC was mainly concentrated in 5-15-year-old age groups, which accounted for 65.99% of the total number of cases. The proportions of school students and farmers were higher than those of other occupation. 20 virus strains were isolated from 28 conjunctival swabs. Sequencing of the VP1 region of these strains identified that coxsackievirus A24 variant (CVA24v) could be the causative agent of the outbreak. Phylogenetic analysis based on the 3C regions showed that the Fujian CVA24v strains belonged to Genotype IV together with the strains isolated from worldwide AHC outbreaks after 2002. Fujian CVA24v strains respectively had 99.4-99.8%, 98.9-99.6% and 98.7-99.6% sequence identify to CVA24v associated with AHC outbreaks in Guangdong of China, India and Pakistan in 2023, and clustered a single linage together with CVA24v in the above 3 regions within Genotype IV.
CONCLUSIONS: CVA24v was identified as the causative agent for the AHC outbreak in Fujian in 2023 and clustered an independent transmission linage together with CVA24v associated with other AHC outbreaks in the world in 2023.
SOURCES OF FUNDING: National key R&D (2021YFF0703801)
Keywords: outbreak;acute hemorrhagic conjunctivitis; coxsackievirus A24 variant;

PP-050
General virology

Deciphering Genetic Modulators of Peripheral Nervous System Involvement in Arbovirus Infections

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AIM: This study aimed to pinpoint genetic variations influencing peripheral nervous system (PNS) complications, such as Guillain-Barré syndrome, after arboviral infection.
METHODS: We employed NGS to analyze a pre-selected panel of variants associated with neuroinflammation and related pathways. The investigation encompassed 97 arbovirus-infected controls without neurological manifestations and 40 cases who developed PNS involvement following arbovirus infection. Fisher's exact test with p-value correction identified statistically significant associations ($p<0.05$) and in silico functional evaluation was carried out.
RESULTS: This investigation revealed distinct genetic variants influencing PNS involvement in arbovirus infections. Controls exhibited a higher frequency of the rs1012334 (A>T/T) variant in the IFNAR1 gene, suggesting a potential protective effect. Conversely, novel variants were identified with higher frequency in cases with PNS complications: (i) chr21:34713752 (A>A/T) in IFNAR1, (ii) chr1:154414062 (A>AG) in IL6, and (iii) chr7:28138175 (T>TA) in JAZF1. Notably, variants (ii), (iii), and (iv) reside within genomic regions potentially regulating gene expression in immune cells and neurons, highlighting their potential functional significance.
CONCLUSIONS: To the best of our knowledge, this is the first study shedding light on genetic contributors in HLA and non-HLA genes to PNS involvement following arbovirus infection. Our findings on novel variants in IFNAR1, IL6, and JAZF1 warrant further investigation to elucidate their precise roles in neuroinflammatory processes triggered by arboviruses. This knowledge could guide the development of personalized prognostic tools and potentially novel therapeutic strategies.
SOURCES OF FUNDING: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).
Keywords: arbovirus, peripheral neurological manifestations, genetic variants

PP-051
General virology

HCMV encoded miRNAs in acute HIV infection and during one year of follow-up

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AIM: To analyze HCMV-encoded miRNA expression in acute HIV subjects, early treated with antiretrovirals, in comparison with HCMV mono-infected individuals. To correlate miRNA expression with viral replication and inflammation markers.
METHODS: Fourteen miRNAs were analyzed in sixteen HIV/HCMV co-infected and six HCMV mono-infected subjects by Real-time-PCRs. HIV-1 RNA, HCMV DNA, VEGF, CXCL-10, and D-dimer levels were quantified before therapy started and during 1-year of follow-up. Statistical evaluation included Fisher and Spearman's analyses.
RESULTS: At baseline, median HIV-1 RNA was 6.6 Log copies/ml (IQR: 5.1-7.1) and HCMV DNA was detected in two out of sixteen HIV patients (<2.2 and 5.5 Log IU/ml). During follow-up, HIV-1 RNA decreased to a median of 1.5 Log copies/ml (IQR: 1.5-1.9), and two transient low-level HCMV-reactivations were revealed. At baseline, while hcmv-miR-70-3p was detected in all subjects, hcmv-miR-UL22A-5p and hcmv-miR-UL148D were more frequently detected in HIV/HCMV co-infected patients than in mono-HCMV individuals. This different prevalence was confirmed during follow-up when hcmv-miR-US33-5p also reached a higher detection frequency in co-infected vs mono-infected subjects. Hcmv-miR-US25-2-3p, hcmv-miR-US5-1, hcmv-miR-UL36-3p, and hcmv-miR-UL36-5p were found only in the plasma of the subject with high HCMV viremia. Except for this latter case, no correlation between miRNAs and HCMV DNA was found. On the contrary, levels of hcmv-miR-22A-5p and hcmv-miR-UL148D correlated with HIV-1 RNA (r=0.573, p<0.001) and VEGF (r=0.316, p=0.0372) respectively.
CONCLUSIONS: In acute HIV infection, HCMV-encoded miRNAs are differently modulated. Since they are involved in host immune evasion or vascular diseases, knowledge of their biology can be crucial for developing new therapeutic strategies.
SOURCES OF FUNDING: Italian Ministry of Health
Keywords: HIV, HCMV, miRNA, inflammation

PP-052
General virology

Circulation of enterovirus types in Europe between January 2018 and August 2023: a retrospective multi-center surveillance study

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AIM: Enteroviruses (EV) can cause severe infections, particularly in young children. Although prevalent, EV infections are not notifiable in most European countries, resulting in substantial variation in surveillance between countries. We collected and analyzed available EV data from participating European countries to assess the current epidemiological and clinical landscape from 2018 to 2023.
METHODS: Aggregated data on EV detected in the study period, was requested from National Focal Points for Surveillance and from the European Non-Polio Enterovirus Network (ENPEN). Number and type of EV detected by month, specimen type, age group, and clinical presentation of the top ten most detected types was collected.
RESULTS: 16,037 EV-positive samples were successfully typed and reported by 26 institutions from 13 countries, identifying 43 types. Most frequently detected EV types were Echovirus-30 (E30; n=1303), followed by coxsackievirus A6 (CVA6), enterovirus D68 (EV-D68), E9, E11, CVB5, E18, EV-A71, CVB4, and E25. Among EV infections with available age information, 6,868 (72%) cases occurred in children under 5 years, and neurological symptoms were reported for 1,367 cases. We observed 57 cases with neonatal sepsis (1.7%), 37 with paralysis (1.1%), and 15 cases with myocarditis (0.4%).
CONCLUSIONS: This study emphasizes the continued widespread circulation of EV among young children, often leading to neurological symptoms. E30, a common cause of viral meningitis, was the most frequently identified type. Despite individual countries collecting extensive EV data, inconsistencies in case definitions and reporting parameters across borders poses challenges to surveillance efforts. Encouraging harmonization of case definition for EV surveillance is recommended.
SOURCES OF FUNDING: No funding.
Keywords: Enterovirus, Laboratory detection, Typing, Europe, Outbreaks

PP-053
General virology

Respiratory viruses during the weeks 04-18/2024 in Northern Greece

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AIM: Viral infections of the respiratory system are most common causes of morbidity in the general population. As the clinical picture of the patients is similar and the differential diagnosis is difficult, laboratory investigation and diagnosis are necessary. The purpose of the study is to detect the presence of respiratory viruses in patients with respiratory syndromes in Northern Greece during the peak weeks 4/2024 to 18/2024.
METHODS: A multiplex PCR system was used to analyse 221 nasopharyngeal swabs from patients with flu-like symptoms, forwarded to the NIC for N. Greece from collection points of the Hellenic epidemiological surveillance network.
RESULTS: 160 (72.4%) of the samples were positive. The pandemic SARS-CoV-2 was detected in 13 (5.9%) samples, RSV virus in 16 (7.2%) samples, and influenza viruses in 41 (18.55%) samples [22 (9.95%) A(H1N1), 6 (2.7%) A(H3N2), 13 (5.88%) B]. At the same time, human coronaviruses (229E, HKU-1, NL63, OC43) were detected in 26 (11.7%) samples, (hAdV) in 10 (4.5%), Rhinoviruses/Enteroviruses in 38 (17.2%), HPVI-3 in 6 (2.7%), hMPV (A and B types) in 7 (3.1%) samples, while 3 (1.3%) samples were positive for a non-viral pathogen, Mycoplasma pneumoniae. Fourteen co-infections were found. A diversity of respiratory viruses was observed, and interestingly, influenza viruses and the rest of the respiratory viruses presented reverse increase-decrease patterns throughout the monitored period.
CONCLUSIONS: The circulation of respiratory viruses seems to make its way back to normality after the recent pandemic adventure.
SOURCES OF FUNDING: Hellenic National Public Health Organisation
Keywords: respiratory infections, viruses, epidemiology, surveillance

PP-054
General virology

AAV2 is a common finding in adenovirus infections in children and adults

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AIM: Recently, both human adenovirus (hAdV) and adeno-associated virus-2 (AAV2) have been implicated in pediatric cases of severe hepatitis. However, these associations were established in a limited number of patients, while extended control data was missing. We sought to determine the presence of AAV2 in a large cohort of patients with hAdV infections.
METHODS: All clinical specimens in which hAdV was detected between January 2022 and September 2023 in our tertiary care hospital, were selected for AAV2 testing. Clinical features, including age, immune status, and clinical presentation, as well as epidemiological characteristics, including seasonal distribution, and hAdV genotype, were recorded.
RESULTS: HAdV was detected in clinical samples from 203 patients, median age at time of infection was 8 years (range 0-87) and 52% of patients were <10 years old. In total, 8,4% of the patients with hAdV detections tested positive for AAV2 in at least one clinical material, 82% of these patients were younger than 10 years. Detection of AAV2 was most prominent in plasma (11.1%) followed by faeces (8,6%) and respiratory material (7,1%). AAV2 was most frequently detected in cases of hepatitis (in 5/7 cases, 71,4%), but it was also found in patients with gastro-enteritis (7,4%) and respiratory infections (4,3%). The highest AAV2 loads (CT 7) were detected in two adult solid organ transplantation patients with hAdV gastro-enteritis.
CONCLUSIONS: Detection of AAV2 is not restricted to hepatitis cases, but is a common finding in hAdV infections in children and adults.
SOURCES OF FUNDING: N/a
Keywords: adenovirus, hAdV, adeno-associated virus 2, AAV2

PP-055
General virology

An outbreak investigation of Hand Foot and Mouth Disease in North India
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AIM: To perform molecular detection of enteroviruses causing HFMD outbreak and to elucidate changes in circulating types through molecular characterization of VP1 gene.
METHODS: Throat swab, swab from vesicles were collected from 174 clinically suspected patients. Samples were extracted using an automated Nucleic acid extraction platform. Extracted RNA was subjected for the detection of Enterovirus (EV) targeting 5'UTR region. For Enterovirus typing, diverged VP1 gene was amplified using Nested PCR from representative (n=29) samples. Amplified products were gel purified and subjected to Sanger sequencing bi-directionally.
RESULTS: Out of 174 clinically suspected HFMD cases, 96 (55.2%) were confirmed positive by PCR. State wise positivity of the samples was: Punjab (64/103) 62.2%, Chandigarh (25/60) 41.7%, Haryana (6/9) 66.7%, Rajasthan (1/1), Uttar Pradesh (0/1). Of the 96 cases, 7 (7.3%) were below one year of age, 18 (18.8%) were between 1-3 years age, 38(39.6%) were between 3-5 years, 23(23.9 %) between 5-10 years, 8(8.3%) between 10-20 years and 2(2.1%) between 20 to 30 years. Most common clinical features were vesicular rash (96,100%), fever (67, 69.8%), loss of appetite (44,45.8%),sore throat-(40, 41.7%),malaise (29, 30.2%). Rash was seen on palms/hands (76,79.2%), soles / feet (61, 63.5%),mouth (60,62.5%), buttocks (34,35.4%), knee (49,51%), genital area (6, 6.3%) Sequencing and Blast analysis concluded prevalence of CV-A-16 strain in Chandigarh, Punjab. However, CV- A-6 strain was found in local outbreak in a school. Another strain CV-B-4 found in Haryana.
CONCLUSIONS: Emergence of new enterovirus strain emphasizes the need for active surveillance of HFMD cases.
SOURCES OF FUNDING: Regional VRDL, PGIMER, Chandigarh
Keywords: Enterovirus, Hand Foot and Mouth disease, outbreak investigation

PP-056
General virology

Virus like particles (VLP) and ultrastructural cell morphology in human cervical carcinoma cells lines: (SiHa - HPV/16 and HeLa - HPV/18)

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AIM: The present study reports the presence of the virus like particles (VLP) and describe ultrastructural cell morphology in several samples. Moreover, demonstrated morphological alterations inside the SiHa and HeLa cell lines ($3x10^6$ cells) described by electron microscopy in previously PCR positive. Few studies have assessed the transmission electron microscopy in different cells lines.
METHODS: For ultrastructural analysis, the specimens (warts samples and SiHa and HeLa cells) were embedded in epoxy resin, fixed in 1% glutaraldehyde and post-fixed in 1% osmium tetroxide. Later steps followed by washes in cacodylate buffer 0.2 M in sodium sucrose 0.7% and distilled water. The ultra-thin sections, 50nm thick, were stained with uranyl acetate and lead citrate, the semi-thin sections, 0.5µm thick, with methylene blue.
RESULTS: Very electrodense cells were detected by electron microscopy presenting well developed mitochondria and rough endoplasmic reticulum, many vesicles and ribosomes in HeLa and SiHa cell lines. Presence of large cellular modifications similar to antigen-presenting cells. Many activated mitochondria. Vesicle transport well preserved and active core. Very rER indicating high cellular activity. Presence of intranuclear virus like virus like particles (VLP), mitochondria, keratin, many ribosomes and cellular junctions like desmosomes. High cellular activity producing keratin.
CONCLUSIONS: These findings suggested a strong cellular activation in these cervical keratinocytes and point to the potential to induce tumors that probably involves mechanisms of oncoproteins to produce virions and differentiation of keratinocytes in the epithelial layers remains to be elucidated. Furthermore, more studies are needed to evaluate the morphological alterations induced by HPV in those cell lines.
SOURCES OF FUNDING: CAPES/CNPq
Keywords: cell lines, HPV, virus like particles, morphological changes, human cervical carcinoma

PP-057
General virology

Genotyping of high-risk human papillomavirus associated with predictor factors to cervical cancer in unvaccinated sexually active women

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AIM: Circular double-stranded DNA genome, around 8 kb non-enveloped belong Papillomaviridae family. The development of vaccines against HPV use recombinant DNA technology, as some viral particles have the ability to self-assembling into virus-like particles. The aim of this study was to evaluate the prevalence of HPV in sexually active women. The demographic and behavior factors were also investigated as co-factors related to cervical cancer.
METHODS: A cross-sectional study was conducted with randomly selected women 15 to 75 years aged presenting normal cytology. Cervical samples collected with a cytobrush were analyzed by PCR amplification of L1 ORF (450bp). HPV-DNA positive samples were detected by consensus (MY09/MY11), Nested PCR (GP5+/GP6+) and high-types specific primers (HPV16/18/31/45). In order to evaluate the viral DNA quality, swab samples collected were amplified by β-globin PCR primers (PC04/GH20). Restriction fragment length polymorphism (RFLP) assay patterns for mucosal HPVs were used for genotyping. Chi-square test was used to analyze the risk factors associated with HPV infection.
RESULTS: Prevalence of 20% positive samples of cervical HPV-DNA was confirmed. HPV-18 was the most prevalent genotype (8%). About 16% reported being smokers and 3% drug users. Of all the participating women, 27% used alcoholic, 40% reported having had at least one abortion, 15% used oral contraceptives, while 71% did not use any type of condom ($p<0,03$). Most women were currently not married (56%) and married or cohabitating (44%) ($p<0.05$).
CONCLUSIONS: This molecular epidemiological study estimates a high prevalence of HPV unimmunized women and it may be to contribute for the optimization of prevention strategies.
SOURCES OF FUNDING: CAPES/CNPq
Keywords: cervical cancer, human papillomavirus, predictor factors, genotyping, clinical virology

PP-058
(Re-) emerging viral infections and one health

Assessing Four Molecular Assays for Robust MPX Virus DNA Detection
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AIM: Monkeypox virus (MPXV) is a zoonotic disease, part of the Orthopoxvirus genus in the Poxviridae family. It spreads to humans through contact with infected animals or humans, or contaminated material. This retrospective and comparative study aimed to assess the detection of MPXV DNA using the VIASURE Monkeypox virus Real-Time PCR Detection Kit in skin lesion/vesicle swabs and serum samples categorized as positive or negative for MPXV DNA. Additionally, it sought to determine the diagnostic accuracy of the qPCR kit compared to routine techniques.
METHODS: A total of 305 leftover specimens (263 skin lesion/vesicle swabs collected in UTM-RT and 42 serum samples) were used in this analysis. The gold standard used for the routine diagnosis was the WHO MPX molecular assay and, in parallel of the VIASURE Kit, the samples were analysed with the Bioperfectus Monkeypox Virus (MPXV) Fast Real Time PCR Kit and with the CDC MPX-specific molecular assay.
RESULTS: The results of these three molecular assays were compared with those obtained in the initial diagnosis/characterisation and compared with each other. All 3 molecular assays reported the same false negative for a sample when compared to the WHO reference assay and all reported a sensitivity of 99%. The specificity of all of them was 100%.
CONCLUSIONS: The study showcased robust overall agreement between the VIASURE Monkeypox Virus Real-Time PCR Detection Kit and the WHO MPX molecular assay, the Bioperfectus Monkeypox Virus (MPXV) Fast Real-Time PCR Kit, and the CDC MPX-specific molecular assay results.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: Monkeypox virus, molecular diagnosis

PP-059
General virology

Bloody Herpesviruses – How the permanence after primary infection complicates molecular diagnosis
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AIM: Identifying clinically relevant herpesvirus infections can be challenging due to their persistent nature post-primary infection. Both primary and recurrent infections can lead to CNS involvement. Virus isolation in cell culture, once the gold standard, is now replaced by PCR-based detection of specific nucleic acids. Blood derivatives have been explored as an alternative to cerebrospinal fluid samples. However, a limitation arises: Whole blood may contain the virus in lymphocytes of healthy individuals, complicating the interpretation of results. The study aimed to assess the feasibility of qualitative qPCR of herpes viruses in blood versus plasma samples.
METHODS: A comparative study was conducted on paired blood and plasma samples from healthy adult donors using VIASURE Viral Meningitis Panel Real Time PCR Detection Kit. Nucleic acid extraction, amplification and analysis was conducted in parallel.
RESULTS: The EBV DNA was detected in 72.7 % of blood samples, HHV-6 DNA in 9.09% and HHV-7 in 54.5 %. Contrary, using the paired plasma samples, no positive result was obtained for any of the analyzed viruses. These results are concurrent with previous studies over healthy blood donors.
CONCLUSIONS: Unlike blood samples, which necessitate establishing a cut-off point or quantifying viral load, plasma samples enable direct interpretation of positive/negative results. This simplifies the analytical validation process, given the limited availability of negative sample matrices. Additionally, plasma samples facilitate multiple determinations through multiplexing methods and allow monitoring of significant changes in viral loads due to the method's semi-quantitative nature.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: herpes virus, whole blood, molecular diagnosis

PP-060
Hepatitis, HIV and other blood borne viruses

Revolutionizing Hepatitis B Detection: Cutting-edge Molecular Assay for Precise Virus Load Measurement in EDTA-plasma

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AIM: Hepatitis B virus (HBV) stands as the predominant cause of viral hepatitis worldwide, casting a shadow over human health. While diagnosis of viral hepatitis typically relies on serological markers in blood, this approach is riddled with limitations. Hence, the adoption of qPCR for disease diagnosis emerges as a beacon of hope, promising a substantial leap forward in diagnostic precision and efficacy. Given the evolving landscape of infectious diseases, there is an urgent need for novel molecular diagnostic tools capable of swift and precise quantification.
METHODS: EDTA-plasma samples characterised as positive or negative and quantified in IU/ml were analysed with VIASURE HBV q Real Time PCR Detection Kit. It was included clinical samples from HBV resolved infection patients (HBsAg and HBeAg negative; anti-HBs/-HBe and anti-HBc positive) and samples from chronic patients (HBeAg negative; HBsAg, anti-HBc and anti-HBe positive), including 10 patients with inactive chronic HBV infection, were analysed.
RESULTS: A total of 310 clinical samples were tested, of which 152 were positive for HBV DNA and 158 negatives. The comparative analysis determined a sensitivity and positive predictive value of >96%, and a specificity and negative predictive value of >99%. For quantification, a correlation value of R2=0.913 was obtained when comparing with the reference assay.
CONCLUSIONS: This clinical evaluation establishes the VIASURE HBV q Real-Time PCR Detection Kit as an exceptional tool for precise clinical diagnosis of the virus, boasting remarkable sensitivity and specificity. Thus, real-time PCR for HBV diagnosis emerges as a definitive solution, surpassing the limitations of serological diagnostic techniques.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: Hepatitis B, viral load, molecular diagnosis

PP-061
Hepatitis, HIV and other blood borne viruses

Expanding Horizons: Dual-Purpose qPCR for Qualitative and Quantitative Detection of Hepatitis B Virus
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AIM: Hepatitis B virus (HBV) infection profoundly impacts global public health, driving rapid advancements in diagnostic and monitoring methods, particularly molecular assays. Qualitative hepatitis B tests have long been pivotal in diagnosis, while viral load serves as a crucial marker for patient monitoring. Amidst numerous kits catering to either qualitative detection or quantitative monitoring, the feasibility of a single assay for both purposes is questioned. This study addresses this query, assessing the clinical efficacy of the VIASURE HBV q Real-Time PCR Detection Kit for dual-purpose use.
METHODS: For quantitative use, 310 EDTA-plasma samples characterised as positive or negative and quantified in IU/ml were analysed and results were compared with those obtained with the molecular reference assays. For qualitative use, 500 EDTA-plasma specimens from blood donors were analysed. All specimens were reported as negative by serological screening for viral-antigen (Alinity) and molecular (Procleix).
RESULTS: The comparative analysis determined a sensitivity >96% and specificity >99%. For quantification, a correlation value of R2=0.913 and R2=0.892 was obtained comparing VIASURE results with cobas® HBV and AltoStar® HBV, respectively. All blood donor clinical specimens were reported by VIASURE as HBV DNA negative, according to initial diagnosis.
CONCLUSIONS: VIASURE assay could be used for both applications, for the quantitation of HBV DNA in human EDTA-plasma samples from HBV-infected individuals and for the qualitative detection of HBV DNA in the screening of individual human blood donors, thus eliminating the need to have different tools for the detection and the quantification of HBV through qPCR in the same laboratory.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: Hepatitis B virus, qualitative and quantitative assay, molecular diagnosis

PP-062
Hepatitis, HIV and other blood borne viruses

Understanding why modelling studies by the Center for Disease Analysis Foundation fail to offer reliable estimates of Hepatitis C prevalence in Switzerland and Germany

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AIM: The Center for Disease Analysis Foundation (CDAF) conducts HCV modelling studies globally. Their Markov modelling uses general population data, supplemented by country-specific subpopulations and mortality information(1,2). In Switzerland and Germany, the general population has low prevalence, with injection drug use (IDU) accounting for most cases(3,4). We conducted this study to assess the accuracy of CDAF's modelling estimates for Switzerland and Germany.
METHODS: Employing the latest models for Switzerland(1) and Germany(2), we evaluated data for estimating prevalence among the general population and subpopulations (including people who inject drugs (PWID), specific foreign-born groups, men who have sex with men living with HIV (MSMHIV), and nosocomially-exposed individuals), along with mortality. We also examined assumptions regarding HCV self-clearance where HCV serology data were used.
RESULTS: For Switzerland, the general population estimate relied on a 2015 assessment, drawing from partly outdated data skewed towards high-risk subpopulations(5). Risks related to IDU and nosocomial transmissions were based on data from 1993 to 2020(1.2). The HCV micro-elimination efforts among MSMHIV in Switzerland, as well as issues of alcohol overuse and excess mortality among certain subpopulations(5), were not considered. Assumptions regarding self-clearance relied on US population data, despite potentially higher rates in Germany and Switzerland(5). An alternative estimate for Switzerland, incorporating additional available data and avoiding the aforementioned shortcomings, indicated a 75% reduction in prevalence(5).
CONCLUSIONS: The Markov modelling method tends to overestimate HCV prevalence in countries with HCV primarily concentrated among people who inject drugs (PWID) with well-established harm reduction measures, and minimal nosocomial transmissions.
SOURCES OF FUNDING: Not applicable
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Keywords: hepatitis C, modelling, prevalence, estimates, Germany, Switzerland

PP-063
Hepatitis, HIV and other blood borne viruses

HIV Detection and Viral Load Measurement in Cerebral Spinal Fluid (CSF) Using the Abbott Alinity M

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AIM: Patients displaying symptoms of central nervous system (CNS) HIV escape currently have limited options for HIV molecular testing in CSF outside of specialist reference services; however, in the Wales Specialist Virology Centre in Cardiff, we describe how we have implemented viral load testing on CSF samples using the Abbott Alinity M.
METHODS: The Abbott Alinity M is a random-access molecular system that removes the need for viral load batch testing. This improves turnaround times and allows for single samples to be tested, lending itself to test requests on unusual samples such as CSF.
Verifying CSF as a suitable sample type is challenging due to the lack of HIV positive CSF, the invasive nature of specimen collection, and the rare instances in which HIV is found in CSF. However, by being able to detect HIV within the CSF, this not only confirms HIV CNS escape but can also facilitate genomic testing for antiviral susceptibility.
RESULTS: The test has been used in a small number of patients, but the results are promising, with viral escape confirmed in at least one case. The CSF was referred for successful antiviral susceptibility testing using whole genome sequencing (WGS) at the Pathogen Genomics Unit, Cardiff.
CONCLUSIONS: In summary, using the Alinity M, we have been able to confirm viral escape by detecting a viral load in CSF and tested that same sample for antiviral susceptibility using WGS. This process therefore offers a solution to testing routinely for HIV CNS escape using a widely used commercial system.
SOURCES OF FUNDING: None to declare.
Keywords: HIV, Molecular testing, CSF, Viral Load, Antiviral susceptibility, CNS escape.

PP-064

Hepatitis, HIV and other blood borne viruses

Microbiological infections in people living with HIV and relationship with CD4 cell count in the era of antiretroviral therapy

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AIM: To evaluate the relationship between bacterial infections in people living with HIV (PLHIV) and CD4 cell count.
METHODS: A retrospective evaluation of bloodstream infections (BSI), respiratory infections (RI), urinary tract infections (UTI) and tuberculosis infection (TB) in 2067 PLHIV from 2018 to 2023 was conducted. The absolute CD4 cell count (cells/mcL) was performed by cytofluorometric analysis of whole blood samples.
RESULTS: Sixty-three (3%) BSI, 46 (2,2%) RI, and 67 (3,2%) UTI were observed. Interestingly, among these, 67% with BSI, 43% with RI, and 52% with UTI were virologically suppressed at the time of infection. Additionally, 11 individuals tested positive for TB and 2 of them had undetectable HIV viremia. Median CD4 cell count in PLHIV with UTI, RI, BSI and TB was 428 cells/mcL, 226 cells/mcL, 149 cells/mcL, 109 cells/mcL, respectively. PLHIV with UTI showed higher CD4 cell count than BSI (P=0,0134) and PLHIV with RI had higher CD4 cell count than TB (P=0,0121). As expected, PLHIV with TB had the lowest median CD4 cell count.
CONCLUSIONS: Despite the success of ART, hospitalization and severe infections remain a concern among PLHIV. The CD4 cell count is a critical parameter in HIV management, serving as a reliable indicator of a patient's immune status and guiding clinical treatment decisions. Moreover, assessing the immunological status is crucial to examine the specific microbiological features of the infection. These data can provide information on the burden of bacterial infections in this population and help prevention, diagnosis and management strategies.
SOURCES OF FUNDING: Not required.
Keywords: HIV, CD4 cell count, bacterial infections

PP-065
Hepatitis, HIV and other blood borne viruses

Human leucocyte G expression after BKV infection
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AIM: our aim is investigating the role of both HLA-G expression and soluble HLA-G in BKV immune evasion.
METHODS: the expression of cell surface HLA-G and soluble plasma HLA-G (sHLA-G) were evaluated in PBMCs from healthy individuals with known BKV status and KTRs with BKV viremia to a BKV peptide pool.The participant urine samples were tested for BKV DNA using quantitative real time PCR (qPCR) and blood samples were collected from health subjects who were urine positive and KTRs with positive plasma, then PBMC isolated and stimulated with BKV peptides for three days before phenotyping the
RESULTS: Our study demonstrates that higher proportions of monocytes express HLA-G than other lymphocyte subsets.
Furthermore, patients with active BKV infection showed dramatic increases in HLAG expression in comparison to recipients who did not encounter infection post transplantation. And finally, no difference was observed in plasma sHLA-G levels among the tested groups.
CONCLUSIONS: Our data revealed that no significant difference was observed in plasma sHLA-G levels in healthy subjects with BKV Ab, either with BKV DNA or without. However, sHLA-G levels were found to be higher in renal transplant patients in comparison to the healthy subjects.
SOURCES OF FUNDING: Prince Sattam University.
Keywords: BKV, HLA-G,sHLA-G, Immunocompromised, Renal transplants

PP-066
Hepatitis, HIV and other blood borne viruses

Evaluation of the diagnostic performance of two fully automated hepatitis E virus antibody test: the Ghent experience

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AIM: Accurate measurement of antibodies against the hepatitis E virus is of high importance in the diagnostic workup of suspected hepatitis E infections. Recent methodological developments allow hepatitis E antibodies measurements in a fully automated way.
METHODS: Using biobanked samples, we retrospectively evaluated the diagnostic performance of the Roche® Elecsys, Liaison® and BioMérieux Vidas® anti-HEV tests. Results were compared against the Mikrogen® anti-HEV assays.
RESULTS: In total 22 HEV positive samples and 11 HEV negative samples (Mikrogen®) were included. For anti-HEV IgM, Liasion®, Roche® Elecsys and Vidas® showed identical results. There was 88 % concordance with the results obtained by Mikrogen®. Four samples tested positive (though close to the cut-off) on the Mikrogen® assay, though negative on the Liaison®, Roche® and Vidas®. As such, the three evaluated tests showed a sensitivity of 79% and a specificity of 100% to detect anti-HEV IgM. For anti-HEV IgG the concordances of Liaison® XL, Roche® Elecsys and Vidas® with Mikrogen® were 100%, 91% and 97%, respectively. The sensitivity was 100% for Liasion® and 94% for Roche® Elecsys and Vidas®
CONCLUSIONS: The evaluated fully automated hepatitis E virus antibody tests show acceptable sensitivity and specificity. As such, they show promising potential.
SOURCES OF FUNDING: None to report
Keywords: Hepatitis E, antibodies, automation

PP-067
Hepatitis, HIV and other blood borne viruses

Analysis of the nationwide HIV screening test data performed at Public Health Centers in Korea (2011-2022)
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AIM: As vital HIV screening institutions in Korea, public health centers provide free HIV testing to local residents and socially vulnerable populations. This study aims to evaluate the current status and implications of HIV testing in these public institutions by analyzing annual HIV testing data from public health centers.
METHODS: We used the nationwide HIV test data from 260 health centers between 2011 and 2022. The results were analyzed by gender, age, region, nationality, test purpose, and seroprevalence. The statistical significance of each variable was assessed through regression analysis.
RESULTS: The average annual number of HIV tests conducted at health centers from 2011 to 2019 was 443,609. Amidst the COVID-19 pandemic, the numbers declined significantly, with 178,653, 104,621, and 141,992 tests conducted from 2020 to 2022, respectively. When examined yearly, age trend analysis showed a decline in teens and twenties, and an increase in thirties ($p<0.001$). Regarding the purpose of testing, there was a significant increase in the proportion of prenatal tests ($p<0.001$), whereas the proportions of tests conducted for physician referrals and voluntary purposes decreased ($p<0.05$). Seroprevalence analysis revealed that HIV prevalence was three times higher among foreigners (0.23-0.55%) compared to nationals (0.08-0.19%). Men exhibited higher seroprevalence than women, and foreigners had a higher seroprevalence than nationals ($p<0.001$).
CONCLUSIONS: Free HIV testing at public health centers, voluntary testing, and screening for foreigners and STI checkups are all crucial aspects of HIV prevention efforts. Additionally, it is essential to analyze the trend of HIV testing at public health centers following the pandemic.
SOURCES OF FUNDING: Unrelated.
Keywords: HIV, Screening test, Public health centers

PP-068
Hepatitis, HIV and other blood borne viruses

The survey about implementing the national 2019 HIV diagnosis algorithm
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AIM: In the survey, we aimed to reveal the problems faced by Medical Microbiologists working in different healthcare centers implementing the national 2019 HIV diagnosis algorithm which was updated according to CDC recommendations and new algorithm.
METHODS: A self-administered questionnaire consisting of 7 descriptive data, 7 information and counseling, 15 HIV screening tests, 15 confirmatory tests, and 7 questions about their recommendations.
RESULTS: 35 centers participated in our survey. When we evaluated the survey results; We found that in 74.3% of the centers, no pretest counseling was performed. It was observed that the information and counseling after the confirmation test were carried out in approximately 70% of the centers. It was determined that the 2019 HIV diagnosis algorithm was applied in almost all centers. While confirmation tests were carried out in 3 of them, the other 32 centers sent samples to the National Reference Laboratory. The average time to receive confirmation test results was 24 hours to 7 days in 17 centers and 7-15 days in 18 centers. The most important suggestions regarding the diagnostic algorithm were: Delay in confirmation results in cases where urgent diagnosis is required was defined as the most problematic situation in the HIV diagnosis and 45.7% said that test centers should be accredited if they meet certain standards based on screening and confirmation tests.
CONCLUSIONS: Although the new algorithm was applied, the diagnosis time was prolonged because there was no decentralization according to the survey results.
SOURCES OF FUNDING: No need for funding
Keywords: HIV diagnosis, algorithm

PP-069
Hepatitis, HIV and other blood borne viruses

Three-Year Analysis Of HIV Diagnostic Data

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AIM: With the National Human Immunodeficiency Virus (HIV)/ AIDS Diagnosis and Treatment Guide updated in 2019 in Turkey, the three-stage CDC HIV test algorithm has begun to be used in the diagnosis of HIV infection. This study is aimed to evaluate the prevalence of people living with HIV diagnosed in two hospitals between 2021-2023 in Istanbul
METHODS: A total of 3 years numbers of peope living with HIV covering the years 2021-2022-2023 were classified according to age, nationality, and the units they were sent from.
RESULTS: The three-year results obtained according to the the updated test 2019 algorithm used in the diagnosis of HIV/AIDS were included in the study. The results detected recurrent reactive results by 4. generation HIV screening test confirmed by HIV1-2 differentiation assay and HIV-1 RNA PCR. In the second hospital, we had the chance to detect 10 cases in the acute HIV (% 0.004). Prevalence of HIV was found to be 0.12% and 0.21% in the first and second hospitals respectively. No statistical difference were seen in the total seroprevalence rate between the hospitals
CONCLUSIONS: This retrospective analysis gave a prevalence rate lower than the general estimated prevalence of HIV all over the world which is given as 0.7% by WHO. We should also mainly follow-up prevalences in key populations and increase the number of anonymous testing centers.
SOURCES OF FUNDING: A retrospective study from routine laboratory
Keywords: HIV, Diagnostic data

PP-070
Hepatitis, HIV and other blood borne viruses

Reliable and sensitive quantification of plasma HDV RNA: A European multicenter study

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AIM: The aim of this study was to improve the comparability of HBV DNA quantification using the CE/IVD-labeled RoboGene HDV RNA Quantification Kit 3.0 (Roboscreen GmbH) with different manual or automated nucleic acid extraction protocols/platforms and amplification/detection devices.
METHODS: For harmonization of HDV RNA concentrations, correction factors (CF's) were determined using the 1st WHO International Standard (IS) for HDV RNA. The limit of detection (LOD) was determined by analyzing dilution series of the IS for HDV RNA. For accuracy testing, reference material was used. Furthermore, clinical samples were analyzed and results compared.
RESULTS: Depending on the combination of NA extraction and amplification procedure, CF's ranged from 14 to 10000 and the LOD's from <10 to approximately 1000 IU/ml. When accuracy was tested, external quality control samples containing low HDV RNA concentrations were not detected by that protocol with the highest LOD. When dilutions of clinical samples were tested, the lower the HDV RNA concentration, the less quantifiable data sets were obtained.
CONCLUSIONS: To ensure reliability in quantification of HDV RNA, any modification of the extraction and amplification/detection protocol validated by the manufacturer requires revalidation. With the IS for HDV RNA, CF's could easily be calculated in this study leading to harmonization of quantitative results. This is essential for both accurate monitoring of response to existing anti-HDV treatment and comparability of study results investigating novel anti-HDV drugs.
SOURCES OF FUNDING: This work was supported by RoboScreen GmbH through providing parts of reagents required for this study.
Keywords: Hepatitis D virus, HDV RNA, Quantification, Harmonization, WHO International Standard

PP-071
Hepatitis, HIV and other blood borne viruses

A peculiar HBsAg isoforms composition characterizes chronic HDV coinfection respect to HBV mono-infection, with higher middle-/large-HBs levels paralleling HDV activity

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AIM: To investigate HBs isoforms (Large- [L-HBs], Middle- [M-HBs] and Small-HBs [S-HBs]) levels in the setting of chronic HBV mono-infection (CHB) and HDV coinfection (CHD).
METHODS: 262 plasma samples from HBeAg-negative patients: 143 CHD and 119 CHB. Total HBsAg is measured by COBAS HBsAg II assays (Roche), HBs forms by ad hoc designed ELISAs (Beacle) and HDV-RNA quantification (Robogene).
RESULTS: CHD and CHB patients have comparable age and rate of NUC treatment. CHD have lower HBV-DNA (median [IQR]: 1.3 [0.0-2.3], 1.6 [1.2-3.4] logIU/ml; P=0.002), higher ALT (median [IQR]: 79 [50-113], 36 [22-63]U/L; P<0.001) and total HBsAg levels (median [IQR]: 5206 [827-8555], 1776 [354-6936]IU/ml; P=0.008). Median(IQR) HDV-RNA is 5.2 (3.4-6.0)logIU/ml. CHD correlate with higher M/L-HBs respect to CHB (median [IQR]: 1127 [145-2301] vs 142 [25-707] and 2.0 [0.2-6.3] vs 0.06 [0.2-0.5]ng/ml); P<0.001), despite similar S-HBs levels (median [IQR]: 3221 [587-6497], 1039 [239-5438]ng/ml). Datum confirmed by multivariable analysis (OR [95%CI]: 4.7 [1.7-12-4], 6.2 [2.2-17.9]; P<0.002). Among CHD, HBs forms positively correlate with HDV-RNA levels (Rho=0.48, 0.49, 0.43 for S-/M-/L-HBs; P<0.001); in CHB, L-HBs correlates weakly with HBV-DNA levels (Rho=0.29, 0.02). Patients with HDV-RNA>3log U/ml show significantly higher levels of all HBs forms than lowly-replicating HDV (median [IQR]ng/ml: 4431 [1251-6950], 274 [25-2640] S-HBs; 1404 [191-2484], 127 [4-1242] M-HBs; 3.3 [0.2-7.8], 0.3 [0.04-1.1] L-HBs; P<0.001). M-HBs>200ng/ml is the best cut-off predicting altered ALT (70% M-HBs>200ng/ml vs 30% M-HBs<200ng/ml has ALT>40U/L; PPV=70%, NPV=76.9%; P=0.04).
CONCLUSIONS: HBsAg form composition differs in CHD vs. CHB, with CHD showing higher M-HBs and L-HBs, along with HDV replication, potentially reflecting a variation in the proportion of circulating viral and subviral particles for CHD and CHB. Overall, HBs forms can aid in prioritizing treatment against liver disease progression.
SOURCES OF FUNDING: No funding dedicated
Keywords: Chronic Hepatitis Delta, Chronic Hepatitis B, HBsAg isoforms, Non-invasive biomarkers

PP-072
Hepatitis, HIV and other blood borne viruses

IgG avidity analysis reveals patterns of Hepatitis E virus reinfection in viremic patients

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AIM: Several studies have suggested that Hepatitis E virus (HEV) reinfection may occur. Our objective was to assess the frequency reinfection in viremic patients using IgG avidity. Low avidity IgG are produced in primary infection and are replaced by IgG with higher avidity, also produced during reinfection.
METHODS: HEV-RNA positive serum samples from routine diagnosis in 2010-2012 and in 2020-2023, and -80°C-frozen stored at the Biological Resource Center of Paris-Saclay were selected for the study. Age, Sex, immunosuppressed status, and nature of HEV infection (acute or chronic) were available for most patients. The Wantai HEV IgG kit was used for IgG status, and with minor modifications, for IgG avidity: low avidity was <60% and high avidity was >70%.
RESULTS: Among 592 patients, 443 had available sera for serological testing. Patients with chronic infection, or unknown status were excluded. Among the remaining 347 patients, HEV IgG was negative in 32.6% (n=113); avidity was <60% in 52.2% (n=181), 60-70% in 2.0% (n=7), and >70% in 13.2% (n=46). There was no difference in the distribution of IgG classes according to collection period, sex or age (mean age ranging from 53 to 56). Genotype 1-infected patients had a higher frequency of high avidity compared to zoonotic HEV-infected patients (54.5% vs. 12.2%, p<0.0001). There was no difference among zoonotic HEV-infected patients.
CONCLUSIONS: Primary HEV infection could be usually asymptomatic at younger ages and mostly symptomatic around the age of 50 whether as a primo infection or reinfection. Further studies are needed to determine the characteristics of these reinfections.
SOURCES OF FUNDING: None
Keywords: Hepatitis E Virus (HEV); reinfection; avidity of IgG anti-HEV antibody, IgG avidity index

PP-073
Hepatitis, HIV and other blood borne viruses

Chemiluminescent immunoassay for diagnosis of acute HEV infections
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AIM: Hepatitis E is a liver disease caused by the hepatitis E virus, which is a non-enveloped, positive sense single-stranded RNA virus. HEV is transmitted through the faecal-oral route due to contamination of drinking water. In developed countries a foodborne transmission occurs by ingestion of undercooked meat. It is usually a self-limiting infection and resolves within 4 to 6 weeks. Occasionally, a fulminant form of hepatitis may develop.
METHODS: The recomCLIA HEV is a chemiluminescent immunoassay for the detection of antibodies against HEV in human serum and plasma using the fully automated random-access analyser KleeYa®. The sensitivity is defined as relative positive agreement. This implies that samples are defined by clinical and/or laboratory data and also by positivity by other assays. Double positives or borderlines were preselected as true positives, considering the time course of IgM and IgG appearance in sporadic acute HEV infections where generally IgM titers develop first and are accompanied by IgG antibodies within 1 or 2 weeks. Specificity is defined as test negativity in the clear absence of a HEV infection. This includes specimens with potentially interfering antibodies.
RESULTS: The diagnostic sensitivity of recomCLIA HEV IgG and IgM should be between 95 and 100%. The specificity should lie between 98 and 100%. The WHO-standard is used to determine true IgG-antibodies quantitatively.
CONCLUSIONS: The test system recomCLIA HEV performs with top most sensitivity and specificity in acute viral hepatitis routine samples. Therefore, it is ideally suited for the detection of acute HEV infections as well as for HEV epidemiology studies.
SOURCES OF FUNDING: ---
Keywords: Hepatitis E virus, KleeYa, recomCLIA, immunoassay, HEV

PP-074
Hepatitis, HIV and other blood borne viruses

Viral load measurements with results below detection limit in HBV samples with high or low Hepatitis B surface antigen titres

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AIM: Although other biomarkers show a more consistent correlation to the amount of HBV cccDNA (covalently closed circular DNA) than Hepatitis B surface antigen (HBsAg), high levels of HBsAg are usually associated with high levels of cccDNA. In this study we investigate whether the level of HBsAg has an influence on HBV viral load measurement with the NeuMoDx HBV Quant Assay (QIAGEN).
METHODS: 120 fresh routine plasma samples tested HBV DNA below LLOD with the test of record (Alinity m HBV, Abbott) were retested without any freeze thaw cycles within 24 hours with the HBV Quant Assay on the NeuMoDx system (QIAGEN). HBsAg values measured either with the qualitative Access HBsAg assay (Beckman Coulter) (n=80) and/or with the quantitative LIAISON® XL MUREX HBsAg Quant assay (DiaSorin) (n=48) were taken into account. Values greater than 3000 S/CO or greater than 150 IU/mL, respectively, were considered high positive, values below this were considered low positive.
RESULTS: Neither HBsAg values above 3000 S/CO (n=46) in the qualitative assay nor values above 150 IU/mL (n=23) in the quantitative test had an influence on the measured HBV viral load. All of the samples that were not detectable with the TOR or <10 IU/mL pre-tested were “DNA not detected” when tested with the NeuMoDx HBV Quant Assay.
CONCLUSIONS: The level of HBsAg had no influence on HBV viral load measurement with the NeuMoDx HBV Quant Assay in high positive HBsAg samples as was reported for other assays.
SOURCES OF FUNDING: QIAGEN GmbH
Keywords: HBV, HBsAg, viral load

PP-075
Advancements in diagnostics & clinical cases

CMV specific T-cell activity determines the risk of clinically significant CMV reactivation following letermovir prophylaxis after allo-HSCT

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AIM: Despite the use of letermovir, a CMV-specific antiviral that reduces clinically significant CMV infections (Cs-CMVi) post-transplantation, late CMV reactivation occurs after stopping letermovir. This study aimed to identify patients at risk for late CMV reactivation on day 100 post-transplantation by measuring CMV-specific T-cell responses.
METHODS: Forty-nine allo-HSCT recipients (10 with lymphoid and 39 with myeloid disease) were prospectively monitored for CMV-specific T-cell immunity using the Elispot-CMV assay (T-SPOT®.CMV, Oxford Immunotec) over 200 days. Analyses were conducted at defined time points post-transplantation (day 100, day 200, at CMV reactivation, 1 and 3 months after reactivation).
RESULTS: While the number of CMV reactivations during letermovir prophylaxis was comparable to the number of reactivations after discontinuation of letermovir (n=18 vs. n=21), significantly more Cs-CMVi were observed after stopping letermovir (n=2 (11%) vs. n=8 (38%)) confirming the protective activity of letermovir. Elispot analysis showed that patients with CMV reactivation during prophylaxis had a significantly higher spot count at day 100 than those with no CMV reactivation (IE 1 spots: 46 and 3, p=0.0066; pp65 spots: 618 and 34, p=0.0004, respectively), also associated with a lower risk of CMV reactivation post-letermovir (OR = 0.062, 95%CI 0.08-0.470, p=0.008).
CONCLUSIONS: CMV reactivations occur despite prophylaxis post-allo-HSCT. However, the risk of Cs-CMVi is significantly reduced. Patients with abortive CMV replication (blips) during letermovir prophylaxis are likely to develop protective immunity, resulting in a lower rate of Cs-CMVi after discontinuing letermovir.
SOURCES OF FUNDING: The project was supported by Oxford Immunotec (Revvity).
Keywords: CMV, HSCT, letermovir, T-cell immunity, Elispot, reactivation

PP-076
Immune response and vaccines

Comparison of immune responses to SARS-CoV-2 spike after Omicron infection and Omicron BA.4/5 vaccination in kidney transplant recipients

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AIM: The emergence of new SARS-CoV-2 variants poses a challenge for maintaining protective immunity, especially in kidney transplant recipients (KTRs). Seasonal boosters with adapted vaccines are recommended. Despite vaccination, the incidence of SARS-CoV-2 infections increased in KTRs during the Omicron wave. This study compared variant-specific immune responses after bivalent Omicron BA.4/5 vaccination or Omicron infection in KTRs to evaluate current vaccine recommendations.
METHODS: The study enrolled SARS-CoV-2 naïve KTRs after four vaccine doses. Before and after a fifth antigen exposure either with the bivalent BA.4/5 vaccine or as a natural Omicron infection, we quantified spike-specific T cells using Elispot and assessed neutralization activity against the Wuhan strain, BA.5, and XBB.1.5 variants.
RESULTS: Although immune responses were similar before the fifth antigen contact, three and six months post-Omicron infection, spike-specific T cell response and neutralization activity against SARS-CoV-2 were higher compared to BA.4/5 vaccination, reaching levels similar to healthy controls. Vaccination modestly boosted neutralization against the Wuhan strain and BA.5 variant but not XBB.1.5. Tixagevimab/Cilgavimab treatment led to robust neutralization of the Wuhan strain in KTRs but was ineffective against XBB.1.5.
CONCLUSIONS: The BA.4/5 vaccine enhances neutralization against the Omicron BA.5 variant but not XBB.1.5 in KTRs. Conversely, Omicron infection more robustly boosted spike-specific T cells and humoral responses, offering better protection against XBB.1.5. Thus, infection-induced immunity may better protect KTRs against future SARS-CoV-2 variants than vaccination, having implications for recommendations of annually adapted vaccines.
SOURCES OF FUNDING: Ministry of Labour, Health and Social Affairs of North-Rhine Westphalia (CPS-1-1A), Federal Ministry of Education and Research (01KX2021).
Keywords: SARS-CoV-2, Omicron BA.5, Omicron XBB.1.5, bivalent vaccination, kidney transplant recipients (KTRs)

PP-077
Immune response and vaccines

Neutralization of emerging SARS-CoV-2 XBB and JN.1 lineages is improved by repeated Omicron exposures
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AIM: Novel SARS-CoV-2 variants, including the recent Omicron XBB and JN.1 lineages, continue to cause global infection waves. In this study, we investigate the immune escape properties of these new variants in large serum panels from well-defined SARS-CoV-2-infected and vaccinated cohorts, representing the range of different immune backgrounds in Austria.
METHODS: The study was carried out with vaccinated individuals who received an updated monovalent XBB.1.5 or a BNT162b2 bivalent vaccine booster and/or experienced an Omicron breakthrough infection. We assessed the neutralizing antibody response against SARS-CoV-2 wild-type and Omicron BA.1, BA.2, BA.5, XBB.1.5, and JN.1 variants, using well-established live-virus neutralization assays.
RESULTS: Our study demonstrates that individuals who received an adapted vaccine booster or had an Omicron breakthrough infection had high antibody levels against previous Omicron variants, but JN.1 effectively evaded antibodies after a single Omicron exposure. However, a second Omicron exposure, either by a second Omicron infection, or by Omicron-adapted boosters markedly enhanced neutralization against JN.1, indicating that repeated Omicron exposures are necessary to broaden immunity against newly emerged Omicron variants.
CONCLUSIONS: Our data indicate that novel variants, such as JN.1, strongly escape antibody responses after a single exposure to a previous Omicron variant, although this is mitigated by repeated Omicron exposures. These findings support recommendations for the use of updated booster vaccines in individuals after recovery from Omicron infections and highlight the importance of evaluating novel variants in the context of diverse immune backgrounds.
SOURCES OF FUNDING: Medical-Scientific fund of the mayor of the federal capital Vienna (#23096)
Keywords: SARS-CoV-2, Omicron, neutralization, antibody, variant, vaccination

PP-078
Immune response and vaccines

Assessment of Antibody Responses in Patients With MERS-CoV Infection and SARS-CoV-2 Vaccination
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AIM: Data describing how preexisting MERS-CoV immunity may shape the response to SARS-CoV-2 following infection or vaccination are lacking. We aimed to characterize the antibody responses (binding, neutralizing, ADCC, and ADE) in patients exposed to MERS and SARS-CoV-2 through infection and vaccination, respectively.
METHODS: This cohort study involved samples collected from patients with MERS-CoV infection and COVID-19 vaccination
RESULTS: Out of 16 MERS patients, nine demonstrated ADE against SARS-CoV-2. The two patients with acute infection also reported ADE in their sera, regardless of the sampling time points. However, out of the seven patients exposed to both SARS-CoV-2 and MERS-CoV, only one patient- acutely in-ected with MERS-CoV and previously vaccinated against SARS-CoV-2- showed ADE activity for SARS-CoV-2. However, none of the samples had an ADE activity against MERS-CoV. Subsequent exposure to SARS-CoV-2 S anti-gen resulted in diminished ADE activity while inducing a broadly reactive response in some patients. ADCC activity was measured to assess anti-S and anti-N antibodies with Fc effector function activity. Overall, no significant difference was shown against both N and S proteins after vaccination.
CONCLUSIONS: This cohort study found a significant boost in cross-reactive NAbs in some patients exposed to MERS-CoV and SARS-CoV-2 antigens. These findings suggest that isolation of broadly reactive antibodies from these patients may help guide the development of a pancoronavirus vaccine by targeting cross-reactive epitopes between distinct strains of hu-man coronaviruses.
SOURCES OF FUNDING: Qatar University
Keywords: MERS, CoV, Vaccine, BnAb

PP-079
Immune response and vaccines

Impact of obesity on the immune response to Measles-Mumps-Rubella vaccination in children

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AIM: Understanding the impact of obesity on adaptive immunity post vaccination will assist in provision of public health strategies catering to an increasingly overweight global population. The aim of this study was to determine the vaccine response rate in obese children after 2 doses of measles-mumps-rubella (MMR) vaccination.
METHODS: Vaccine response was measured in a cohort of 36 obese (BMI >25 kg/m2) and 25 lean (BMI <25 kg/m2) children (Mean age 12.9±0.4 years). Virus specific IgG to measles, mumps and rubella was measured using commercial manual and automated ELISA assays. Antibody avidity was also investigated.
RESULTS: The highest number of vaccine responders were of normal weight with 77.8%, 77.8%, and 88.9% showing detectable IgG responses to measles, mumps and rubella respectively, compared to 52.4%, 57.1% and 66.7% for the obese cohort. The lean cohort had slightly higher mean titer levels than the obese counterparts for all viruses. High-avidity antibodies developed against rubella and measles virus for most vaccine responders (75.6% and 96.7%, respectively). In contrast, responders to the mumps had low-avidity antibodies, with only 5.3% developing high-avidity antibodies. The response rate for all viruses was higher in females (range 75-85.7%) compared to males (57.6-66.7%). No significant correlation was observed between participant BMI and IgG levels.
CONCLUSIONS: Our findings suggest that obesity may be associated with a higher risk of vaccine failure and could inform future public health vaccination strategies.
SOURCES OF FUNDING: National Virus Reference Laboratory, UCD and Kathleen Lonsdale Institute for Human Health Research, Maynooth University
Keywords: Children, Obesity, Vaccine response

PP-080
Immune response and vaccines

Assessing a multivalent vaccine containing VP8* proteins from different rotavirus genotypes in mice

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AIM: Rotavirus remains a significant public health threat, especially in low-income countries, where it is the leading cause of severe acute childhood gastroenteritis, contributing to over 128,500 deaths annually. Although the introduction of Rotarix and RotaTeq vaccines in 2006 marked a milestone in reducing mortality rates, approximately 83,158 preventable deaths persist, underscoring ongoing challenges in vaccine accessibility and effectiveness. To address these issues, we have developed and assayed in the mouse model a novel parenteral vaccine formulation targeting multiple rotavirus genotypes.
METHODS: A parenteral vaccine based on nine VP8* proteins expressed in Escherichia coli from six distinct rotavirus geno-types (P [4], P [6], P [8]Wa, P [8]] lineage III, P [8] lineage IV, P [9], P [11], P [14], and P [25]) was constructed. Monovalent and polivalent vaccines were administered subcutaneously at a concentration of 5 µg/mouse to C57BL/6J mice. Aluminum hydroxide hydrogel was used as an adjuvant. Immunizations were given three times with 3-week intervals. Immunogenicity was determined by measuring anti-VP8* IgG in sera by ELISA.
RESULTS: Immunized mice developed IgG antibodies against six of the nine VP8* proteins. Significantly higher antibody titers against VP8* P [8]Wa were observed in mice immunized with the nonavalent vaccine compared to mice specifically immunized against this genotype alone, indicating the potential for broader protection against rotavirus infections.
CONCLUSIONS: The development of parenteral vaccines targeting multiple rotavirus genotypes represents a promising strategy in mitigating the global burden of rotavirus-related morbidity and mortality, offering new avenues for disease prevention and control.
SOURCES OF FUNDING: Grant PI20/00801 from the Carlos III Health Institute, Spanish Ministry of Health
Keywords: rotavirus, vaccines, genotypes, lineages, immunogenicity

PP-081
Immune response and vaccines

Difference in sero-prevalence of CVA EV-C types in children from South Sudan and the Netherlands

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AIM: Non-polio enteroviruses (NPEV) are worldwide circulating viruses. They are divided in four EV species A-D. In the Western world, EV-B followed by EV-A and EV-D are the most dominant. In sub-Saharan Africa (SSA) NPEV types belonging to EV-C are the most frequently reported genotypes. EV-C types are only sporadically encountered in clinical samples from Europe although they occur at a higher frequency in wastewater studies. This difference could be partially explained by the fact that many studies from SSA contain fecal samples from asymptomatic individuals. Sero-surveillance, by measuring nAb in serum, can largely overcome this sampling bias as it can provide information to which NPEV types a population has been exposed. However, seroprevalence data on NPEV-C are scarce. Our goal was to compare population immunity against NPEV-C types between two countries from SSA and Europe.
METHODS: We measured nAb titres against five of the most frequently reported NPEV-C types (CVA13, CVA20, CVA24, EV-C99, EV-C116) in 325 serum samples from children 3-18y matched for age and sex, and two pooled blood samples from South Sudan and the Netherlands (IVIG), collected between 2018-2019 and 2016-2017 respectively.
RESULTS: We found nAb titres against all included EV-C types in samples from South-Sudan. We found nAb titres against EV-C99 and EV-C116 but not against CVA types CVA13, CVA20, and CVA24 in samples from the Netherlands.
CONCLUSIONS: Our study shows a difference in population immunity against the included CVA EV-C types providing additional evidence for a difference in circulation between South Sudan and the Netherlands.
SOURCES OF FUNDING: None
Keywords: NPEV, EV-C, sero-surveillance, CVA, South Sudan, Netherlands

PP-082
Immune response and vaccines

High-risk HPV but not HPV16/18 are frequently detected in a French cohort of vaccinated women

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AIM: Increasing HPV vaccination coverage may change the prevalence and distribution of HPV genotypes. Here, we aimed to estimate the prevalence and distribution of HPV in a cohort of vaccinated French women with a cervical sample collected for cervical cancer screening (CCS).
METHODS: Adult women who had received at least one dose of HPV vaccine and had a cervical specimen collected and tested for HPV genotyping at our institution from 2018 to 2023 were eligible. High-risk HPV testing, detecting 13-14 hrHPV, was performed by Hybrid Capture 2 (Qiagen) or Alinity m assay (Abbott). HPV genotyping, detecting 32 HPVs, was performed using the INNO-LIPA technique (Fujirebio).
RESULTS: 553 vaccinated women (mean age: 28.4y) were included. Cytology, performed in 469 of them was abnormal (≥ASC-US) in 18.34% of cases. hrHPV testing was performed in 334 women and was positive in 27.8% of cases. HPV genotyping, performed in all women, was positive in 56.4% of cases. Among HPV-positive women, 22% had a multiple infection. At least one HPV type covered by the bivalent, quadrivalent, and nonavalent vaccines was detected in 3.8%, 7.8%, and 25.5% of patients, respectively. The most common hrHPV detected were HPV52 (7.05%), HPV51 (5.97%), and HPV56 (5.06%). HPV16/18 were detected in only 3.80% of cases and accounted for 3 of the 4 women with a high-grade lesion.
CONCLUSIONS: hrHPV are still frequently detected with, however, a low prevalence of HPV16/18 in our cohort of women mostly vaccinated before the introduction of the nonavalent vaccine.
SOURCES OF FUNDING: None
Keywords: HPV; vaccination; genotyping

PP-083
Immune response and vaccines

Humoral and T cell-mediated immune responses to the mRNA COVID-19 vaccine in lung and liver transplant recipients

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AIM: The aim of this prospective studio was to assess the humoral and T cell immune response to the mRNA COVID-19 vaccine in a cohort of lung transplant (LUT) and liver transplant (LT) recipients to evaluate the immunogenicity and efficacy of vaccine.
METHODS: Samples were collected 60 days post-second and third dose of the vaccine from March 2021 to December 2022 to quantify Anti-SARS-CoV-2 Spike RBD antibody using an electrochemiluminescence immunoassay and analyze the SARS-CoV-2-specific T-cell response using an IFN-γ ELISpot assay
RESULTS: 244 LT and 120 LUT recipients were enrolled. The third dose increased antibody titres in LT recipients (from a median value of 131 to 5523 IU/mL, p<0.001) and LUT recipients (from 14.8 to 1729 IU/mL, p<0.001). T cell-mediated response also increased in LT recipients (from 8.5 to 23 IFN-γ SFU per 250,000 PBMC, p<0.001) and LUT recipients (from 8 to 15 IFN-γ SFU per 250,000 PBMC, p<0.001). Previous SARS-CoV-2 infection was significantly associated with higher antibody titers in both LT and LUT recipients and with higher INF-γ SFU among LUT recipients. The third dose was associated with higher immune responses in both LT and LUT recipients
CONCLUSIONS: Both humoral and T cell-mediated responses were elicited after the three-dose schedule of the mRNA COVID-19 vaccine in LT and LUT recipients. The risk of severe COVID-19 post-vaccination was low in this population. As expected, humoral and T-cell mediated responses were higher among LT recipients compared to LUT recipients
SOURCES OF FUNDING: (partially) RC 260/01/2022/Ministero della Salute per Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano
Keywords: humoral response, T cell-mediate response, lung transplant, liver transplant, mRNA COVID-19 vaccine

PP-084
Immune response and vaccines

Antibody detection in the context of vaccination against tick-borne encephalitis virus using a newly designed ELISA

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AIM: This study investigated seroconversion in human serum samples between vaccine doses and after complete vaccination against tick-borne encephalitis (TBE), which requires 3 vaccine doses for basic immunization.
METHODS: Panel A contained single sera from 50 individuals obtained after ≥1 vaccine dose against TBE. Panel B contained 54 sera from 9 individuals (6 per person) obtained before and after first and second vaccine dose and 6 sera from one individual (#51) with sampling after second and third vaccine dose. Samples were analyzed for anti-TBE virus IgG antibodies using the newly designed ELISA.
RESULTS: Results of both panels showed that the positivity rate for specific antibodies increased with continued vaccination. 8 of 10 individuals from panel B had negative results in the ELISA before the first dose and antibody titers above the cut-off after the second, which then decreased again. Individual #51 had a high antibody titer (>200 RE/ml) after the third dose, which corresponds to an antibody level to be expected after completion of basic immunization. Individual #55 had specific antibodies before the first dose, which might be due to prior infection with TBE virus or undocumented vaccination against TBE.
CONCLUSIONS: The observed antibody dynamics are plausible in view of the ongoing vaccination and indicate the necessity of the third vaccine dose for basic immunization. Overall, the data show that the ELISA is suitable for both detection of specific antibodies and determination of immune status.
SOURCES OF FUNDING: None to declare.
Keywords: ELISA, tick-borne encephalitis virus, TBE, vaccination, IgG, immune response

PP-085
Pandemic preparedness and health services research

Automatization of CDC Influenza H5 assays into commercial integrated platforms: a strategic approach to pandemic preparedness

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AIM: The recent COVID-19 pandemic highlighted the global challenges diagnostic laboratories faced, which made it difficult for these environments to quickly adapt to fast-arising needs of global health threats. This study aimed to evaluate the performance of two automated systems for specific Influenza A H5 detection to support surveillance for effective Influenza pandemic preparedness.
METHODS: CDC H5 PCR assay performance specific for Influenza A H5 was evaluated on two independent, automated IVD molecular systems: 1) a closed customized cartridge and 2) an open mode Laboratory Developed Test. Analytical performance and sensitivity were optimized using artificial gene fragments designed for H5N1 harbouring HA-gene, and efficiency and specificity were validated using inactivated H5N1 viral culture supernatant specimens.
RESULTS: Both platforms yielded target-specific positive results for viral H5N1 samples, with sensitivity levels of 2.56E+4 (system 1) and 2.56E+2 copies/ml (system 2), demonstrating suitable performance at viral titers typical of symptomatic patients. In both devices, similar sensitivity values were obtained for the respective CE-IVD Influenza A assay (generic detection of Influenza A RNA discrimination), confirming the efficacy of the dual amplification strategy for Influenza A subtypes for correct diagnostics.
CONCLUSIONS: The successful implementation of the CDC Influenza A H5 assay on both automated systems underscores their potential utility in surveillance for pandemic preparedness. This offers laboratories a fast and efficient identification of Influenza A H5 RNA, facilitating a correct H5 subtype detection. This highlights the importance of automated, rapid molecular systems in swiftly adapting to emerging global health threats.
SOURCES OF FUNDING: None
Keywords: Influenza A, H5N1, Pandemic preparedness

PP-086
Pandemic preparedness and health services research

Successful implementation of large-scale regular health checkups in a university in Japan during the COVID-19 pandemic

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AIM: In the COVID-19 pandemic, we successfully conducted large-scale regular health checkups. To evaluate the impact of the COVID-19 pandemic on regular health checkups, we analyzed changes in venue size, hourly attendance, and checkup rates before and during the pandemic.
METHODS: Participants: students who underwent checkups from 2019 to 2023. Statistical analyses included: checkup rate = attendees / total students; attendance per hour = attendees / checkup hours. Two-way ANOVA assessed academic year and department effects on checkup rates; one-way ANOVA with Tukey-Kramer tests analyzed attendance in each academic year. Significance level was set at 0.05 using JMP17.0.
RESULTS: Venue size increased approximately 4.2 times during the 2020 pandemic compared to pre-pandemic levels in 2019, and further expanded about 9.0 times from 2021 to 2023. Attendance per hour decreased to 0.22-0.38 times during the 2020 pandemic compared to pre-pandemic levels, and remained low in subsequent years (0.37, 0.35, 0.31 times). Significant inter-year differences were observed in attendance per hour. Significant differences in attendance per hour were observed when comparing to 2019 for 2020 pre-declaration, 2020 post-declaration, 2021, 2022, and 2023. However, no differences were observed in other combinations. Significant differences in checkup rates were observed between departments and across years.
CONCLUSIONS: Successful large-scale health checkups during the COVID-19 pandemic were attributed to the significant increase in venue size (4.2-9.0 times) and reduction in hourly attendance (0.22-0.38 times), alongside rigorous infection prevention measures. We advocate for the establishment of preparedness measures to safely conduct health checkups in pandemic occurrence.
SOURCES OF FUNDING: JSPS21K11552
Keywords: COVID-19, Regular health checkups

PP-087
Pandemic preparedness and health services research

Transmission of SARS-CoV-2 among health care workers and patients in a hospital ward outbreak revealed by deep sequencing

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AIM: To investigate whether deep sequencing of viral strains could be used to clarify transmission patterns in a hospital outbreak of SARS-CoV-2 involving both patients and hospital staff.
METHODS: We analyzed an outbreak at a surgical ward with single-occupancy rooms only, which mostly affected health care workers (HCWs). Deep sequencing of SARS-CoV-2 by Ion Torrent was performed on nasopharyngeal swab samples from 28 HCWs and 8 patients collected over a period of 4 weeks during late autumn 2020. For comparison, we analyzed 14 outpatient samples and 32 sequences from the same time and region, downloaded from the GISAID database of SARS-CoV-2 strains. The sequences were were subject to phylogenetic analysis by CLC genomic workbench. Onset of symptoms were collected from patient records and/or questionnaires.
RESULTS: Sequencing of 24 HCW and 7 patient samples was successful. A vast majority (96%) of the samples from the ward formed a separate cluster (bootstrap values ≥90%). Only two outpatient sequences and one GISAID-database sequence clustered with the ward sequences. The index patient was identified as a staff member with a high viral load at the time of sampling (Ct-value of 12). All patients developed symptoms of SARS-CoV-2 infection during the hospital stay.
CONCLUSIONS: Phylogenetic analysis support extensive transmission within the ward. The relatively low number of cases among patients indicates that the risk of being infected at the ward was lower among patients than among staff. More detailed outbreak analysis with sequencing, including both patients and HCWs, is needed to understand and prevent outbreaks of transmissible disease.
SOURCES OF FUNDING:
Keywords: Healthcare associated infections (HCAI), SARS-CoV-2, Next-generation sequencing

PP-088
Respiratory viruses

Respiratory syncytial virus genetic diversity and lineage changes in Ireland pre- and post-COVID-19 pandemic

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AIM: Human respiratory syncytial virus (RSV) is a common cause of lower respiratory tract infections globally. Newly-licensed prophylactic vaccines and monoclonal antibodies are anticipated to alleviate this burden. However, such interventions may exert selective pressures on RSV evolution that could impact effectiveness, highlighting the importance of robust genomic surveillance.
METHODS: Whole genome sequencing was performed on RSV-A (n=121) and RSV-B (n=108) collected in 2022-2024 from cases in community and acute hospital settings. In addition, G gene sequences, RSV-A (n=141) and RSV-B (n=141), collected in 2015-2019 were examined. Phylogenetic analyses was performed and lineages assigned.
RESULTS: Phylogenetic trees inferred with the G gene and whole genome were consistent. Changes in lineage prevalence post-COVID-19 reflected the impact of non-pharmaceutical interventions introduced to reduce SARS-CoV-2 transmission. RSV-A lineages A.D.1 and A.D.5 were dominant, while B.D.E.1 was dominant for RSV-B. Similar trends were also observed in prevalent lineages in the European region. Also, the emergence of a new lineage was identified as descended from A.D.1 with eight distinctive amino acid substitutions in proteins G, F and L. Other circulating lineages with amino acid substitutions in the F glycoprotein could impact the binding sites of Nirsevimab.
CONCLUSIONS: We provide insights into RSV transmission and evolution within Ireland over the last decade through the selective forces created by the measures introduced during the COVID-19 pandemic. This study provides a foundation for future public health surveillance employing pathogen genomics to enable an evidence-based assessment of the impact of pharmaceutical interventions on RSV evolution and disease severity.
SOURCES OF FUNDING: National Virus Reference Laboratory
Keywords: Respiratory syncytial virus, whole genome sequencing, genetic diversity, lineages, evolution

PP-089
Respiratory viruses

FluReD: a new bioinformatic tool for enhancing reassortment and zoonosis detection in influenza surveillance

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AIM: Reassortment is a frequent genetic mechanism of evolution in seasonal influenza and commonly observed in non-human natural hosts, conferring pandemic potential. FluReD is a newly developed bioinformatic tool that aims to identify reassortments, including zoonotic segments detection, in a user-friendly way.
METHODS: The pipeline assigns genetic clades based on distance-based methods. The implementation in nextflow allows fast and easy reassortment identification. Sequences of the week with the highest incidence (accessed at https://erviss.org/) for influenza A/H1pdm09 (W04/2024), A/H3 (W03/2024) and B/Vic (W08/2024) obtained from GISAID were used for validation. Clade assignment was based on ECDC reference sequences for 2023-2024 season. Results from FluReD were confirmed through phylogenetic analyses. Additionally, a dataset of 100 human influenza sequences (50 A/H1pdm09 and 50 A/H3) with 18 avian and swine influenza segment sequences was created for testing.
RESULTS: For A/H1pdm09 (417), A/H3 (163) and B/Vic (45) strains, results were obtained in less than 30", 15" and 7", respectively. FluReD clade assignment was achieved for more than 99.5% of sequences per segment, except for A/H1pdm09 MP classifying 89.7% of the sequences and A/H3 NP with 93.2%, due to their low mutation rate. Results obtained from FluReD were consistent with those from phylogenetic analyses for all genotyped segments. Inter-clade reassortant strains were detected at 11.7%, 100% and 0% for A/H1pdm09, A/H3 and B/Vic, respectively. For the zoonotic dataset, segments were correctly classified in less than 15".
CONCLUSIONS: FluReD enhances influenza genomic surveillance by facilitating rapid and straightforward segment genotyping, identifying reassortant strains, and detecting zoonotic segments effectively.
SOURCES OF FUNDING: No funding
Keywords: bioinformatics, influenza, one health, surveillance

PP-090
Respiratory viruses

An updated molecular protocol to determine community prevalence of influenza C virus during the 2023/2024 influenza season in Wales

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AIM: Influenza C virus (ICV) is historically understudied due to difficulties in isolation by viral culture. Whilst infections are generally believed to cause milder illness than other influenza viruses, reports of severe lower respiratory tract infections and acute encephalopathy highlight the need for prompt and accurate diagnosis. ICV is not tested for in Wales and there are no studies determining prevalence amongst the Welsh population. Our aim is to determine prevalence of active infection amongst community patients during the 2023/2024 influenza season, as well as provide a framework for the in-house development of molecular diagnostic tests for understudied viruses.
METHODS: Advancements in molecular diagnostics have resulted in ICV RT-PCR protocols. We have modified an existing protocol according to conserved changes found in the primer/probe binding region. Our new protocol will test oropharyngeal swabs collected from outpatients with respiratory symptoms presenting to general practitioners and pharmacies across Wales between week 50 2023 and week 16 2024.
RESULTS: Results are currently pending, but we intend to screen upwards of 500 samples collected during the period and expect to find some positivity.
CONCLUSIONS: Positivity indicates ICV transmission in Wales. Previous molecular-based studies largely focused on hospitalised patients, creating a bias towards severe cases believed to be in the minority, which may result in a higher positivity in our study. However, previously demonstrated wide bi-annual variations in prevalence may impact our results if our study period occurs during the off-season. Therefore, negative results will not preclude transmission in Wales, but rather highlight a need for extended studies.
SOURCES OF FUNDING: None.
Keywords: influenza, influenza C virus, molecular diagnostics, respiratory virus

PP-091
Respiratory viruses

Overview of Adenovirus respiratory infections: preliminary findings of a multicentre study in Italy, 2022-2023

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AIM: Human Adenovirus (hAdVs) typically causes mild to severe respiratory infections. This study was promoted by the Working Group on Respiratory Virus Infections of the Italian Association of Clinical Microbiologists, aiming to investigate hAdV respiratory infections among adults and children and assess the prevalence of different hAdV genotypes during the study period.
METHODS: This study included 34112 samples collected from eleven Italian clinical laboratories (January 2022-June 2023). Samples were screened to detect hAdV-DNA and sequencing of the hexon gene was performed to determine their genotype.
RESULTS: 5.2% (1772/34112) of all samples were hAdV-positive. Among them, 988 were males and 773 females (55.7% and 44.3%, respectively), median age of 3 years (range 24 days-98 years). In 867/1772 cases (75.7%) at least one other respiratory virus was detected, including HRV, RSV and MPV (51.8%, 9.2% and 7.5%, respectively). hAdV typing was performed on 158/1772 (8.9%) collected samples between July 2022 and June 2023. The most common strain was hAdV-B3 identified in 62% of cases (98/158), followed by hAdV species C strains, including hAdV-C2, -C1 and -C5 (17%, 12% and 4.4%). During the study period, a shift in circulating genotypes has been observed. From July 2022 to January 2023, hAdV-C strains were the most commonly detected while from February 2023 hAdV-B3 was the prevailing strain.
CONCLUSIONS: hAdV accounts for 5% of all respiratory infections, with children as the most susceptible. hAdV was present throughout the study period, with increased cases during the spring of 2023, sustained by the hAdV-B3 strain.
SOURCES OF FUNDING: EU funding (Project no. PE00000007, INF-ACT Foundation)
Keywords: Adenovirus, respiratory infections, epidemiology

PP-092
Respiratory viruses

Comparative Analysis of Flu, COVID-19, and RSV Infections in Pediatric Admissions
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AIM: This study assesses the clinical presentations and epidemiology of common respiratory infections — flu, COVID-19, and RSV in children in Bialystok, Poland.
METHODS: A retrospective analysis was conducted on medical records of children hospitalized from September 2022 to September 2023, involving 818 admissions. One-hundred and ten cases were randomly selected for detailed examination of infection progression.
RESULTS: Seasonal peaks were identified with over 90% of flu cases occurring from December to March; specifically, flu A from December to February, and flu B from February to April. RSV cases were most frequent from November to January, comprising 90% of infections during that period. COVID-19 cases were highest in March (30%) but persisted year-round. The youngest patients were those with RSV (median age: 5 months), followed by COVID-19 (10 months) and flu (58 months). Each virus presented distinct symptoms: flu cases often had elevated CRP levels (median 9 mg/L) and sore throat (70%); RSV cases displayed significant respiratory distress, including shortness of breath (60%), chest indrawing (43%), nasal congestion (93%), and crepitations (87%). COVID-19 showed milder symptoms. Systemic antibiotics were used in 53% of COVID-19 cases, 50% RSV cases, and 35% flu cases.
CONCLUSIONS: This study highlights the unique epidemiological patterns and symptomatic profiles of common respiratory infections in children. The less frequent use of antibiotics in flu cases suggests the potential benefits of antiviral treatments, underscoring the need for similar advancements in therapies for RSV and COVID-19 to reduce antibiotic reliance.
SOURCES OF FUNDING: Medical University of Bialystok, Poland
Keywords: flu, COVID-19, RSV

PP-093
Respiratory viruses

Detection of causes of respiratory viral infections in animals in Kazakhstan in 2023-2024
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AIM: Many viruses that cause severe respiratory diseases in humans and animals, especially influenza viruses, evolve rapidly through mutation (drift or shift). Accordingly, the aim was identification of the respiratory viral infection pathogens circulating in pigs and dogs in Kazakhstan.
METHODS: Oropharyngeal swabs (n = 555) were investigated by RT-PCR with reagents from the Central Research Institute of Epidemiology of Rospotrebnadzor (Russia) using a Rotor-Gene Q6plex device (QIAGEN, Germany). HI assay was used to detect antibodies to influenza virus in serum samples (n = 180). ELISA was performed using the IDEXX Influenza A test system (USA).
RESULTS: As a result of RT-PCR, genetic material of IAV was detected in 41 samples (7.39%), of the total number of samples studied,of which A/H1N1pdm RNA was detected in 20 (3.60%) and A/H3N2 in one sample (0.18%). Coronavirus, bocavirus, and adenovirus were not detected in the studied samples. The results of HI assay showed the presence of antihemagglutinins for influenza A/H1N1pdm virus in 6.11% (11 samples) of the total number of samples, for A/H3N2 in 9.44% (17 samples). Antibody titers amounted to 1:40–1:160. ELISA has revealed the presence antibodies of IAV nucleoprotein for 5.56% (10 samples). Thus, the results of screening biological samples in RT-PCR, HI assay and ELISA revealed the presence IAV A/H1N1pdm and A/H3N2 in animals.
CONCLUSIONS: Monitoring the circulation of influenza viruses and other acute respiratory viral pathogens in Kazakhstan provides insight into viral diversity and the risk of zoonotic infection.
SOURCES OF FUNDING: The work was carried out within the framework of the grant funding project AP19677931.
Keywords: Virus, influenza, PCR diagnostics, nucleoprotein, antibody

PP-094
Respiratory viruses

Severe influenza during the 2023/24 SARS-CoV-2 pandemic season. Restoration of the Influenza infections dynamics

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AIM: We analyze the circulation of influenza viruses in emergency admissions for influenza-like illness (ILI) and severe acute respiratory infection (SARI) in the Valencia Region, Spain, during the last 2023/24 SARS-CoV-2 pandemic season.
METHODS: Prospective active-surveillance hospital-based test-negative design study on respiratory infections. ILI/SARI cases were screened in 5 tertiary-care hospitals (22% of the inhabitants), from Nov. 2023 to March 2024. Swabs were tested by RT-PCR. Whole genome (WG) of influenza-positive isolates was sequenced and phylogenetic analyses were performed.
RESULTS: During the 2023/24 study period, 348 Influenza positive cases were detected. Influenza A dominated the season (n=347) reaching the peak of infection in late-Dec.2023-Jan. 2024. Only 1 Influenza B was detected in Nov. 2023. A/H1N1 and A/H3N2 were present throughout the season, with A/H1N1 being the predominant subtype. One hundred and sixteen WG were obtained H1N1 (n=86) and H3N2 (n=30). The H1N1 viruses belong to two clades 6B.1A.5a.2a and 6B.1A.5a.2a.1 represented by A/Sydney/5/2021 and A/Victoria/4897/2022. All H3N2 sequences belong to subclade 3C.2a1b.2a.2a.3a.1, represented by A/New York/66/2022.
CONCLUSIONS: After the SARS-CoV-2 pandemic, the 2023/24 influenza season is the first to present dynamics in influenza infection comparable to influenza seasons prior to the SARS-CoV2 pandemic, beginning in late autumn and peaking in winter. H1N1 dominated in 2023/24, but coexisted with H3N2. 43% of the patients were vaccinated and 77,17% were >60 years old. Eight were admitted to the ICU, 305 needed supplemental oxygen and 13 died.
SOURCES OF FUNDING: VANHSI network
Keywords: Influenza, H3N2, H1N1, respiratory viruses

PP-095
Respiratory viruses

Decrease in Severe SARS-CoV-2 cases in 2023-24 season, Valencia Hospital Surveillance Network for the Study of Influenza and Other Respiratory Viruses (VAHNSI), Spain

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AIM: To characterize SARS-CoV-2 cases in emergency admissions for influenza-like illness (ILI) and severe acute respiratory infection (SARI) in the Valencia Region, Spain, in the 2023-24 season.
METHODS: Prospective active-surveillance hospital-based test-negative design study on respiratory infections. ILI/SARI, cases were screened in 5 tertiary-care hospitals serving to 22% of the 4,860,874 inhabitants. Swabs were tested by RT-PCR and whole genome sequencing; phylogenetic analysis and variant characterization was performed in SARS-CoV-2 positive isolates.
RESULTS: From November 2023 to March 2024, 4009 patients were admitted with ILI/SARI. Only the 4,64% tested positive for SARS-CoV2. The 87,63% of these positives were collected from patients admitted from Nov. 2023 to Jan 2024. All detected variants were Omicron. XBB.1, XBB.1.5 and BA.2 coexisted in Nov. 2023, but since Dec. 2023 XBB.1 and XBB.1.5 disappeared and BA.2 become dominant. 88,2% of the patients were >60 years old. One was admitted to the ICU, 139 needed supplemental oxygen and 10 died.
CONCLUSIONS: : In our area, SARS-CoV-2 infections decreased sharply since Feb. 2024. Most patients were > 60 years old, 10 (6,1%) of whom died. All the infections occurred in 2023-24 season were due omicron variants, with the BA.2 variant prevailing since Dec. 2024.
SOURCES OF FUNDING: COVIDRIVE
Keywords: Respiratory viruses, SARS-CoV-2, BA.2, XBB.1, XBB.1.5

PP-096
Respiratory viruses

Clinical Impact of Current Variants in COVID-19 Patients

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AIM: Although the symptoms caused by various SARS-CoV-2 variants may be similar, some variants are associated with increased severity of infection or high infectiousness. We aimed to investigate the relationship between the SARS CoV-2 variants we detected and the severity of the disease by using the whole genome sequence analysis method in patient samples diagnosed with COVID-19 in our hospital, in the period from the announcement of the EG.5 variant until the end of 2023, and to contribute to SARS CoV-2 surveillance.
METHODS: The study included 68 patient samples that were found to be SARS CoV-2 positive by polymerase chain reaction. And then whole genome sequence analysis was performed, on samples suitable for sequence analysis using Illumina next-generation sequencing platform.
RESULTS: EG.5 variant was detected in 15 (21%) of the samples included in the study, while different sub-variants other than EG.5 were detected in 53 (77.9%) samples. XBB.1.16, EG.5.1 and FL.1.5.1 were the most frequently detected variants respectively. There were 20 patients who were hospitalized and followed up, and 14 of them had moderate-severe clinical status according to the WHO clinical progression scale. In the statistical evaluation, no significant difference was found between the current subvariants in terms of age group, clinical symptoms and disease severity.
CONCLUSIONS: Strengthened genomic analysis capacities will enable risk assessments to be made by better understanding the evolution and circulation of pathogens. Thus, public health measures will be shaped by genomic surveillance systems that will form the basis for the development of vaccines, therapeutics and diagnostic analyses.
SOURCES OF FUNDING: No funding
Keywords: EG.5, SARS CoV-2, Sequence, Variant

PP-097
Respiratory viruses

Sequence Analysis of RSV Positive Samples in the Pediatric Patient Group at Ankara Bilkent City Hospital Following the COVID-19 Pandemic

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AIM: Following the COVID-19 pandemic, we examined the epidemiology and clinical severity of RSV with whole genome sequencing (WGS).
METHODS: Between November 2023 and January 2024, 27 samples with RSV A/B detected by the Respiratory Tract RT-qPCR (Bio-Speedy, Bioeksen, Turkey) kit and suitable for sequence analysis (Ct<20) at Ankara Bilkent City Hospital were included in the study. WGS was performed by the extracted RNA using RNA Prep kits and DNA/RNA UD Indexes (Illumina, USA) - Illumina Technologies.
RESULTS: While the sequence analysis resulted as RSV A in 22 of the patients, the sequence result could not be determined in 5 patients. RSV B was not detected in any patient. The median age of the patients was 3.5 months, and the age range was between 1 month and 5 years. The most common presenting symptoms were cough, wheezing and shortness of breath. Pneumonic infiltration was detected in 9 (45.0%) of 20 patients who underwent imaging. There were 15 patients who were hospitalized and monitored, and almost all of them (n=14) had low saturation and oxygen need. Three children, two one-month-old and one 12-years-old (diagnosed with cerebral palsy and epilepsy), required intensive care. All patients were discharged with recovery from RSV.
CONCLUSIONS: Only RSVA was detected in our current patient group. In our country, RSV A was also reported to be more dominant in studies conducted before the COVID-19 pandemic. Local RSV molecular epidemiology requires detailed sequence data and provides important information in the development and implementation of vaccine strategies.
SOURCES OF FUNDING: No funding used
Keywords: RSV, Sequence

PP-098
Respiratory viruses

Impact of the RSV passive immunisation campaign in infectivity and variant circulation

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AIM: Children's RSV immunisation with Nirsevimab, despite its benefits, comes with the risk of selection and spread of resistance mutations. The objective was the global surveillance of RSV variants circulating in the 2023-24 season.
METHODS: A previously described protocol (Davina-Nunez C et al., Int J Mol Sci. 2023) compatible with Illumina CovidSeq (Illumina, San Diego, USA, iSeq, NextSeq) was used. Variant distribution was studied by age, gender and geographical origin. For the phylogenetic analysis (MAFFT, MEGA11.0), all GISAID sequences for the same period were included.
RESULTS: A total of 688 samples from two Spanish regions were sequenced in this study.
In Galicia, a majority of the samples were RSV-B (470/568; 82.7%) with clade B.D.E.1 being dominant (448/568). Notably, in the Canary Islands RSV-A was dominant (106/120; 88.3%), with clade A.D.1 being dominant (88/120). Regarding mutations in the Nirsevimab binding area, mutations in residue 208 were detected in 3 samples from Galicia but not from any other region around the globe. These mutations were only previously detected in vitro and associated with the complete loss of Nirsevimab binding. A cluster of samples from the Canary Islands showed the mutation N63S.
CONCLUSIONS: Mutations in the binding site of Nirsevimab were uncommon in the first season after immunisation rollout. Nonetheless, global RSV molecular surveillance remains at a low level. Active surveillance is key to monitor the effect of the immunisation campaigns.
SOURCES OF FUNDING: Consellería de Sanidade, Galicia and ECDC/HERA incubator - GRANT 2021/PHF/23776
Keywords: Respiratory Syncytial Virus, Immunisation, Viral Genetics, Epidemiology

PP-099
Respiratory viruses

Impact of genetic diversity of influenza A in the effectiveness of the vaccine

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AIM: We estimated the influenza vaccine effectiveness (VE) by season, influenza type, subtype and clade. We described the amino acid substitutions that might impact vaccine effectiveness.
METHODS: We detected influenza by RT-PCR in respiratory samples of the influenza season 2022-23 and 2023-24 (weeks 40 - 20). Influenza clades were studied (Lin et al. 2023).
Hemagglutinin/neuraminidase phylogeny was performed. Vaccination status was recovered from the Galician vaccine registry. Patients vaccinated <14 days before diagnostic were excluded.
VE (vaccine efficacy) = 1 - (odds ratio (OR)) * 100.
RESULTS: 2022-23: Mean age (57.9 ± 28.9), 1081 influenza/15852 samples.
H1N1pdm09 (n=123, VE 36.9%)
H3N2 (n=679, VE 53.4%)
B (n=299, VE 90.6%)
Sequenced A(H1N1) (n=11):
A/Victoria/2570/2019 (n=10)
A/Norway/25089/2022 (n=1)
Sequenced A(H3N2) (n=109):
A/Denmark/3264/2019 (n=16,VE 40.8%)
A/Slovenia/8720/2022 (n=41, VE 36.9%)
A/Bangladesh/4005/2020 (n=52, VE 27.7%)
2023-24: Mean age (57.1 ± 30.0), 1918 influenza/16161 samples.
H1N1pdm09 (n=930, VE 56.7%)
H3N2 (n=317,VE 30.1%)
B (n=66, VE 84.9%)
Sequenced A(H1N1) (n =169):
A/Victoria/4897/2022 (n=16, VE 42.7%)
A/Sydney/5/2021 (n=153, VE 72.7%)
Sequenced A(H3N2) (n=78):
A/Thailand/8/2022 (n=78)
No novel glycosylation sites were detected in the samples sequenced, although the H3N2 variant dominant last season (2a.3) includes mutation HA1:N96S, associated with a new glycosylation site (NSS)
CONCLUSIONS: We showed the genetic diversity of circulating influenza viruses and the need for continuous antigenic and molecular surveillance.
SOURCES OF FUNDING: Conselleria de sanidade and ECDC/HERA incubator - GRANT 2021/PHF/23776
Keywords: Influenza, Epidemiology, Vaccine Efficacy, Genetic Surveillance

PP-100
Respiratory viruses

Validation of a lab-developed Influenza-A HA typing multiplex-PCR assay for detection of H1pdm09, H3 and H5 on a high-throughput system

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AIM: The aim of this study was to develop, validate and implement an Influenzavirus-A typing qPCR-assay on a high-throughput platform to rapidly differentiate circulating human A(H1pdm09N1), A(H3N2) and avian A(H5Nx) in clinical samples.
METHODS: Suitable assays (Panning et al., Terrier et al., WHO) were optimized for inclusivity and adapted to cobas5800/6800/8800 chemistry. Internal 2'O-methly RNA bases were used to delay primer dimer formation. Internal Quenchers were used in Taqman probes to depress baseline fluorescence.
RESULTS: Lower-limit of detection (LoD) was determined for each assay by serial dilution (2-fold, 8 repeats, 8 steps) by probit analysis: Influenza-A M-gene: 45.2 cp/ml, H1pdm09: 754 cp/ml, H3 148 cp/ml, H5: 156 cp/ml. Linearity was verified over 4-6 log-steps depending on target (Repeatability: M-gene: 0.18ct, H1pdm09: 0.479ct, H3: 0.289ct, H5: 0.376ct). No cross-reactivity between H1/H3/5 was observed and no false positives occurred in the inclusivity/exclusivity set (n=45). In a set of 40 clinical samples (collected in different Influenza-seasons), 78% of typing results were concordant with the reference assay (Seegene RP1A). In 15% of samples, the reference assay failed to generate a typing result, whereas in 7% of cases, the lab-developed assay failed to generate typing results. There were no cases of contradictory typing.
CONCLUSIONS: The assay presented in this study showed good analytical and clinical performance in detecting and differentiating Influenza-A H1pdm09 and H3 in clinical samples, as well as H5 in reference material. Implementation on a fully automated sample-to-result platform allows for easy scaling of capacity and fast turn-around-times in case of outbreaks.
SOURCES OF FUNDING: Institutional only.
Keywords: RT-qPCR, Influanza-A, HA-typing, cobas x800

PP-101
Respiratory viruses

Detection and differentiation of influenza A/B virus, respiratory syncytial virus, and SARS-CoV-2 during 2021-2023, as part of integrated laboratory surveillance in Bosnia and Herzegovina

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AIM: Respiratory infections caused by SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) can manifest with similar symptoms and also possible mutual co-infections This indicates a need for diagnostic tests that can detect and differentiate between SARS-CoV-2, influenza, and RSV in clinical laboratories. In addition, according to ECDC and WHO, it is crucial, that all patients with acute respiratory symptoms are tested for SARS-CoV-2 and influenza, especially during influenza season. The objective of this study was to simultaneously detect and accurately distinguish the viruses SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) to implement sentinel surveillance.
METHODS: The study respiratory specimens tested by multiplex real-time polymerase chain reaction (rtPCR) for simultaneously detected influenza, RSVA&B, and SARS-CoV-2 in a one-run. Testing was done in the Clinical Center of the University of Sarajevo, OU Clinical Microbiology, Sarajevo, Bosnia and Herzegovina.
RESULTS: There were 472 nasopharyngeal swab (NPS) specimens from sentinel surveillance (SARI, ILI) during two seasons; 64 (13,56%) samples were positive for influenza viruses, 27 (5,72%) for RSV A&B, and 39 (8,26%) for SARS-CoV-2. Among them, 74/275 (26,90%) positive cases were SARI, while 56/197(28,42%) cases were ILI.
CONCLUSIONS: Our results show the importance of integrated surveillance of respiratory pathogens in the influenza season, especially due to the continued presence of SARS-CoV-2 and the very similar clinical manifestation of the disease. Improving diagnostic capabilities during respiratory outbreaks enables timely intervention and improves surveillance of SARI and ILI.
SOURCES OF FUNDING: No source of funding.
Keywords: SARS-CoV-2; RSV; Influenza; RT-PCR;

PP-102
Respiratory viruses

National surveillance for the epidemiology of respiratory viruses in Korean children before and after the COVID-19 pandemic

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AIM: We aimed to characterize the epidemiology of respiratory viruses in Korean children during post-COVID-19 pandemic years compared to those before the pandemic.
METHODS: A nationwide prospective ongoing surveillance study has been conducted for detection of respiratory viruses between January 2017 and June 2023. We analyzed the weekly detection frequency of individual viruses and the age distribution of the affected children. The study period was divided into prepandemic (2017-2019) and postpandemic (2021-2023) periods.
RESULTS: A total of 19,589 and 14,068 samples were collected in the pre- and postpandemic periods, respectively. The overall detection rate of any virus throughout the study period was 63.1%, with the lowest occurring in the 2nd half of 2020 (50.6%) and the highest occurring in the 2nd half of 2021 (72.3%). Enveloped viruses (HCoV, HMPV, IFV, PIV, and RSV) almost disappeared, but nonenveloped viruses (AdV, HBoV, and HRV) were detected even during the peak of the COVID-19 pandemic. The codetection rate increased from 15.0% prepandemic to 19.1% postpandemic ($P < 0.001$). During the postpandemic period, a large out-of-season PIV and HMPV epidemic occurred, but the usual seasonality began to be restored in 2023. The mean age of children with each virus detected in 2023 was significantly greater than that in prepandemic years ($P = 0.003$ and 0.007 for AdV and HCoV, respectively; $P<0.001$ for others).
CONCLUSIONS: With the relaxation of NPI measures, several seasonal respiratory viruses cocirculated with unusual seasonal epidemic patterns and were associated with increasing age of infected children.
SOURCES OF FUNDING: KDCA (grant No. 6300-6332-304).
Keywords: COVID-19, Epidemiology, Respiratory viruses, Immune debt

PP-103
Respiratory viruses

Five-year (2017-2022) evolutionary dynamics of human coronavirus HKU1 in southern France with emergence of viruses harboring the spike H512R substitution

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AIM: The evolution and diversity of HCoV-HKU1 have been poorly studied worldwide with (as of 04/12/2023) only 106 complete genomes avaialble in GenBank. Here, we investigated the diversity and genomic evolution of HCoV-HKU1 over 5 years.
METHODS: The study was retrospectively conducted on samples diagnosed HCoV-HKU1 RNA-positive between 2017-2022 (CSE23-39 AP-HM). Next-generation sequencing used an Illumina Novaseq 6000 instrument and an adapted CovidSeq protocol post-whole genome PCR amplification with a primer systems' sets designed using Gemi and primal scheme. Genome assembly and analyses used CLCGenomics and Mafft, BioEdit, Nextstrain, Nextclade, MEGA, iTol and RDP4 bioinformatic tools. Spike molecular modelling and dynamics simulations used Molegro Molecular Viewer/Hyperchem.
RESULTS: 28 PCR primer systems were designed for whole genome amplification pre-NGS.158 genomes were obtained: 69 and 89 of genotype A and B, respectively. Both genotypes co-circulated during the whole study period but one after the other predominated. 1,683 amino acid substitutions including 80 in ≥10 genomes were detected relatively to a 2004 reference genome. H512R in spike, first detected in 2009 worldwide and reported as involved in antibody neutralisation, was found in all genotype A genomes, combined with V387I and K478N, and it was predicted here to significantly improve TMPRSS2 binding. 1,802 amino acid substitutions including 64 in ≥10 genomes were detected relatively to a 2005 reference genome.
CONCLUSIONS: Overall, the present work enriched by 2.5-fold the HCoV-HKU1 global genome database and improved HCoV-HKU1 evolution knowledge with the description of an emerging mutation pattern and its putative impact on viral replication.
SOURCES OF FUNDING: National Agency for Research, Méditerranée-Infection 10-IAHU-03.
Keywords: Coronavirus, HKU1, respiratory virus, genome, evolution, molecular epidemiology

PP-104
Respiratory viruses

Etiologic spectrum and clinical characteristics of respiratory viral infections and co-infections in Bulgaria during the first two seasons of the post-COVID-19 era

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AIM: This study aims to explore the etiology and clinical significance of viral acute respiratory infections (ARIs) and coinfections in Bulgaria during the period 2022-2024.
METHODS: Epidemiological and clinical data and nasopharyngeal swabs were prospectively collected from patients aged 0-16 years with ARIs between October 2022 and April 2024. Real-time PCR for 13 respiratory viruses was performed.
RESULTS: Of the 3406 patients examined, 1661 (48.8%) tested positive for at least one respiratory virus: 845 (52.9%) in the first (2022-2023) post-pandemic season, 774 (44.9%) in the second (2023-2024) and 42 (48.8%) inter seasons. Influenza A(H1N1)pdm09 was the most frequently detected virus (15.5%), followed by RSV (9.6%). SARS-CoV-2 had a low prevalence (3.3%). Coinfections between two or three respiratory viruses were detected in 130 (3.8%) patients, including 13 with SARS-CoV-2. The frequency of co-infections was higher in patients aged 0–4 years (6%). Proportions of influenza viruses detected in patients with tracheobronchitis, bronchiolitis, pneumonia, and neurological complications were 20.4%, 10.2%, 16.9%, and 12%, respectively; regarding seasonal non-influenza viruses, the proportions were 33.3%, 45.4%, 29.8%, and 18%, respectively. RSV was the most common virus found in children aged <5 years with bronchiolitis (32%) and pneumonia (19%).
CONCLUSIONS: This study revealed a strong resurgence of seasonal viral ARIs in the period after the lifting of anti-COVID-19 measures. Influenza viruses and RSV had the greatest clinical burden. Diagnostic testing for respiratory viruses using molecular methods may lead to the reduced use of antibiotics and may assist in measures to control infection.
SOURCES OF FUNDING: Project КП–06-Н73/7-05.12.2023; КП-06-Н43/5
Keywords: RSV, SARS-CoV-2, Influenza, respiratory viruses

PP-105
Respiratory viruses

Development and Characterization of an Aerodynamic System for Pulmonary Delivery of Influenza Vaccine
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AIM: The name influenza is Italian meaning "*influence*", Commonly referred to as flu, is an infectious disease caused by RNA viruses of family Orthomyxoviridae, that affects birds and mammals. The aim is to develop and characterize aerodynamic systems with recombinant H1N1Ags for safely deposition in alveoli to enhance bioavailability and control release of influenza antigen after pulmonary administration in animal model. This induces not only systemic humoral (IgG) responses, but also cell¯mediated (IL¯4,IFN¯gamma) and mucosal immune responses (IgA, IgG), non¯invasive, propellant & needle free delivery of vaccine.
METHODS:Chitosanmicroparticleswerepreparedbyionicgelationmethodofchitosanwithtripolyphosphate(TPP). Formulations were optimized on basis of particle size, tap density & entrapment efficiency. External morphology of optimized formulations was studied by TEM & SEM. Zeta potential was determined along with stability studies at accelerated tempreatures. In-vivo studies involved determination of antibody titres in serum & mucosal secretions & uptake studies by fluorescence microscopy.
RESULTS: The results show that, as preparation was reduced to lyophilized form which increased stability as compared to conventional liquid formulations. Microparticles of uniform size distribution were obtained owing to repulsion between positively charged particles. The fluorescence images show the uptake of microparticles by various organs and the ELISA results shows comparable IgG responses along with IgA.
CONCLUSIONS: Thus, it can be concluded that antibody production was found to be more in pulmonary route as compared to other routes so with further studies with this method can be implemented for vaccine delivery.
SOURCES OF FUNDING: NA
Keywords: influenza, Microparticles

PP-106
Respiratory viruses

PlexPlus® mass multiplex assay detects 14 viral respiratory targets in a single well
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AIM: PlexPlus® technology doubles standard qPCR readout capabilities. The technology uses universal probes which fluoresce at either low or high temperatures, with fluorescence acquired at two temperatures across multiple wavelengths. Data obtained within each channel at each temperature is discrete, with no cross talk between temperatures. Using the RespiV PlexPlus® prototype assay as a model of the technology, the aim was to assess the capacity to simultaneously detect 14 respiratory viral targets in one well.
METHODS: Fluorescence data were acquired at 52°C and 76°C in six channels during qPCR cycling on the QuantStudio™ Real-Time PCR system. The assay was characterized by retrospective analysis of 680 nasopharyngeal swab samples using SpeeDx's PlexPCR® RespiVirus and PlexPCR® Flu/RSV/SARS-CoV-2 tests as comparators, with a subset of non-concordant results resolved by sequencing.
RESULTS: The assay had the capacity to detect down to 5-20 copies of target across the entire panel in preliminary analytical studies. Analysis of the nasopharyngeal swabs showed overall concordance of 96% or greater for all 14 targets. Further, the assay successfully detected and discriminated Human Rhinovirus and Enterovirus in the nasopharyngeal specimens and in a QCMD 2023 Rhinovirus RNA EQA Programme (RVRNA23S) panel.
CONCLUSIONS: The single-well, highly multiplexed assay delivered comparable results on 680 samples compared to two current IVD cleared assays. The assay expedites high throughput analysis, allowing determination of up to 1,302 individual results relating to the presence or absence of 14 respiratory viral targets in 93 patient samples, analysed simultaneously in one 96 well plate on a standard PCR instrument.
SOURCES OF FUNDING: SpeeDx Pty Ltd
Keywords: respiratory virus, nucleic acid technology, enterovirus

PP-107
Respiratory viruses

Investigation of respiratory viruses, Greece, 2021-2024
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AIM: Besides specific testing for SARS-CoV-2 and/or influenza viruses, several respiratory samples are sent to the laboratory for syndromic testing, as after relaxation of protective measures against the risk of SARS-CoV-2 spread, an increase in the incidence of viral respiratory infections was observed. Aim of the study was to identify viruses responsible for respiratory infections and further analyze them genetically.
METHODS: During January 2021 up to date, 305 respiratory samples were tested either by BioFire FilmArray Pneumonia Panel applied directly on clinical samples, or by the RespiFinder panel applied on extracted genetic material. For typing of respiratory syncytial virus (RSV) and adenoviruses, conventional PCRs combined with Sanger sequencing were applied, while de novo Nanopore NGS was applied on selected positive samples followed by phylogenetic analysis.
RESULTS: At least one viral pathogen was detected in 78/305 (25.6%) samples. Besides influenza virus (20, 6.55%) and SARS-CoV-2 (8, 2.62%), RSV was detected in 45 samples (14.75%), rhino/enteroviruses in 20 (6.56%), adenovirus in 13 (4.26%), bocavirus in 4 (1.31%) and parainfluenza virus (PIV)-3 in 6 samples (1.96%). Eleven co-infections were observed. In spring 2023 adenovirus 2 was replaced by adenovirus B3, while in December 2023 RSV-B was replaced by RSV-A. The RSV-A and RSV-B sequences clustered within the ON1 and BA genotypes, respectively.
CONCLUSIONS: Besides SARS-CoV-2 and influenza viruses, the most frequently detected virus was RSV. Syndromic testing combined by NGS and phylogenetic analysis is the best approach to gain a good insight into the epidemiology of respiratory infections
SOURCES OF FUNDING: The study was funded by Horizon 2020 VEO (874735).
Keywords: respiratory viruses, syndromic testing, Nanopore, Greece

PP-108
Respiratory viruses

Validation of facial tissues as an alternative to nasopharyngeal swabs to diagnose viral respiratory infection

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AIM: Used facial tissues (UFT) are an alternative method to collect nasopharyngeal secretion and sputum from patients with respiratory tract infection. Self-sampling without exposure of a third party and mail shipping are few assets of this approach. We aimed to assess the feasibility of this method to diagnose viral respiratory infection after shipment of UFT to a remote laboratory.
METHODS: Nasal discharge with or without sputum was collected through UFT from sixty patients with a viral respiratory infection (VRI) documented by a positive syndromic point-of-care (POC) performed on a naso-pharyngeal swab in the emergency room (ER). UFT were mailed out to a remote virology lab and analyzed 23 days [8-87] later using the same POC and a syndromic confirmatory PCR assay.
RESULTS: Overall, the ER diagnosis was confirmed for 80% of the analyzed UFT with detection of SARS-CoV-2 (43%), rhinovirus (27%), RSV (7%) but also influenza, PIV, AdV and CoV. Absence of cellular DNA on 8 (13%) UFT reflects inappropriate blowing and was considered as a non-conformity. For the 27 samples positive on both ER and remote POC, Ct values obtained for each virus were not different (median=34 [21-38]). Failure to detect virus on UFT was not linked to the delay before analysis. Adding sputum to UFT did not improve the rate of detection.
CONCLUSIONS: Despite a slight lower sensitivity compared to swabs, UFT confirm their potential to collect and ship samples for VRI diagnosis. Comparable Ct-values obtained after 23 days of storage demonstrate the stability of viral genomes on inert dry matrices.
SOURCES OF FUNDING: None
Keywords: Inert matrices, sensitivity, stability, nucleic acid, diagnosis

PP-109
Viruses, tumors and immunocompromised hosts

Is it time to change the guidelines for preventing HSV infections in liver transplantation?
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AIM: Guidelines to prevent cytomegalovirus (CMV) infection after liver transplantation (LT) are well defined, but those to prevent herpes simplex virus (HSV) infection are less clear. Some, based on older literature, suggest introduction of HSV prophylaxis in HSV-seropositive recipients (R) who do not benefit from CMV prophylaxis. But none for HSV-seronegative recipients, while HSV infection can be transmitted by HSV-seropositive donors (D). As the number of viruses screened in donors is increasing and HSV seroprevalence is decreasing, HSV serological testing should be considered in donors.
METHODS: Based on our experience with LT in 2023, we estimated how many donors would be tested if we focused on HSV-seronegative recipients without CMV prophylaxis (CMV R+ or D-/R-).
RESULTS: Among 122 patients who underwent LT, 77.0% (94/122) were HSV-seropositive, 19.7% (24/122) were seronegative and 3.3% (4/122) had uncertain immunity. Of the 28 patients at risk of donor-transmitted HSV infection, 11 were CMV D+/R- receiving valganciclovir prophylaxis effective against HSV. Almost 14% (17/122) of donors could have been screened for HSV. One patient was diagnosed with a donor-transmitted HSV infection, fortunately with a positive outcome.
CONCLUSIONS: Fatal outcome after HSV infection is rare but could be avoided with appropriate care. Knowing HSV donor status of a HSV-seronegative recipient without CMV prophylaxis would be of added value in guiding the management of HSV D+/R- patients after LT with preemptive follow-up or HSV prophylaxis. This would represent less then 20% of the donors at our centre. Recommendations for HSV prevention should also be reviewed for other types of transplantation.
SOURCES OF FUNDING: None
Keywords: Guidelines, liver transplantation, Herpes simplex virus, prevention

PP-110
Viral infections in pregnancy

A cross-sectional survey of Congenital Cytomegalovirus Infection Awareness among Medical Faculty Students

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AIM: Cytomegalovirus (CMV) is the most frequent cause of congenital infection causing severe morbidity in children. The aim of this study was to raise awareness about CMV among medical students, to understand that congenital CMV (cCMV) infection is preventable with accurate knowledge about transmission route and clinic, and to benefit future practices in educating students about existing treatments and strategies.
METHODS: We performed a questionnaire-based cross-sectional study. Medical students from all grades of a medical faculty in Antalya, Turkey were enrolled for the survey. A printed, 11-question survey was prepared to investigate awareness and knowledge about cCMV infection.
RESULTS: Of the 1075 respondents, 43,3% considered themselves never heard of cCMV infection before. More than half of the participants did not know some basic features of cCMV infection including populations at risk, transmission routes, what conditions cCMV infection causes, drug treatment of cCMV infection in newborns, and existence of a licensed vaccine. The grade of the medical school especially third and fourth grades had a positive impact on the level of knowledge.
CONCLUSIONS: Our study indicates an inconsistency and insufficiency at the level of knowledge and awareness among medical students. The data will contribute to the processes of determining different educational strategies to be used in different educational years in the curriculum within the scope of program evaluation. Educating medical students about preventive strategies for populations at risk like the care of pregnant women should be the main effort in medical education.
SOURCES OF FUNDING: No fund
Keywords: Congenital CMV Infection, knowledge, awareness, medical students, program evaluation

PP-111
Viral infections in pregnancy

Viral load considerations in relation to sample type and age of a cytomegalovirus-infected group of patients

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AIM: The study aimed to analyze cytomegalovirus (CMV) infection based on the age of the tested persons, sample type, and viral load.
METHODS: Between May 10, 2023, and April 8, 2024, 493 samples—297 urine samples and 196 plasma samples—collected from 415 participants were analyzed for CMV infection using automated quantitative polymerase chain reaction (qPCR) equipment.
RESULTS: From 16.9% (70/415) of the participants, blood and urine samples were taken simultaneously. 5.3% (22/415) of the patients tested positive for CMV DNA in both samples, and the 1-2-year-old age group was mostly affected (14.0%, 6/43). The highest percentage of urine (+)/blood (-) CMV DNA results (16.3%, 7/43) was seen in the same age group of patients. The blood (+)/urine (-) result was observed in 0.7% (3/415) of tested persons, with the highest proportion in the age group of 3–11 years (3.8%, 2/52). The population over the age of 18 showed the largest percentage of blood (+) results, while young children aged 1-2 years accounted for the majority of single urine (+) results (44.2%, 19/42). The highest average viral load was noted in the group of infants (22 days–12 months) with 120112.5 IU/ml of CMV DNA in urine and 729.8 IU/ml in blood samples. A suspected congenital CMV infection was noticed in 2 newborns (aged 0–21 days) with urine (+) samples and an average viral load of 8417 IU/ml.
CONCLUSIONS: The prevalence of CMV infection and the monitoring of viral load suggest the need for a CMV vaccine to prevent the virus from causing illness.
SOURCES OF FUNDING: None.
Keywords: CMV, viral load, urine, blood, qPCR

PP-112
Viral infections in pregnancy

Increased incidence of Parvovirus B19 in Barcelona: comparative analysis from 2019 to 2024

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AIM: Parvovirus B19 (PVB19) typically cause an exanthematous disease in children, but in pregnant women can cause fetal damage. After COVID-19 pandemic, outbreaks have been reported in few countries (France, The Netherlands, Israel). The objective is to analyse PVB19 incidence in Barcelona from 2019 to 2024 among January-April period.
METHODS: A retrospective study was conducted at Microbiology Department of Vall d'Hebron University Hospital, which receive samples from inpatients and primary healthcare centers in Barcelona. All positive and borderline results of PVB19 IgM determination (LIAISON® Parvovirus B19 IgM, Diasorin, Italy) were included to determine the incidence of this infection. Age and sex distribution of positive IgM was assesed.
RESULTS: In 2019, positivity rate of PVB19-IgM was 6.9% (21/304). A decrease was observed between 2020-2023: 3.1% (9/287), 5.1% (17/331), 1.1% (5/471) and 3.4% (14/409), respectively. In 2024, this percentage increased to 13.2% (62/471). Within the study period, 65.6% of episodes occurred in women. Median age was 36.5 years old (IQR 12.3-45.1), but the groups with the highest incidence were between 5-15 (18.0%) and 35-45 (27.3%). No differences in age and sex were observed by year.
CONCLUSIONS: An increased incidence of PVB19 infection has been detected in Barcelona during the first months of 2024. A limitation of the study was counting borderline results as positive and false positives due to cross-reactivity, potentially overestimating new infections. The most affected population were women and children. Therefore, it would be advisable to implement screening protocols and protective measures during the outbreak, especially for high-risk groups like pregnant women.
SOURCES OF FUNDING: No funding
Keywords: Parvovirus B19, outbreak, serology, exanthematous, pediatric, pregnancy

PP-113
Viromics and virus discovery

Impact of micro-organisms RNA extraction from respiratory samples on microbiome analysis using Nanopore NGS technology

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AIM: The respiratory microbiome and its involvement in respiratory diseases have been increasingly studied since its discovery in the 2010's. The low biomass in respiratory samples makes it difficult to study by next generation sequencing (NGS). Here, we assess two types of micro-organism RNA extraction from nasopharyngeal swabs (NPS) and bronchoalveolar lavages (BAL) for optimizing metatranscriptomic analysis based on long-read sequencing.
METHODS: Two kits, Quick-DNA/RNA Miniprep Plus (Zymo-Research) and NucleoSpin Virus (Macherey-Nagel) were tested on 200µL or 400µL of 4 NSP and 4 BAL pools. Metatranscriptomic NGS librairies were prepared and sequenced on a MinION sequencer (Oxford Nanopore Technologies). Taxonomy classification was performed using a combination of Kraken2 and Bracken softwares.
RESULTS: RNA extraction with the Zymo-Research kit gave significant reads count results compared to the Macherey-Nagel kit (p=3.085e-07) but did not showed better N50 mean reads length (p=0.4468). Using a larger volume of sample did not permit to improve the reads count or N50 reads length obtained for each sample, neither with the Zymo-Research kit (p=0.1287 and p=0.9011 respectively) or the Macherey-Nagel kit (p=0.0726 and p=0.5125 respectively). Taxonomy classification showed similar profiles regarding RNA extraction protocol and sample volume. Virus detection was not more efficient using the NucleoSpin Virus kit.
CONCLUSIONS: These results show that an RNA extraction protocol including bead beating (Zymo-Reasearch kit) provides a large amount of bacterial information, without compromising its quality. Furthermore, this protocol doesn't appear to have a deleterious effect on the viral information amount, making it compatible with microbiome analyses, including virome specific studies.
SOURCES OF FUNDING: U1311 INSERM DYNAMICURE
Keywords: Respiratory microbiome, RNA extraction, Metatranscriptomics, Taxonomic classification, Next-Generation Sequencing, Nanopore

PP-114
Viromics and virus discovery

Rapid development of drug-resistant associated mutations in herpes simplex viruses type 1 after single passage in the presence of Acyclovir

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AIM: The development of Acyclovir (ACV) resistant HSV-1 strains, subverting the drugs' therapeutic effect, poses a major problem in immunocompromised patients. We aim to understand how phenotypic drug resistance develops on a HSV genome level and how to predict drug resistances on the basis of whole genome data.
METHODS: We compared replication kinetics of i) a bacterial artificial chromosomes (BAC) derived viruses, ii) HSV-1 patient isolates, and iii) plaque purified clinical isolates in the presence of up to 62 µM ACV and measured ACV sensitivity before and after ACV treatment. Moreover, we developed a whole genome sequencing (WGS) workflow to correlate mutations to phenotypic data.
RESULTS: ACV-sensitive and ACV-resistant HSV 1 isolates and BAC-derived viruses replicated in the presence of increasing ACV concentrations to normal titers showed a significant time delay. Also in BAC-derived viruses, WGS revealed multiple immediately emerging mutations in the thymidine kinase (UL23). Unexpectedly, we also observed additional mutations in various other genes. The clonal origin of BAC-derived viruses demonstrates the fast development of de novo mutations as a cause of ACV resistance apart from positive selection of minority variants conferring ACV resistance potentially preexisting in patient's isolates.
CONCLUSIONS: Our data indicates that ACV-sensitive HSV-1 viruses rapidly adapt to drug selection pressure in cell culture by developing de novo mutations in ACV resistance-associated genes like UL23 and potentially other genes. Currently, we are evaluating the contribution of defined UL23 mutations to ACV treatment failure as well as potential compensatory mutations outside of known ACV resistance loci by reverse genetics.
SOURCES OF FUNDING: No funding.
Keywords: Resistance testing, Next-generation sequencing, Resistance mutations, HSV-1, Acyclovir

PP-115
Viromics and virus discovery

Evaluation of measles whole genome sequencing protocols and metagenomic pipelines using Nanopore and Illumina sequencing

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AIM: Here we describe the evaluation of three different protocols for measles whole genome sequencing: ARTIC tiled-amplicons, overlapping long amplicons, and unbiased metagenome sequencing.
METHODS: The ARTIC tiled-amplicon based approach consisted of two pools of 26 primer pairs each developed by the ARTIC Network and distributed through Github that produced 52 overlapping amplicons of 400 bp length covering the whole measles genome. In addition, we evaluated PCR's producing six overlapping 2-4 kb long amplicons based on a publication by Schulz et al., J Virol Methods 299 (2022). We also developed an unbiased metagenomic approach using the Transplex Complete Whole Transcriptome Amplification kit from Sigma-Aldrich. All three approaches were tested on the most common measles genotypes B3 and D8 with libraries sequenced on MinION and Illumina. Metagenomic analysis of reads was done with Epi2Me/Kraken2 and CZ-ID. Finally, we applied a new metagenomics protocol specifically designed for Oxford Nanopore Flongle sequencing based on a publication by Pichler et al., J Virol Methods 320 (2023).
RESULTS: We were able to generate measles whole genome sequences with good coverage with all three approaches on both sequencing platforms. These protocols were applied to two clinical measles cases with unknown epi-link. We were able to show that both measles genomes were identical indicating that these cases seemed to be epi-linked.
CONCLUSIONS: The evaluated WGS protocols are suitable for measles whole genome sequencing and can assist to determine chains of transmission.
SOURCES OF FUNDING: This work was supported by institutional funds from Canterbury Health Laboratories.
Keywords: Measles virus, whole genome sequencing, metagenome sequencing

PP-116
Viromics and virus discovery

Discovering viruses in diverse settings: a public health perspective
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AIM: Viruses, including those with potential public health implications, are all around us. Yet, only a select few undergo specific targeting in the clinical diagnosis of patient samples or routine surveillance of environmental samples. Our goals include identifying potential undiagnosed viral causes in patients with unknown etiology, comparing viral populations in treated and untreated wastewater samples, and characterizing viruses found in bats in Israel.
METHODS: Metagenomics approaches play a pivotal role in the identification of viruses in diverse settings. At the national virology laboratory, we design and implement metagenomic sequencing along with bioinformatic methods for various clinical and environmental samples.
RESULTS: Application of our metagenomic approaches was successful in identifying viruses in a myriad of clinical, animal and environmental samples.
CONCLUSIONS: By comprehensively examining genetic material from such diverse sources, metagenomics contributes significantly to aiding patients, understanding of viral diversity and the potential emergence of infectious diseases, thereby supporting public health initiatives and strategies.
SOURCES OF FUNDING: None.
Keywords: virome, metagenomics, sequencing, public health, viruses

PP-117
Viruses, tumors and immunocompromised hosts

A case report: Cytomegalovirus(CMV) meningoencephalitis in a patient receiving immunosuppressive therapy for treatment of malignant glioma

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AIM: In our study, we aimed to emphasize the risk factors that may develop in the treatment of CNS malignancies by examining CMV meningoencephalitis in a patient who underwent routine treatment for malignant glioma.
METHODS: CSF and blood samples were analized with multiplex meningoencephalitis panel and singleplex quantitative PCR tests, respectively. Patient's clinical course was evaluated with the clinicians who followed the patient.
RESULTS: A 60-year-old woman, who was diagnosed with glioblastoma a year ago and had relapsed despite repeated surgeries and subsequent radiochemotherapy, presented with complaints of worsening consciousness, wound dehiscence and discharge. The last treatment protocol including surgery, radiotherapy and temozolomide was completed shortly before the hospitalization. She has been also utilizing long term corticosteroid treatment. Neurological examination revealed GCS11(E3,V3,M5),meningeal irritation findings positive. CMV DNA positivity was detected in the meningoencephalitis panel. Serologic results were anti-CMV IgG(+)/IgM(-).CSF and plasma quantitative CMV PCR results were 58,900 and 66,410 copies/mL, respectively. Ganciclovir treatment was started and then changed to valganciclovir due to side effects. During treatment, plasma CMV-DNA levels were 3569/449/negative at one-week intervals, respectively. Unfortunately, the patient died due to a newly developed Acinetobacter spp.infection.
CONCLUSIONS: Due to the nature of the treatment protocol (radiotherapy, temozolamide, long-term corticosteroid therapy) patients with glioma are at risk of CMV infection.The main conclusions to be drawn from this case are the need to increase disease awareness in at-risk groups, include CMV diagnosis and follow-up in the standard of care for patients with CNS cancer, and focus on preventive antiviral regimens.
SOURCES OF FUNDING: None
Keywords: Cytomegalovirus, Central Nervous System(CNS) infection, İmmunocompromised host

PP-118
Viruses, tumors and immunocompromised hosts

Cytomegalovirus infection and emergence of resistance during letermovir salvage therapy in a pediatric patient with severe combined immunodeficiency

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AIM: The terminase inhibitor letermovir is approved for cytomegalovirus (CMV) prophylaxis and can be used off-label as a therapy for resistant or refractory CMV. We describe the emergence of drug resistance mutations in a pediatric patient with severe combined immunodeficiency (SCID) receiving letermovir salvage therapy.
METHODS: We prospectively analyzed blood and cerebrospinal fluid (CSF) specimens during a 140-day time-period for drug resistance mutations in the CMV genes UL54 (DNA polymerase), UL97 (protein kinase), and UL51, UL56, UL89 (terminase subunits). Retrospectively, targeted next-generation sequencing of the UL54 and UL56 genes and direct viral whole-genome sequencing (WGS) with probe-based target enrichment was performed on blood specimens.
RESULTS: The patient was initially treated with ganciclovir and foscarnet. Prospective resistance analysis identified a compartmentalized CMV infection, with wild-type virus in CSF and drug-resistant viruses in blood [UL54 mutations V715M (foscarnet-resistance), V781I and A809V (ganciclovir/foscarnet-resistance)], after which treatment was shifted to cidofovir and letermovir. CMV became undetectable in CSF and the UL56 C325F letermovir-resistance mutation emerged rapidly in the backbone of the V781I virus, resulting in an increased viral load. A stable genotype conveying resistance to ganciclovir, foscarnet, and letermovir was established (UL54-V781I and UL56-C325F). CMV replication remained uncontrolled and the patient ultimately died. Analysis of highly variable genes through WGS indicated a highly stable CMV genotype throughout the infection.
CONCLUSIONS: Multidrug resistance was rapidly established in a neonatal patient affected by SCID. Therapeutic letermovir use for resistant/refractory CMV infections appears to be limited, particularly with high viral loads, due to its low barrier to resistance.
SOURCES OF FUNDING: Sciensano.
Keywords: Human cytomegalovirus, drug resistance, next-generation sequencing, severe combined immunodeficiency

PP-119
Viruses, tumors and immunocompromised hosts

Predictive value of EBV viremia for lymphoma diagnostic in kidney transplant patients?

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AIM: Epstein-Barr virus (EBV) is an oncogenic virus that remains latent in B-lymphocytes after primary infection. During the latency phase, EBV viremia is frequently detected, and its predictive value for a tumor process, especially post-transplant lymphoproliferative disorder (PTLD), is not well known. Interpretation of such viral loads remains tricky.
Our objectives were to evaluate the predictive value of EBV viremia for lymphoma diagnostic in kidney transplant patients.
METHODS: Between 2015 and 2022, hospital records of adult patients of the nephrology ward with viral loads ≥ 5 log10 copies/ml (Artus, Qiagen), were reviewed. In parallel, Uniform Hospital Discharge Data Set (UHDDS) was queried to identify kidney transplant patients with a lymphoma diagnostic during the same period in our hospital; then their EBV viral loads were analysed.
RESULTS: Among 1768 nephrology patients who had an EBV PCR, 87 kidney transplant recipients had a high viral load, among which we identified 8 patients (9%) with an EBV-associated lymphoproliferation. On the other hand, the UHDDS query identified 9 kidney transplant patients with a discharge diagnosis of EBV-associated lymphoproliferation. When compiling these data, the total number was 12 adult patients with both a kidney transplant and a PTLD (5 patients were identified by both methods). Half of them had a high EBV viral load at diagnosis. The diagnosis was made based on a clinical symptom for all of them.
CONCLUSIONS: EBV viral load is a low-sensitive and low-specific marker for PTLD in adult kidney transplant recipients. Improvements can be made to regulate its prescription.
SOURCES OF FUNDING: None
Keywords: Epstein-Barr virus, kidney transplant, post-transplant lymphoproliferative disorder

PP-120
Viruses, tumors and immunocompromised hosts

Occurrence and frequency of Human Herpesviruses (HHV-1 to 6) in broncheoalveolar lavage fluid (BALF) from various kind of patients

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AIM: Occurrence of human herpesviruses in BALF is associated with clinical pictures like e.g., viral pneumonia[1]. A cohort of 210 BALF samples from patients with various diseases were tested retrospectively for human herpesviruses (HHV-1 to -6) to evaluate the frequency of alpha-, beta- and gamma- herpesviruses. 48 patients out of this BALF sample collection had a known *Pneumocystis jirovecii* pneumonia (PJP).
METHODS: BALF samples were collected and stored at -20°C. HSV-1, HSV-2, CMV, varicella zoster virus (VZV), Epstein–Barr virus (EBV), and human herpesvirus (HHV) 6 were detected using commercial methods (AltoStar® Herpesvirus assays, altona Diagnostics, Germany).
RESULTS: Out of 210 BALF samples 72 samples (34.3%) were tested negative for CMV, EBV, HSV1/2, HHV6 and VZV, whereas 138 samples (65.7%) were tested positive for a least one herpesvirus. Out of 138 positive tested samples 69.6% were infected by two or more herpesvirus types. The highest prevalence in this cohort of samples showed EBV (49%). The infections with CMV (29.1%) and HSV-1 (28.1%) equaled. No sample was tested positive for HSV-2. 58 BALF samples out of 210 (27.6%) were tested positive for HHV6, but in 84.5% in combinations with EBV and/or CMV and/or HSV-1. Only 2 samples showed a positive PCR signal for VZV.
CONCLUSIONS: In this study, mainly EBV, CMV, HSV-1 and HHV-6 from the human herpesvirus family were detected. HHV-6 shows very low quantitative results. The high percentage (69.6%) of co-infection with more than one herpesvirus type in this cohort is significant.
SOURCES OF FUNDING: Funded by altona Diagnostics GmbH
Keywords: BAL, herpesvirus, pneumonia, PJP

PP-121
Viruses, tumors and immunocompromised hosts

Workflow and result comparison of alpha herpesvirus detection in cerebrospinal fluid (CSF) samples
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AIM: Alpha herpesvirus central nervous system infections caused by herpes simplex virus (HSV) types 1 and 2 (HSV-1, HSV-2) and varicella zoster virus (VZV) have similar clinical and laboratory manifestations. Next to the qualitative analysis quantitative results for treatment follow up especially in immunocompromised patients might be necessary. Automated workflows and results of alpha herpesvirus DNA detection in cerebrospinal fluid (CSF) samples were evaluated using commercial alpha Herpesvirus single and multiplex PCR kits.
METHODS: Frozen CSF samples from 163 patients were retrospectively analysed for the presence of HSV-1, HSV-2 and/or VZV-specific DNA by commercial PCR assays. Additionally, 25 negative CSF samples were spiked with either HSV-1 or HSV-2 positive swab material from individual patients to evaluate correlation of quantification on at least 25 results each.
Nucleic acids were extracted from each sample and PCR set-up for AltoStar® assays was automatically done for all samples on the AltoStar® Automation System AM16 in one run set-up. In parallel, the same samples were extracted with the NUCLISENS® easyMAG® and PCR set-up was done manually for all R-gene® assays.
RESULTS: Diagnostic sensitivity and diagnostic specificity between the respective assays were for all 3 alpha herpesviruses greater than 95%. Quantification correlated highly: The r-values were ≥ 95% for all analyses.
CONCLUSIONS: The AltoStar® Automation System AM16 allows parallel PCR set-up from the same sample for multiple AltoStar® assays and thus simplifies the daily routine.
SOURCES OF FUNDING: Funded by altona Diagnostics GmbH
Keywords: CSF, herpesvirus, automation

PP-122
Viruses, tumors and immunocompromised hosts

Frequency of CMV and of co-infection of CMV and P. jirovecii in bronchoalveolar lavage fluid (BALF) samples

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AIM: In immunocompromised patients Cytomegalovirus (CMV) is often detected from respiratory samples like bronchoalveolar lavage fluid (BALF) with other pathogenic agents, particularly *Pneumocystis jirovecii*[1]. CMV has immunomodulatory effects and inhibits the immune response generated against *P. jirovecii*, contributing to the delayed clearance of the *P. jirovecii* from patients[2]. BALF samples were tested for CMV and *P. jirovecii* by commercial real-time PCR assays (altona Diagnostics) to evaluate the frequency of CMV in BALF and the frequency of co-infection of CMV in *P. jirovecii* positive BALF samples.
METHODS: 188 BALF samples were collected from patients with various diseases and from patients with a known *P. jirovecii* pneumonia (PJP). Samples were retrospectively tested for *P. jirovecii* and for CMV by PCR.
RESULTS: 51 out of 188 BALF samples were tested positive for CMV. Viral load varied from low positive (not quantifiable) up to 1.00E+06 IU/ml. 63% of the CMV positive samples had a quantitative result higher 1.00E+03 IU/ml. 62 samples were tested positive for *P. jiroveccii*, and 26 (42%) out of them were tested positive for CMV. 101 BALF samples were tested negative for both pathogens.
CONCLUSIONS: 27% of the BALF samples were tested positive for CMV, 63% of them with a viral load ≥1.00E+03 IU/ml. 42% of positive tested *P. jirovecii* samples showed a co-infection with CMV. Since CMV remains latent after primary infection and can potentially reactivate due to any inflammatory response, CMV detection in BAL specimen may not indicate acute CMV pulmonary disease.
SOURCES OF FUNDING: No funding.
Keywords: Immunocompromised; CMV; {Pneumocystis jirovecii}; BAL

PP-123
(Re-) emerging viral infections and one health

Monitoring of the circulation Hepatitis A virus in France: tools and challenges
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AIM: Hepatitis A virus (HAV) is a ubiquitous virus, transmitted in an oro-faecal manner. Probability and severity of symptoms increase with age (50% of adult cases are hospitalised in France). In France, the circulation of HAV is low and monitored to prevent the occurrence of an epidemic. It is compulsory to declare each case to the authorities (ARS); positive isolates may be sent to the reference laboratory (CNR) for sequencing; and for inpatients, diagnosis data are available (PMSI). Our objectives were to compare the data obtained from different sources, to assess their completeness and the need for other tools.
METHODS: We collected data from 2016 to 2023 from the ARS, the number of positive isolates sent to the CNR and the number of HAV-infected inpatients obtained with the PMSI.
RESULTS: Data of all origin show an increase in the number of cases in 2017 (in connection with a global emergence among men who have sex with men reported that year) followed by a decrease in 2020 (in connection with the cessation of travels during CoViD-19). Comparison of data between different sources show a lower number in CNR compared to other sources, and a number from PMSI above half of the ARS number.
CONCLUSIONS: Hepatitis A virus cases are probably under-declared in France and most strains are not sent to the CNR, which impairs monitoring. Studies were also conducted in wastewater samples and healthy blood donors, and show differences in the circulation of HAV compared to what is evidenced with our monitoring.
SOURCES OF FUNDING: None
Keywords: Hepatitis A virus, monitoring, reference laboratory,

PP-124
(Re-) emerging viral infections and one health

Performance evaluation of a multiplex RT-PCR assay for detection and differentiation of Dengue, Zika and Chikungunya viral RNA in serum samples

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AIM: Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV) are closely related mosquito-borne viruses with potential to cause large-scale outbreaks. Comprehensive and timely testing strategies to detect and differentiate these viruses can limit disease spread and enable swift public health responses. We assessed the performance of multiplex PCR TaqMan™ Arbovirus Triplex Kit (ZIKV/DENV/CHIKV) (TaqMan™ Arbovirus kit) for ZIKV/DENV/CHIKV detection.
METHODS: In total 370 serum samples comprising 99 DENV-, 99 CHIKV-, 62 ZIKV-positive, and 110 negative samples for the three viruses were tested in parallel with TaqMan™ Arbovirus kit and RealStar® Chikungunya RT-PCR Kit 2.0, RealStar® Dengue RT-PCR Kit 3.0, and RealStar® Zika Virus RT-PCR Kit 1.0. Positive (PPA) and negative percent agreement (NPA) between the tests were calculated and limit of detection (LoD) of the tests compared using viral isolates.
RESULTS: TaqMan™ Arbovirus Kit showed comparable performance to RealStar® singleplex kits on serum samples. For ZIKV detection, PPA and NPA were 100%. For DENV and CHIKV, PPA were 100% while NPA were 94% and 95%, respectively. Genomic sequencing confirmed viral presence in 6 DENV and 7 CHIKV samples positive only on TaqMan™ Arbovirus Kit, resulting in a sensitivity and specificity of 100%. TaqMan™ kit demonstrated higher analytical sensitivity (LoD 40 copies/ml lower) than the comparator assays for all isolates, except ZIKV where LoD was comparable.
CONCLUSIONS: The TaqMan™ Arbovirus Kit has excellent sensitivity and specificity for detecting DENV, CHIKV and ZIKV in a single reaction. Worldwide arbovirus outbreak spikes necessitate rapid and reliable testing solutions to support timely outbreak investigations.
SOURCES OF FUNDING: ThermoFisher Scientific; Ministry of Health-Kenya
Keywords: Dengue, Chikungunya, Zika, arbovirus, PCR, multiplex

PP-125
(Re-) emerging viral infections and one health

One Health paradigm for Surveillance of Climate Sensitive Vector Borne Diseases in Yadadri Bhuvanagiri District Telangana, India

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AIM: to conduct comprehensive surveillance of Climate Sensitive Vector Borne Diseases (CSVBD) in Yadadri Bhuvanagiri District, Telangana, India
METHODS: Considering the above we intend to conduct surveillance of Climate Sensitive Vector Borne Diseases (CSVBD) in Yadadri Bhuvanagiri District of Telangana to begin with
Study Design: Prospective Community-Based - Operational Model
Implementation Plan:
1. To conduct sero-surveillance of Climate Sensitive Vector Borne Diseases (CSVBD) in Yadadri Bhuvanagiri District of Telangana
2. To study the distribution of various vectors in rodents, animals and in the environment in the region
3. To assess the changes in vector population in based on climate change indicators
4. To perform Metagenomic Next Generation Sequencing to study the pathogens identified in the vectors and the environment
RESULTS: Expected RESULTS:
1. Identification of hotspots and risk mapping of CSVB diseases
2. Improved understanding of the infection and transmission dynamics and ecology of CSVB diseases
3. Formulation of disease prevention and control strategies for CSVB diseases
4. Impact of climate change parameters on CSVB diseases will be known
CONCLUSIONS: The district selected for the study is relatively-moderately vulnerable to climate change. Data related to Climate Sensitive Vector Borne Diseases if collected appropriately, analyzed thoroughly, and complemented by sequencing. Prediction of outbreaks/epidemics, control and prevention of such events in the future could be possible. Developing community resilience against Climate Sensitive Vector Borne Diseases will no longer remain a slogan. It will take form into action.
SOURCES OF FUNDING: National center for disease control
Keywords: CSVBD,NCDC,One health

PP-126
(Re-) emerging viral infections and one health

Magnitude and Determinants of Zoonotic Enteric Disease in Under Five Children of Rural Telangana

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AIM: To study the prevalence of zoonotic enteric virus carriage among children less than five years with diarrhoea and their association with domestic animal exposure.
METHODS: We selected four Mandal with high animal density in Yadadri-Bhongir district. From each Mandal, one village was chosen. A door-to-door survey identified 122 households with domestic animals and children under 5 who had diarrheal illness in the past 3 months. An urban control group of 122 households without domestic animals was also included, meeting the same criteria. All the samples were tested using multiplex PCR primers (Altona diagnostics) and processed using real time PCR
RESULTS: A total of 244 (Rural 122, Urban 122) samples from under 5 children were tested for Zoonotic enteric viruses namely Rota virus, Adeno virus, Astrovirus, Noro virus and Sapovirus. Of these Adeno virus,& Astrovirus were predominant. (n=46, n=29)
A total of 448 rectal swabs were collected from the domestic animals of the same households. Rotavirus was tested positive in 7 animals (cows) and Adenovirus in 11 animals (5 cows, 6 sheep). We have detected same viruses (Rota virus - 5,Adeno virus - 8 from the both the children and animal.
CONCLUSIONS: This pioneering study on asymptomatic carriage of zoonotic enteric viruses in rural and urban children found Rotavirus and Sapovirus exclusively in rural children, while Adenovirus was isolated solely in urban children. Follow-up is crucial to monitor potential health impacts like diarrhea, growth or mental retardation.
SOURCES OF FUNDING: National center for disease control (NCDC)
Keywords: Zoonotic disease, Rota Virus, Adeno Virus, Astro Virus, Noro Virus, Sapo Virus

PP-127
(Re-) emerging viral infections and one health

Measles whole genome phylogenetic analysis in the ongoing outbreak in Romania
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AIM: Romania is facing a measles outbreak with a number of 18146 during the last twelve months. Most cases were in unvaccinated children. This study characterized epidemic viral strains by performing phylogenetic analysis on whole genome sequences.
METHODS: 168 patients diagnosed with measles in 2024 at the National Institute for Infectious Diseases, Bucharest were included. Nasopharyngeal swabs, clinical, epidemiological data were collected from all patients. Real-time PCR was performed and samples with ct values below 30 were further processed for whole genome sequencing as previously described. The reads were mapped on a reference measles genome by using Geneious software. Phylogenetic analysis was performed with ML FastTree. Mutations in HA were evaluated by comparing the Romanian sequences with vaccine and subtype specific reference sequences.
RESULTS: Whole genome sequencing was successfully performed for a number of 125 samples. Subtype analysis assigned most sequences to D8 subtype; one sequence was B3. Phylogenetic analysis identified a large local transmission network (n=108). Another small transmission cluster was identified (n=14) with sequences from patients with no apparent epidemiological links. At the root of this cluster are sequences from Western-Europe. The analysis of HA sequences revealed D8 specific mutations detected in the large local cluster; the smaller cluster had a different mutation profile that affect also epitopes regions.
CONCLUSIONS: Subtype D8 is the main circulating subtype. Several HA mutations, some in major epitopes, were observed; some were subtype specific, others unique to the smaller local transmission cluster. Molecular surveillance may help monitor vaccine efficacy and the viral evolution.
SOURCES OF FUNDING:
Keywords: Measles, outbreak, HA mutations, WGS, phylogenetic analysis

PP-128
(Re-) emerging viral infections and one health

A rational approach for the selection of samples for testing for rat hepatitis E virus (Rocahepevirus) in a large virus diagnostics laboratory

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AIM: Rocahepeviruses (RocaHEV) are zoonotic viruses linked to human hepatitis. Prevalence data is missing and most studies are based on incidental findings or do not have well-defined inclusion criteria in cases of negative results. We aim towards specifying criteria for archived samples most likely to test RocaHEV positive.
METHODS: We assed potential factors associated with RocaHEV-RNA detection, such as liver disease, exposure to rats, Paslahepevirus-antibody results, and immune disorder.
RESULTS: Given the absence of data on liver disease for archived samples, we recommend requests for Paslahepevirus-PCR testing as a surrogate criterion for zoonotic hepatitis. As most RocaHEV-cases did not report contact with rats, we excluded this criterion. Furthermore, we did not consider positive Paslahepevirus-antibody testing for sample selection due to uncertainties regarding cross-reactivity between Roca- and Paslahepevirus antigens, with Paslahepevirus antigens used in diagnostic tests. While RocaHEV has been detected in both immunocompetent and immunocompromised individuals, we focus on the latter group, which may be more prone to chronic infection and thus more likely to yield positive RocaHEV results. We screened all 2,520 samples submitted for Paslahepevirus-PCR to our laboratory from 2022 and 2023. We excluded follow-up and Paslahepevirus-RNA positive samples. Following our inclusion criteria, we selected samples from hematology/oncology, nephrology/rheumatology, and transplant surgery (n = 857). These subsets will undergo RocaHEV-RNA testing using specific RT-PCR.
CONCLUSIONS: Inclusion criteria in retrospective-studies on zoonotic viruses are key, particularly if negative results should be beneficial to guide decision-making. Our approach for selecting samples requires minimal contextual information and thus might be of use across diagnostic labs.
SOURCES OF FUNDING:
Keywords: Zoonoses, Hepeviridae, Hepatitis

PP-129
(Re-) emerging viral infections and one health

FlaviSeq: a combined RT-PCR and Nanopore sequencing approach for the rapid detection and determination of human flavivirus infections

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AIM: Flaviviruses are a large group of RNA viruses that can infect humans, causing a wide spectrum of clinical presentations varying from asymptomatic infections to severe haemorrhagic fever and death. Here, we present a novel approach for the broad-range detection followed by rapid determination of flaviviruses in clinical specimens using Nanopore sequencing.
METHODS: We optimized a published flavivirus RT-PCR (1), enabling flavivirus determination using a conserved 260-basepair NS5 region. To acquire full-length transcripts, the Nanopore cDNA kit was modified to shorten the library prep time, allowing the FlaviSeq approach to be completed within a single workday. We used cultured viruses to evaluate the technical sensitivity and specificity. For clinical validation, we assessed 208 clinical specimens obtained from returning travellers, that were previously tested by dengue and/or Zika virus specific RT-PCR.
RESULTS: The FlaviSeq technical sensitivity on cultured strains was less than 0.01 TCID50 of flavivirus per RT-PCR reaction. Sequence analysis showed accurate determination, including the dengue virus geno-/serotype. Using clinical specimens, we demonstrate comparable sensitivity to both the dengue virus and zika virus specific RT-PCR using a single assay.
CONCLUSIONS: We present a novel approach allowing for rapid and broad-range detection and determination of flaviviruses. The FlaviSeq approach is highly useful and cost-effective for testing (a)symptomatic returning travellers, avoiding parallel testing using multiple RT-PCR's.
SOURCES OF FUNDING: This research received no specific grant from any funding agency.
1: Patel P, Landt O, Kaiser M et al. Development of one-step quantitative reverse transcription PCR for the rapid detection of flaviviruses. Virol J. 2013 Feb 14;10:58.
Keywords: flavivirus, diagnostics, Nanopore, sequencing

PP-130
(Re-) emerging viral infections and one health

Establishment of a hepatitis E virus genotype 3 (HEV-3)-specific target enrichment panel resolves complete genomes but still requires additional sequencing of the hypervariable region (HVR) to enable correct determination

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AIM: HEV is the leading cause of acute viral hepatitis globally. In Europe, >99% of the cases are caused by HEV-3, which is mostly identified with the unified HEVnet protocol. This is suitable for subtyping, but much information is lost as only 493 bp of the 7.2 kb genome are sequenced. Our aim was to establish an HEV-3-specific target enrichment panel.
METHODS: Probes were designed based on >1 kb *Hepeviridae* sequences. The panel was condensed by excluding probes due to repeats, redundancy or affinity to human DNA. The final panel consisted of 2,997 HEV-3-specific probes. Library preparation was performed according to the Illumina RNA Prep enrichment tagmentation protocol and sequenced on a MiniSeq. Reads were mapped to specific reference sequences.
RESULTS: A median whole genome coverage of more than 10k reads per site was achieved. However, we saw drops in the 4.6–5.8 kb genome region. By including an HEV strain harbouring an insertion in the HVR, we found that reference-based mapping and de novo assembly would not detect the insertion. This prompted us to design HEV-3 specific primers to amplify the HVR for conventional sequencing revealing a novel recombination of an HEV-3 strain downstream of the HVR.
CONCLUSIONS: HEV whole genome sequencing has been challenging, as HEV RNA concentrations in diagnostic samples are too low for direct RNA sequencing. By applying the HEV-3-specific enrichment panel, we generated almost completely covered HEV whole genomes. A second version of the panel has already been designed to target coverage drops in the 5 kb region.
SOURCES OF FUNDING: None
Keywords: hepatitis E virus (HEV), Paslahepevirus balayani, target enrichment, next generation sequencing (NGS), hypervariable region (HVR), recombination

PP-131
(Re-) emerging viral infections and one health

Optimization of a whole genome amplicon sequencing protocol for Clade I Monkeypox Virus

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AIM: In 2022, the world witnessed an outbreak of mpox, an infectious disease presenting with skin lesions caused by the Monkeypox virus (MPXV, of Clade II). Currently, mpox cases are rising sharply in the Democratic Republic of the Congo (DRC) (WHO[1]), caused by the more pathogenic MPXV of Clade I. Viral whole genome sequencing (WGS) has become indispensable to understand outbreak epidemiology. Hybrid-capture probe-based virome sequencing (Twist®) generated evidence of sustained human-human (sexual) transmission of MPXV Clade I in the Kamituga mining region, in Eastern DRC (Vakaniaki[2]). For upscaling MPXV Clade I genomic surveillance, we aim to generate a less-complex and -costly WGS protocol by adjusting a tiled amplicon MPXV sequencing protocol established during the Clade IIb worldwide mpox outbreak (Welkers[3]).
METHODS: The original tiling PCR primers failing to anneal with publicly available MPXV clade I genomes (using PrimerMap) were redesigned (with Primer-BLAST) and spiked into the original tiling PCR primer pools before sequencing of the PCR amplicons using Oxford Nanopore® technology.
RESULTS: Using an in-house developed pipeline (Debaetselier[4]) to analyze the sequencing data, the Clade I-optimized tiling protocol improved the horizontal clade I MPXV genome coverage from 95% to 98% (approximating the 98,5% obtained for clade IIb) in DRC mpox patient samples collected in 2021-2022.
CONCLUSIONS: Final verification of the Clade I-optimized protocol using patient samples collected during the ongoing Kamituga outbreak illustrates a functioning Clade I MPXV sequencing protocol based on tiled amplicon PCR enrichment, a technique compatible with decentralized genomic surveillance (Kinganda-Lusamaki[5]).
SOURCES OF FUNDING: Belgium_Directorate-General for Development Cooperation
(1)https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON493
(2)https://www.medrxiv.org/content/10.1101/2024.04.12.24305195v1
(3)https://www.protocols.io/view/monkeypox-virus-whole-genome-sequencing-using-comb-ccc7sszn
(4)https://www.nature.com/articles/s41591-022-02004-w
(5)https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8549801/pdf/nihms-1728333.pdf
Keywords: monkeypox virus, mpox, emerging infection, genomic surveillance

PP-132
(Re-) emerging viral infections and one health

Torque Teno Virus (TTV) and Cytomegalovirus (CMV) Viral Load Dynamics: Which Emerges First During Immunosuppression?

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AIM: To date, the precise relationship between TTV and CMV viral loads has been only marginally addressed. The aim is to verify whether a correlation exists between the two viruses and clarify whether the increase in TTV viral load precedes or follows the reactivation of CMV.
METHODS: With the above aim, TTV viremia was precisely examined in a large cohort of transplanted immunocompromised patients (192 hematological and 60 solid organ transplant recipients) being monitored for CMV reactivation.
RESULTS: TTV load was measured in 2,612 plasma samples from 448 patients. The results revealed a significant increase in TTV viral load approximately 14 days following CMV reactivation in SOT patients. No recognizable difference in TTV load was noted among hematological patients during the entire timeframe analyzed. Furthermore, a temporal gap of approximately 30 days was noted between the viral load peaks reached by the two viruses, with TTV preceding CMV.
CONCLUSIONS: It was not possible to establish a correlation between CMV reactivation and TTV viremia in the hematological patients. On the other hand, the SOT patient cohort allowed us to analyze viral kinetics and draw intriguing conclusions. Taken together, the data suggest, to our knowledge for the first time, that CMV infection itself could potentially cause an increase in TTV load in the peripheral blood of patients undergoing immunosuppressive therapy.
SOURCES OF FUNDING: Italian Ministry of University and Research: PRIN 2022 [grant number 2022ZRNTHW] to Guido Antonelli and EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (project no. PE00000007, INF-ACT).
Keywords: Cytomegalovirus, Torquetenovirus, Immunosuppression, SOT, Biomarker.

PP-133
(Re-) emerging viral infections and one health

Emergence of B1c Genotype of Coxsackievirus A16 in Heilongjiang Province of China in 2022
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AIM: Coxsackievirus A16 (CVA16) is one of the important agent for hand, foot, and mouth disease (HFMD) which caused severe burden to children. Genotype B1b is the dominant cluster in the mainland of China during the continuous the national HFMD pathogen surveillance since 2008. To the best of our knowledge, only 2 B1c CVA16 were reported in the mainland China.
METHODS: According to the national HFMD surveillance system, there were 178 HFMD cases in Jixi city of Heilongjiang province in 2022. Real-time RT-PCR method was used to directly detect the enterovirus in clinical samples. There were 95 laboratory-confirmed patient with laboratory evidence of enteroviruses(EVs) infection. The positive samples were inoculated into human rhabdomyosarcoma cells and human laryngeal epidermoid carcinoma cells. We randomly selected 65 isolates for sequencing and phylogenetic and evolutionary analysis.
RESULTS: In 2022, there was an outbreak of B1c genotype CVA16 in Jixi city of Heilongjiang province for first time, and 15 B1c CVA16 were isolated. The VP1 sequences of these B1c CVA16 strains isolated from this study showed high similarity, with 99.7–100% identity. The 2022 Chinese strains were closely related to the 2018 Indian strains. Markov jumps and rewards also showed that Chinese CVA16(B1c) might be imported from India. Recombination analysis found intraspecies recombination in the non-structural regions with EV-As.
CONCLUSIONS: This study provides a further insight into the B1c CVA16 outbreak and transmission and illustrates the importance of valuable surveillance in the future.
SOURCES OF FUNDING: National Key Research and Development Program of China(2022YFC2305302)
Keywords: Coxsackievirus A16(CVA16), B1c genotype, Phylogenetic analysis, Evolutionary analysis

PP-134
(Re-) emerging viral infections and one health

Tick-Borne Encephalitis infection among patients with neurological acute and sub-acute disorders living in Saint-Etienne area in France between 2021 to 2023

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AIM: To determine the prevalence of TBEV infection among patients with neurological acute and sub-acute disorders living in Saint-Etienne and its area, Auvergne-Rhône-Alpes, France.
METHODS: This retrospective study includes patients aged over 2 years old presenting neurological acute and sub-acute disorders from 2021 January 1st to 2023 December 31st, living in Saint-Etienne and its area and for whom residual CSF specimen was stored at -80°C. When available, and if CSF specimen were either positive or in not enough quantity to be analyzed, serum specimens were tested. Clinical specimens were analysed for TBEV antibodies using ELISA Virion/Serion IgG and IgM kits (Eurobio, France). The local ethic committee approved this study.
RESULTS: During this 3-year period, 544 patients were included. They were 7 cases (1.3 %) of confirmed TBEV infections including one child; 4 other possible cases with positive IgM CSF specimens and 12 patients presenting with IgG only, are still under investigation with seroneutralization due to potential cross-reactions. All the confirmed cases lived in rural areas, 5 presented with meningoencephalitis, one with meningoencephalomyelitis and one with meningitis. One patient had not been previously diagnosed for TBE. None died, and 3 were briefly hospitalised in Intensive Care Unit.
CONCLUSIONS: In Saint-Etienne area, TBEV infection should be systematically evocated in case of neurological evocative symptoms, even in children. Vaccination may be discussed for people living in specific rural areas at risk.
SOURCES OF FUNDING: Pfizer financially supported the purchase of ELISA kits without interfering in the design of the study nor the data interpretation.
Keywords: Encephalitis, arbovirus, climate change, virus emergence, serology, seroneutralisation

PP-135
Advancements in diagnostics & clinical cases

EBV DNA detection in plasma of COVID 19 patients

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AIM: The potential role of Epstein-Barr virus (EBV) and its reactivation in the context of SARS-CoV-2 infection severity and long or post COVID syndrome is evident in the literature. The aim of our study was to detect EBV reactivation in patients with acute/ongoing COVID-19.
METHODS: Our study included 71 cases with inpatient care and positive SARS-CoV-2 N-antigen in blood. Control group of 29 patients had negative plasma N-antigen test. Alinity m System (Abbott) was used to quantitate EBV DNA in patient and control plasma specimens. The SARS-CoV-2 N-antigen detection (Biohit Healthcare Hefei) in peripheral blood was used to determine the ongoing COVID-19 disease.
RESULTS: We have detected EBV DNA in 34 out of 71 patients with ongoing COVID-19 disease (48%). In patients with negative N-antigen test, EBV DNA was detected in six out of 29 (20%). EBV DNA concentration detected in both groups ranged between 20-700 IU/ml. Patients of 71 years and older had the EBV reactivation occurring more often than younger patients. We did not observe any difference among patients infected with the delta and omicron variants of the SARS-CoV-2.
CONCLUSIONS: EBV DNA was detected significantly more often (OR; p=0.0147) in patients with acute SARS-CoV-2 infection, in comparison with control group. These findings suggest EBV reactivation may occur soon after or with COVID-19 infection and COVID-19 inflammation-induced EBV reactivation may contribute to long COVID symptoms.
SOURCES OF FUNDING: Supported by MH CZ - DRO (FNBr, 65269705).
Keywords: EBV, Real-time PCR, N-antigen, COVID-19

PP-136
Advancements in diagnostics & clinical cases

Evaluating RT-LAMP Versus RT-PCR and WGS for Co-Detection of SARS-CoV-2 and Influenza in Respiratory Specimens

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AIM: The gold standard for Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) detection is Reverse Transcription Polymerase Chain Reaction (RT-PCR). With the rapid global spread of COVID-19, alternative diagnostic methods like antigen tests and Loop-Mediated Isothermal Amplification (LAMP) assays have emerged. This study evaluates a new RT-LAMP assay's sensitivity and specificity, comparing it against RT-PCR and whole-genome sequencing (WGS).
METHODS: We conducted the study with168 nasopharyngeal/oropharyngeal swabs tested using RT-LAMP. These samples were also subjected to RT-PCR, with WGS performed on 76 samples having Cycle threshold (Ct) values ≤ 25.
RESULTS: The LAMP assay demonstrated a sensitivity of 0.80 and a specificity of 1.00 for SARS-CoV-2 compared to RT-PCR. Its accuracy peaked at 100% for samples with RT-PCR Ct-values ≤ 17, but detection of false negatives increased for Ct values > 35. Additionally, the assay reliably detected all SARS-CoV-2 lineages present in the WGS-analyzed samples. Despite lower accuracy than RT-PCR, the new RT-LAMP assay demonstrated good sensitivity and effectively identified various SARS-CoV-2 lineages.
CONCLUSIONS: Faster turnaround, ease of use, and portability make this RT-LAMP assay a potential rapid "sample to result" solution. Finally, with noteworthy caution of its limitations in its sensitivity and accuracy, it could be a useful diagnostic technique as a near point-of-care testing (POCT) for the rapid detection of SARS-CoV-2 and influenza.
SOURCES OF FUNDING: None.
Keywords: RT-LAMP, COVID-19, Influenza; whole-genome sequencing (WGS), point-of-care testing (POCT)

PP-137
Advancements in diagnostics & clinical cases

Human Papillomavirus Genotypes in Self-Sampling Women in Portugal
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AIM: Early detection and treatment of cervical precancerous lesions can reduce the burden of cervical cancer. In Portugal, some population does not have a general practitioner or access to the screening programmed; self-sampling strategies to detect Human Papillomavirus infection have been adopted to reduce the number of cancer cases.
METHODS: Women who go to the health centers were invited to participate in a self-sampling screening for HPV. Self-samples were collected by swab by the women themselves, and HPV genotyping was performed by the Allplex™HPV28 Detection (Seegene Inc., Korea).
RESULTS: Between May 2022 and December 2023, 1626 (mean age 42.9) women were screened. HPV was detected in 40.3% (655/1626) of the women. Of those, 75.7% (496/655) corresponded to the high-risk (HR) genotypes, with HPV 52 (8.1%) the most frequent, followed by HPV 31 (9.0%). HPV 16 and 18 were detected in only 7.5% and 5.2% of the cases, respectively. Low-risk genotypes were detected in 49.9% (327/655) of the positive women, with HPV 54 being the most common (15.9%) followed by HPV 42 (12.1%). Possible HR-HPVs were detected in 39.5% of the positive cases, with HPV 53 (13.6%) being the most frequent. 46.9% (307/655), had mixed HPV infections.
CONCLUSIONS: In our self-sampling population, the frequency of HR-HPV is high. Without this sampling strategy, these women will be excluded from cervical cancer screening. Our results reinforce the importance of self-sampling in achieving the goal of eliminating cervical screening and show that healthcare policies should take this type of sampling into consideration.
SOURCES OF FUNDING: Not application
Keywords: Human papillomavirus, Self-sampling, HPV Genotypes, Cervical cancer

PP-138
Advancements in diagnostics & clinical cases

Multimodal Analysis of ADEM-Like Presentation as first clinic episode of Highly Active Multiple Sclerosis Following COVID-19 Vaccination

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AIM: One month after COVID-19 single-dose RNA-vaccine, a 19-years-old female presented lower limb paresthesia, quickly evolving into tetraplegia. She had OCB on CSF, cervical myelitis and brain MRI suggestive of ADEM. She reached EDSS-9.5, but remission was achieved post-natalizumab treatment (EDSS-1.5).
METHODS: We performed NGS analysis and assessed plasma biomarkers at various stages of clinical progression using SIMOA, Luminex, and ELISA assays.
RESULTS: Neurodegeneration biomarkers NfL, GFAP, Tau and UCHL1 were remarkably increased during the attacks (EDSS-9.5), treated with Methylprednisolone and plasmapheresis. Natalizumab onset was followed by neurological and biomarkers improvement. Conversely, peripheral inflammatory biomarkers CRP, CD-14, TLR4, IL-1β and IL-17A remained increased even after remission. Neuroprotective cytokine TGFβ levels were low. It was identified black-holes on MRI suggesting previous subclinical disease. Additionaly, it was found genetic risk factors sorted by TNF(rs1800629), SOD2(rs4880) and FCGR2A(rs1801274) variants.
CONCLUSIONS: This case highlights the importance of precision medicine utilizing multimodal analysis to guide therapeutic management decisions. Previously, this patient may have been diagnosed and treated as having post-vaccinal recurrent ADEM. However, the new MS criteria incorporating OCB biomarkers enabled early diagnosis and treatment. Monitoring NfL, GFAP, Tau, and UCHL1 levels also influenced therapeutic decisions, leading to the use of natalizumab, a biological-barrier-drug inhibitor of alfa-4-integrin that presents a rapid action mechanism. The role of vaccination remains uncertain, perhaps a combination of genetic susceptibility and the disruption of immunologic tolerance triggered by vaccination explains this case.
SOURCES OF FUNDING: Fundação Carlos Chagas de Amparo a Pesquisa do Estado do Rio de Janeiro Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Keywords: multiple sclerosis; neuroinflammation; mRNA vaccine; ADEM; genetic susceptibility; biomarkers.

PP-139
Advancements in diagnostics & clinical cases

Simplified Respiratory Screening: Self-Collected Nasal and Saliva Samples with Flocked Swab
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AIM: The recent COVID-19 pandemic has shown that the use of alternative samples has facilitated access to screening campaigns and improved sample management, especially when laboratories are centralized. The study aims to evaluate whether self-collected nasal and saliva swabs, transported dry, can be used for screening respiratory infections, thus facilitating sample transportation and handling.
METHODS: Between January and February 2022, 72 samples (33 nasal swabs and 39 saliva swabs) were self-collected by 25 employees who underwent company screening for possible SARS-CoV-2 infection. For nasal collection, participants were asked to rotate the swab (FLOQSwabs 552C) 4 times in both nostrils and then return the swab into the tube. For saliva collection, the swab was inserted into the oral cavity for 10 seconds to collect saliva. The swabs were stored dry at -80°C for two years and subsequently eluted in UTM before analysis with Xpress SARS-CoV-2. Nasopharyngeal swab was collected as reference sample.
RESULTS: 94 samples were tested without cartridges error. Of these, 11 individuals were positive in both nasopharyngeal, nasal and saliva swabs while 7 were tested positive for SARS-CoV-2 only in nasopharyngeal and nasal sample. The remaining 7 employees were negative and there were not sample tested positive only in the saliva specimens.
CONCLUSIONS: The study demonstrates the effectiveness of FLOQSwabs in preserving nucleic acids for extended periods when the sample is self-collected from either the nostril or the oral cavity. This tool could simplify the collection procedure during screening campaigns and in those contexts where a nearby laboratory is not available.
SOURCES OF FUNDING: Copan Italia
Keywords: Respiratory viruses, Self-collection, Nasal swab, Saliva swab, Long time stability

PP-140
Advancements in diagnostics & clinical cases

An unusual presentation of Seoul virus infection triggering hemophagocytic lymphohistiocytosis (HLH)
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AIM: To illustrate the heterogeneity of clinical manifestations attributed to hantavirus infections. A 17 year-old male was admitted with 2-days vomiting, fever and malaise. Clinical examination revealed right upper quadrant pain and splenomegaly, while elevated CRP, 5-fold increase in transaminases and mild thrombocytopenia (Plts 110,000/μl) were detected. An upper abdominal ultrasound showed the presence of pericholecystic fluid. Empiric treatment with ceftriaxone and metronidazole was initiated. Serologic tests for hepatrotropic viruses were negative. His condition deteriorated and a CT scan revealed the presence of pleural effusion and ascetic fluid. A decline in platelet count (Plts 60,000/μl) and kidney function (creatinine 1.3mg/dl) and elevated ferritin levels (9,480ng/ml) were detected. A diagnosis of HLH based on HScore was assumed. Pulse corticosteroid therapy combined with IVIG was initiated with subsequent patient's improvement. A detailed medical history revealed repeated contact with rodents and hantavirus infection was suspected.
METHODS: ELISA and IFA were applied to detect IgM and IgG antibodies in patient's serum, while RT-nested PCRs were applied to amplify fragments of the S and M RNA segments of hantaviruses. De novo next generation sequencing was also applied and isolation of the virus in cell culture was attempted.
RESULTS: Hantavirus- specific IgM antibodies were detected by both ELISA and IFA, with borderline IgG antibodies. Sequencing of the PCR products confirmed a Seoul hantavirus infection.
CONCLUSIONS: The information of patient's contact with rodents led to the laboratory testing and confirmation of hantavirus infection. HLH is only rarely associated with Seoul virus infection.
SOURCES OF FUNDING: The study was partially supported by EU4H-2021-PJ4 DURABLE (101102733).
Keywords: Hantavirus, Seoul virus, hemophagocytic lymphohistiocytosis

PP-141
Advancements in diagnostics & clinical cases

Detection of immune response to Rubella antigen using an anti-Rubella IgM ELISA and a newly designed anti-Rubella IgG ELISA

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AIM: Rubella infections during early pregnancy may cause congenital rubella syndrome or foetal death. Vaccination can prevent the disease, but rubella outbreaks still occur in countries with limited access to vaccines. This study analyses the performance of the Anti-Rubella Virus Glycoprotein IgM ELISA and a newly designed recombinant-antigen-based Anti-Rubella IgG ELISA in comparison with established ELISAs.
METHODS: Quality assessment samples (IgM, n=49; IgG, n=188) and consecutive samples (n=100) from 15 vaccine recipients grouped in the periods 0-20 days (T1), 21-30 days (T2), 31-40 days (T3), and 41-70 days (T4) were analysed for presence of anti-Rubella IgM and IgG as well as for IgG avidity.
RESULTS: Using the quality assessment samples, sensitivity and specificity of the anti-Rubella IgM and IgG ELISAs were 100%. In all vaccine recipients, anti-Rubella IgM and IgG were present: Using the anti-Rubella IgM and the established ELISA, IgM was detected in 18.8% and 12.5% (T1), 93.8% and 75.0% (T2), 100% and 86.2% (T3), and 100% and 58.3% (T4) of samples, respectively. Using the novel anti-Rubella IgG and the established ELISA, IgG was detected in 12.5% and 30.8% (T1), 78.8% and 93.3% (T2), and both 100% (T3, T4) of samples, respectively. In one individual, high-avidity IgG indicated previous rubella antigen exposure.
CONCLUSIONS: The anti-Rubella IgM and the novel anti-Rubella IgG ELISA revealed high sensitivity and specificity. Samples from naïve vaccinees showed early and long-lasting IgM. IgG seroconversion was comparable to established assays. Thus, the combination of assays provides an excellent diagnostic tool for the detection of an anti-Rubella immune response.
SOURCES OF FUNDING: no funding
Keywords: Rubella infections, diagnostics, ELISA, anti-Rubella IgM and IgG

PP-142
Advancements in diagnostics & clinical cases

X-ray irradiation of SARS-CoV-2 Omicron BA.4 and comparison of the sensitivity of live and irradiated virus in a lateral flow test assay format

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AIM: The 2020 COVID-19 pandemic demonstrated the need for inactivated SARS-CoV-2 reference material, suitable for use in diagnostic assay development conducted in Containment Level 2 laboratories. The National Collection of Pathogenic Viruses (NCPV) contributed to the development of X-ray irradiated SARS-CoV-2 Omicron BA.4 for distribution clinical laboratories internationally. This study describes the irradiation process for SARS-CoV-2 Omicron BA.4 and the efficacy on Omicron BA.4 and other variants in a Lateral Flow Device (LFD) assay format.
METHODS: SARS-CoV-2 Omicron BA.4 was grown in Vero/hSLAM cells (ECACC 04091501). The virus was titred and sterility tested. 60 ml of virus was subject to 9.7 KGy of X-ray irradiation at 4 °C. To confirm virus inactivation, 10% of the volume of the irradiated virus was serial passaged three times in Vero E6 cells and tested for evidence of viral growth. The LFD assays were carried out according to the manufacturer's instructions.
RESULTS: Live and irradiated Omicron BA.4 were tested in triplicate using the Orient Gene's Rapid Covid-19 (Antigen) Self-Test Lateral Flow Devices assay. Both were detected down to 2 x 101 TCID50/ml. The intensity of the bands was comparable between live and irradiated. Previously irradiated SARS-CoV-2 variants, Wuhan-Hu-1, Alpha and Omicron BA.2 showed equivalent sensitivity.
CONCLUSIONS: X-ray irradiated viruses can be transported and handled at a lower hazard category showing their value in diagnostic assay development and outbreak preparedness. NCPV plans to expand the range of irradiated viruses available to include other high consequence viruses.
SOURCES OF FUNDING: UKHSA, Culture Collections, NCPV.
Keywords: X-ray irradiation, inactivation, diagnostics, SARS-CoV-2 Omicron BA.4, virus

PP-143
Advancements in diagnostics & clinical cases

Detection of antiviral antibodies in urine – noninvasive alternative for serodiagnostics

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AIM: Billions of cases of infectious diseases occur annually. Appropriate management of the severe ones calls for correct diagnosis. In many viral and some other microbial infections this is achieved via measurement of pathogen-specific antibodies (Abs). The current blood sampling is invasive, inconvenient for the patient and requires a medical professional.
METHODS: We have developed a revolutionary non-invasive uro-immunodiagnostic procedure for measurement of Abs from a urine sample – at first against Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Parvovirus B19 (B19V) and SARS-CoV-2.
RESULTS: The antiviral Ab concentrations in urine varied between individuals and between serial samples of an individual, and compared with serum were 10^4-10^5-fold lower, rendering urine as such barely suitable for Ab detection. However, with optimized sample preparation and detection technologies these issues were largely overcome. Among constitutionally healthy individuals (n=35) with long-term immunity, the clinical sensitivities of our uro-Ab-assays were for EBV 100 %, for CMV 96 %, for B19V 96 %, and for SARS-CoV-2 100 %. The respective specificities were 86, 92, 100 and 100 %, compared with serum-derived Ab data. Overall, uro-sensitivity was 97 % and uro-specificity 97 %. Uro-Abs of vaccinees neutralized SARS-CoV-2 and uro-Ab avidity allowed primary infection diagnosis, despite lack of urinary IgM.
CONCLUSIONS: Antiviral Abs occurred in urine ubiquitously, and every individual studied had Abs against at least one virus, suggesting that Abs in urine like those in serum remain detectable for years or decades after infection. These findings hold considerable potential for revolutionizing most if not all Ab-based diagnostics in medicine.
SOURCES OF FUNDING: Presented at ESCV2024
Keywords: antibody, diagnostics, urine, herpesvirus, parvovirus B19, SARS-CoV-2

PP-144
Advancements in diagnostics & clinical cases

Automating SARS-CoV-2 CPE Detection with Deep Learning to Simplify Post-Isolation Processes

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AIM: Cell culture studies are time-consuming and require additional confirmation through various tests. The aim was to detect CPE caused by SARS-CoV-2 using two newly trained artificial intelligence (AI) models immediately upon their occurrence.
METHODS: SARS-CoV-2-Ank1 local isolate, identified by GenBank accession number MT478018, was cultured in VERO cells within the department's ABSL3+. The viral titer was determined using the TCID50 assay. The cells were infected with different multiplicities of infection (MOI), ranging from 0.5 to 10^{-4}. CPE images were captured at magnifications of 5X, 10X, 20X, 40X, and 60X over a period of three days post-infection until all the cells had detached. This dataset was then used to train deep learning models, MobileNetV2 and ResNet18.
RESULTS: SARS-CoV-2-Ank1 was propagated successfully in VERO cell lines and subsequently TCID50 was determined as $5x10^{7}$/ml. CPE was detected at all MOIs. Models were trained and optimized through data enrichment and parameter tuning to accurately detect CPEs and healthy cells. All MOIs CPE was detected after 1 day with AI although the CPE of the local strain usually observed at the third day when the infection day accepted as one.
CONCLUSIONS: Two models gave successful results and demonstrated usability for cell culture studies and SARS-CoV-2 CPE detection. AI detection also reduced the risk of human error and eliminated the need for post-isolation tests. Additionally, we gained an extra day before CPE becomes visibly detectable. Based on these findings, we plan to increase our success rate in future studies by using different models.
SOURCES OF FUNDING: None.
Keywords: Cell Culture, CPE, Deep Learning, Diagnostics, SARS-CoV-2.

PP-145
Advancements in diagnostics & clinical cases

The biomolecular markers for the personalized prediction of the treatment effect in patients with head and neck cancer: pilot study

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AIM: The clinical utility of liquid biopsies as diagnostic, predictive and prognostic biomarkers has been shown for several cancers. Our study focuses on analyzing the dynamics of HPV cell-free DNA levels during the surveillance of patients with oropharyngeal tumors to improve the management of patients. We present preliminary data on the evaluation of plasma collection methods, and their efficiency in subsequent analyses of the dynamics of HPV cfDNA level.
METHODS: Plasma was collected the day before treatment and in intervals during post-treatment follow up. Two methods of plasma collection were used. Isolation of ctDNA from plasma was performed, the quality of extracted ctDNA was evaluated, and HPV cfDNA was detected by ddPCR and NGS.
RESULTS: Fifty-two paired samples from 20 patients were analyzed. The paired profiles on the chip electrophoresis were comparable, with a greater influence of the individual patient´s condition rather than the collection method or time since sampling. We observed a decreasing level of HPV cfDNA after surgery or from the initiation of treatment in long-term follow-up patients.
CONCLUSIONS: In summary, we have shown that both methods of plasma collection are usable for HPV cfDNA analysis and that the level of HPV cfDNA decreases in time from the beginning of treatment to the zero level.
SOURCES OF FUNDING: The research was funded by the Project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU, and by the Ministry of Health of the Czech Republic - DRO (Thomayer University Hospital - TUH, 00064190).
Keywords: cell-free DNA, head and neck cancer, plasma

PP-146
Development of new antiviral strategies

RSV Treatment Beyond Direct Antivirals: Exploring the Host Cells for Potential Targets for Therapeutic Intervention

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AIM: Respiratory Syncytial Virus (RSV) is a leading cause of death in infants under the age of one year, the elderly, and immunocompromised patients. Despite recent vaccine approvals, challenges remain in achieving widespread public acceptance and providing effective protection for immune-compromised patients. Existing antiviral drugs, targeting viral proteins drawback from the potential development of resistance by mutagenesis. An alternative is to compromise proteins in the host cell which are essential for the virus but not for the host. Our approach is to identify host factors, and particularly transcription factors, that have been hijacked by the virus to reprogram gene expression induced by RSV.
METHODS: We used multiple previously published gene expression and host-factor screening data. Followed by gene set enrichment analyses, we select the most prominent pathways to employ gene regulatory network models to identify regulators being responsible for the changed gene expression.
RESULTS: We found immune response and morphogenesis/development to be enriched in upregulated genes due to infection, while cell cycle and metabolism being enriched in downregulated genes. Applying gene regulatory models to genes coding for cytokines led to the prediction of MAFF, IRF3, POU5F1 and AP-2 as regulators of RSV induced changes.
CONCLUSIONS: While experimental validation is needed, these findings may lead to a better understanding of reprogrammed infected host cells by the virus paving the way for new therapeutic options.
SOURCES OF FUNDING: The work was funded by the BMBF (13N15711, 13N15745).
Keywords: Respiratory Syncytial Virus, host factor, knockdown screen, host-directed antiviral, gene regulation

PP-147
Development of new antiviral strategies

The effectiveness of Marecipe AV therapeutics, an herbal compound prescription, for life-threatening viral infections in animals

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AIM: To assess the effectiveness of Marecipe AV therapeutics, an herbal compound prescription, in treating viral infections.
METHODS: The oral administration of Marecipe AV was utilized to treat viral infections with high morbidity and mortality in real-world. The efficacy was measured by comparing mortality between treated and untreated animals.
RESULTS: The mortality was 28.57% versus 100% among treated and untreated pigs with African swine fever, and the mortality was 0% versus 100% for treated and untreated cases when treatment was administered in the early presymptomatic stage.
The mortality was 39.04% versus 100% in treated and untreated ducks with Avian influenza, and 0.86% versus 99.83% for ducks when treatment is given in the early presymptomatic stage. For chickens, the mortality was 4.60% versus 85% for treated and untreated, respectively. All surviving ducks and chickens ultimately achieved complete recovery.
For dogs with canine distemper and/or canine parvovirus, the mortality was 10.11% and 82.88% among the treated and untreated. For dogs infected with parvovirus only, the mortality was 0% for treated and 87.5% for untreated. Among dogs simultaneously infected with parvovirus and canine distemper, the mortality was 15.25% and 96.84% for treated and untreated, respectively. All surviving dogs fully recovered.
CONCLUSIONS: The findings revealed a significant disparity in mortality between treated and untreated. It suggests that Marecipe AV therapeutics can save lives from deadly viral infections and cure these infections. MarecipeAV is a potentially effective therapeutic option against acute viral infections.
SOURCES OF FUNDING: Marecipharm Co.Ltd
Keywords: Viral infectious diseases, Herbal Therapy, African swine fever, Avian influenza, canine distemper, canine parvovirus

PP-148
Development of new antiviral strategies

Identification of a small-molecule Inhibitor of KSHV Lytic Replication
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AIM: To identify a small-molecule inhibitor of KSHV lytic replication
METHODS: A medium-throughput phenotypic ELISA screening assay based on anti-KSHV activity and cell viability was developed to screen 400 compounds in the Medicines for Malaria Ventures Pandemic Response Box. Western blot, qPCR and re-infection assays were used to determine viral protein, viral DNA replication, viral transcript, and viral infection levels.
RESULTS: A non-cytotoxic inhibitor of KSHV lytic replication (which is critical for tumourigenesis) was identified as a hit compound. The identified hit compound inhibited KSHV DNA replication process, reduced the expression of KSHV immediate-early and late lytic genes (including KSHV ORF57 an important mediator of viral RNA nuclear export) and inhibited the production of infectious KSHV particles.
CONCLUSIONS: Taken together, our data identify a novel compound with anti-KSHV activity that may be developed further into future therapeutic agents for KSHV-related malignancies.
SOURCES OF FUNDING: 1. Academy of Medical Sciences Newton Advanced Fellowship and Department of Science and Innovation and National Research Foundation (DSI/NRF) South African Research Chair (SARChI) grant (Grant number: 98566).
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Keywords: KSHV, ORF57, lytic replication, MMV, Pandemic Response Box,

PP-149
Development of new antiviral strategies

Membrane Traps: towards a general, drug-free approach to inactivate viruses by engulfment
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AIM: As viruses mutate and develop resistance to existing drugs, there is an increasingly urgent need for more effective antiviral treatments. We aim at developing a new approach to inactivate viruses that does not requires the use of drugs. Rather than targeting specific biochemical pathways, we are using the physical process of membrane engulfment to trap virions and prevent them from binding to their cellular receptors, thereby preventing infection.
METHODS: We built membrane Traps using synthetic lipids that mimic the physiological composition of human membranes. These Traps can be rendered highly deformable and able to engulf viruses in conditions that recapitulate the physiological environment.
RESULTS: As a proof-of-principle, we functionalized Traps with proteins able to interact with Lentiviruses, and we demonstrated successful capturing of virions. Testing of the efficacy of Traps to prevent infection in cell culture is ongoing.
CONCLUSIONS: Here, we propose that engulfment could be an effective strategy to inactivate viruses. Our approach does not require the use of drugs and it is expected to elicit minimal resistance. Toxicity is also expected to be low, given that membranes are highly biocompatible. Thus, we propose Traps as a novel strategy to inactivate viruses, which can be used in combination with existing drugs and vaccines. This approach could be used to target viruses that infect skin and mucosae, the respiratory tract and potentially even the systemic circulation.
SOURCES OF FUNDING: National Science Center (NCN), Poland
Keywords: Engulfment, virus, membrane, drug-free

PP-150
Development of new antiviral strategies

Identifying compounds to target host dependency factors of SARS-CoV-2
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AIM: Besides vaccines, broad spectrum host-directed antivirals (HDA) are promising therapeutic options for SARS-CoV-2. While CRISPR/Cas9 or RNA interference screens have been employed in numerous studies to identify relevant host factors (HDF), achieving consistent identification poses a challenge, making it difficult to establish reliable results in the identification of host factors.
METHODS: We screened over 27,000 small interfering RNAs (siRNAs) targeting 9,096 human genes in SARS-CoV-2 infected Caco-2 cells. Knocked down genes were not only selected based on their protective effect in infected cells but also by considering their impact on cytotoxicity. To increase the robustness of potential hits, we incorporated a prioritization pipeline considering the expression of the genes in infected cells and cellular network properties derived from generic and virus specific protein-protein interactions to prioritize proteins with high degree and centrality measures. The rationale behind this is that inhibiting central proteins by drugs may cause stronger effects on the virus' life cycle.
RESULTS: The top-ranking host factors are significantly enriched in transmembrane transport, energy metabolism, and the respirasome, suggesting that these processes are of particular importance for the virus. To further evaluate these results, we systematically searched several drug databases and selected the most suitable drugs targeting these processes.
CONCLUSIONS: While experimental validation is ongoing, these findings may pave the way for identifying SARS-CoV-2 HDAs opening new perspectives to improved treatment options.
SOURCES OF FUNDING: The work was supported by Research Grants of Merck KGaA, Darmstadt, the Willy Robert-Pitzer-Foundation, the BMBF 01KI2015OB, 01EO1502, 13 N15711, 13 N15745, 01KI2015OA and the EU (GA 101137311).
Keywords: SARS-CoV-2, host dependency factor, RNAi, knockdown screen, host-directed antiviral, compounds

PP-151
Development of new antiviral strategies

Cellular receptor tyrosine kinase signaling plays an important role in coronavirus infection
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AIM: Coronaviruses are a severe health burden by causing seasonal epidemics and occasional pandemics. The recent COVID-19 pandemic was caused by a new human coronavirus SARS-CoV-2, which has resulted in an estimated 761 million cases and 6.8 million deaths alone. New and effective therapeutics are urgently needed to treat the current and future coronaviral pathogens.
METHODS: Common antiviral therapeutics used to treat viral respiratory infections, including influenza and coronaviruses, typically target specific viral components such as viral proteins, which can lead to rapid emergence of drug resistant mutants. Targeting host signaling pathways and the associated cellular factors that are essential for viral replication and disease development can provide a novel approach toward development of next-generation antiviral drugs, which may overcome antiviral drug resistance.
RESULTS: Receptor tyrosine kinases (RTK) are proteins that activate and control a variety of cellular pathways that have been shown to play a role in respiratory viral replication such as influenza. Using chemical inhibitors we have shown that inhibition of RTK signaling blocks replication of SARS-CoV-2 in two different cell lines Vero and A549-ACE2. We have also shown that SARS-CoV-2 upregulated RTK signaling during viral replication.
CONCLUSIONS: These data support further efforts to characterize the role of host RTKs in regulating respiratory viral infection and disease pathogenesis that might aid the future effort toward the development of a new generation of drugs targeting host signaling pathways as effective antiviral therapeutics.
SOURCES OF FUNDING: NIH R21 AI094133
Keywords: Antiviral, SARS-CoV-2, Receptor Tyrosine Kinase

PP-152
Diagnostic stewardship & Diagnostic test accuracy

In Silico Analysis: Key to Accurate RNA Virus Detection
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AIM: The constant evolution of pathogens presents a significant challenge in RT-qPCR diagnostic procedures, potentially leading to mismatches between primers, probes, and target sequences, thereby reducing assay efficacy and increasing the risk of false negatives. Regular in silico analyses are essential for molecular diagnostic tests to remain effective and aligned with pathogen variations. A post-marketing surveillance plan has been established to track potential cases and includes extensive in silico analysis using the latest sequences from prevalent databases. In this instance, it underscores the significance of periodically reviewing Flu A virus.
METHODS: Influenza A sequences were from the GISAID database spanning from January 2018 to September 2023. Alignment of these sequences with primers and probes was conducted using in-house bioinformatic software. The resulting alignments underwent thorough evaluation, considering factors such as the quantity of mismatches, primer and probe positions within each sequence, and the size of amplification products, in accordance with fundamental PCR design principles.
RESULTS: Influenza A H3N2 subtype includes a new clade named 3C.2a1b.2a.2a.2a.3a.1. Since April 2023, this clade represents more than 50% of the sequences from GISAID database and continues growing to date. This revision confirmed a correct detection of this Influenza A H3N2 new clade with the VIASURE Flu Typing II Real Time PCR Detection Kit.
CONCLUSIONS: Due to the genetic and evolutionary diversity of Influenza A virus, it's crucial to continuously monitor molecular diagnostic products. This involves assessing the compatibility of primer and probe binding regions with evolving sequences, as changes could affect test accuracy.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: Influenza A RNA, in silico analisis, molecular diagnosis

PP-153
Diagnostic stewardship & Diagnostic test accuracy

Validating a Novel Molecular Assay for Precise Epstein-Barr Virus Load Quantification in EDTA-Plasma
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AIM: Epstein Barr virus (EBV) is a herpesvirus in which over 90% of the population worldwide has been infected. This virus infects B lymphocyte cells. Complications of EBV infection are rare in immune-competent individuals but are especially important in a range of hosts with compromised immunity in where EBV is capable of in vitro and in vivo transformation of host cells and associates with specific tumors. Hence, novel tools for swift and precise detection and quantification of the Epstein-Barr virus hold immense clinical value.
METHODS: Plasma-EDTA samples characterized as positive or negative and quantified in IU/ml were analyzed. For comparative analysis, the CE-marked molecular reference assay used was RealStar® EBV PCR Kit 1.0. VIASURE analysis was performed using the following workflow: the MagDEA® Dx SV automated extraction method with magLEAD® 12gC and CFX96™ Opus Real-Time PCR Detection System.
RESULTS: A total of 205 clinical EDTA-plasma samples were tested, of which 105 were positive for Epstein Barr virus and 100 were negative. The comparative analysis determined a sensitivity and specificity >99%. As for quantification, an R2 correlation value of 0.824 was obtained.
CONCLUSIONS: This clinical evaluation demonstrated that the VIASURE EBV q Real Time PCR Detection Kit is a good tool for the clinical diagnostics of the virus as it has good sensitivity and specificity. No false positive and false negative results were observed. It could be concluded that this VIASURE product meets the initially established requirements and is for the purpose and the population for which it is intended.
SOURCES OF FUNDING: Certest Biotec S.L.
Keywords: Epstein Barr virus, virus load, molecular diagnosis

PP-154
Diagnostic stewardship & Diagnostic test accuracy

Validation of cobas 6800 human papillomavirus test in comparison to the cobas HPV test on cobas 4800 system

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AIM: Many international guidelines, including Singapore's, recommend the use of clinically-validated molecular assays for high-risk human papillomavirus (HPV) DNA as the primary test for cervical cancer screening. As the Roche cobas 4800 system will be phased out in year 2028, we evaluated the performance of cobas HPV on the Roche cobas 6800 system in comparison with the cobas 4800 HPV test on the cobas 4800 system.
METHODS: A total of 291 routine cervical (214 negative and 77 positive) specimens, collected in PreservCyt Solution and 52 remnant proficiency test (PT) survey samples were used in this study. These samples were tested on both HPV assay systems following manufacturer's instructions. HPV results obtained were assessed using percentage of overall agreement of qualitative results and type (HPV16, HPV18 and others HPV) agreement. Complete and partial type concordance were evaluated by categorization. A panel of 18 viruses were also included to assess the analytical specificity.
RESULTS: Direct comparison of the cobas 4800 and cobas 6800 results in the same archival samples showed a very high concordance, with 96.9% (n=282/291) of samples having identical results on both platforms. HPV type distribution by hierarchical categorization of all samples were reported. All PT test samples showed 100% concordance to the intended outcome. This study also showed the absence of cross reactivity of the assay with other viruses.
CONCLUSIONS: Our data showed that the cobas 6800 HPV test demonstrated a comparable performance as the cobas 4800 HPV test. This system is suitable for use of large-scale cervical cancer screening.
SOURCES OF FUNDING: Not applicable
Keywords: HPV, cervical cancer screening

PP-155
Diagnostic stewardship & Diagnostic test accuracy

Analytical performance evaluation of Alinity-m quantitative PCR assays for HBV DNA, CMV DNA, HCV RNA, HIV RNA and correlation analysis with Cobas-6800

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AIM: It was aimed to evaluate the analytical performance of the Alinity-m (Abbott Molecular Diagnostics; USA) quantitative PCR assay, as the first experience in Turkey.
METHODS: For precision analysis, 3 positive 3 low positive samples for intra-assay and 1 positive, 1 low positive samples for inter-assay evaluation were tested thrice. For linearity analysis, quality control samples from previous panels (MOTAKK External Quality Control Program, Turkey) were tested in two separate sets at 1/10, 1/100 and 1/1000 dilutions. Additionally, the correlation between Cobas-6800 System (Roche Diagnostics, USA) and Alinity-m results were evaluated in 99 positive patient samples. All analyzes were performed on logarithmic values.
RESULTS: Intra-assay and inter-assay "coefficient of variation" (CV) for all four viruses were ≤5%. In low positive samples, intra-assay CVs for HBV, CMV and HCV were ≤5% and the inter-assay CV for HBV was 6%, for HCV was 9%, for CMV was 4%. For low positive HIV samples, the intra-assay CVs were between 0%-18% and the inter-assay CV was 21% (but all standard deviation values were below 0.32 Log10). In the linearity analysis, all R2 values were found to be ≥0.96. The Pearson correlation coefficient between the results of two systems was determined to be ≥0.9.
CONCLUSIONS: The linearity and precision analysis results of Alinity-m for HBV, HCV, HIV, and CMV were found to be successful. Although higher CV values were detected in low positives, variations in Log10 values were in acceptable values (maximum 0.32 Log10). A high level of agreement was detected with Cobas-6800 System.
SOURCES OF FUNDING: None
Keywords: Alinity-m quantitative PCR, Analytical performance evaluation

PP-156
Diagnostic stewardship & Diagnostic test accuracy

HSV 1 & 2 / VZV – a new qualitative multi-plex PCR test on the Alinity m platform
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AIM: HSV-1, HSV-2 and VZV often cause phenotypically indistinguishable skin blisters or lesions. The objective was to evaluate the Alinity m HSV-1&2/VZV assay, a qualitive multiplex real-time PCR for detection and differentiation of the three pathogens.
METHODS: Residual archived (-20°C) specimens (n=367; Aptima collection-tubes) pre-selected based on results obtained with the AllplexTM Genital ulcer assay (Seegene) targeting HSV-1/2, VZV and 4 additional sexually-transmitted organisms, were re-tested with Alinity m HSV-1&2/VZV assay and Allplex in parallel. A HSV-1&2/VZV/Syphilis-Positive-Swab EQA sample (Microbix-Biosystems Inc.) resuspended in 0.9% NaCl was tested in triplicates with both assays. Assay linearity was assessed by testing 10 replicates of dilutional series prepared from HSV-1/2 and VZV Verification-Panels (Exact Diagnostics).
RESULTS: Overall-agreement between Alinity and Allplex for qualitative detection of HSV-1, HSV-2 and VZV in clinical samples was 95.4% (104/109), 98.4% (126/128), and 100% (30/30), respectively. Alinity identified 4 infections missed by Allplex, 3 infections were only detected by Allplex. All samples pretest-negative for HSV-1/2 and VZV (n=100) tested negative with Alinity. No cross-reactivity for non-targeted sexually-transmitted pathogens present in 219 clinical samples in absence or presence of HSV1/2 and/or VZV was observed with Alinity. Both assays detected the Herpes viruses in the EQA sample at high concentration and demonstrated high dilutional linearity, while overall, precision was higher with the Alinity assay.
CONCLUSIONS: The Alinity m HSV-1&2/VZV assay showed a very good performance in detecting the Herpes viruses without any detectable competitive effects in samples with co-infections. The random-access capability of the Alinity platform is a valuable tool in clinical routine.
SOURCES OF FUNDING: Abbott GmbH
Keywords: Herpes viruses, Varizella zoster virus, HSV-1/2, multiplex PCR

PP-157
Diagnostic stewardship & Diagnostic test accuracy

Viral load measurement in HIV-1 samples with highly mutated Integrases
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AIM: Although being highly conserved, the increasing usage of Integrase inhibitors puts selection pressure on the integrase gene with resistance associated mutations being selected. Some qPCR assays use integrase as one of the targets for their probe-based measurements. In this study we focus on HIV-1 viral load measurement in viruses with highly mutated integrases.
METHODS: As part of a larger comparative study with 500 samples studying the performance of the NeuMoDx HIV-1 Quant assay (QIAGEN) with the more established Alinity m HIV-1 assay (Abbott), we analysed 25 samples of different HIV-1 subtypes with highly mutated integrase-genes. The routine samples were sequenced for resistance testing and stored at -20°C. Afterwards they were quantified in parallel to document possible bias due to non-matching primers/probes in the integrase-gene target-regions of both assays.
RESULTS: The samples distributed across 9 different subtypes (A, A1, A6, B, C, D, F, CRF01_AE, CRF02_AG) showed between 9 and 31 deviations in the approximately 300 amino acids of the integrase compared to the consensus B wild type. The correlation of the results from both tests was high and achieved an R2 of 0.9484. No false quantifications were detected within the viral load range of 2.8 to 6.2 log IU/mL.
CONCLUSIONS: A high correlation between the two assays was demonstrated with a specific focus on highly mutated integrase genes. The true random access capability of the NeuMoDx platform is a valuable tool for clinical routine HIV-1 viral load measurement and performance was not impacted by the highly-mutated samples tested in this study.
SOURCES OF FUNDING: QIAGEN GmbH
Keywords: Integrase, mutations, viral load,

PP-158
Diagnostic stewardship & Diagnostic test accuracy

Evaluation of the performance of the NeuMoDx MPXV Test
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AIM: Timely testing of m-pox virus (MPXV) is important for patient care, contact tracing, and decreasing transmission. The aim was to evaluate the accuracy of the new NeuMoDx MPXV Test in detecting MPXV from swabs compared with an in-house-assay developed to begin of the global outbreak 2022
METHODS: We tested 247 MPXV-positive and 50 MPXV-negative archived (-20°C) residual samples in parallel on the NeuMoDx with the MPXV Test strips (Qiagen) and with an in-house PCR (LDT, primer and probes from TibMolbiol, cfx96 Biorad; extraction Nimbus, Seegene) to estimate the correlation between both tests. In a dilution series from a sample quantified by digital PCR, the 95% hit-rates were determined by probit analysis.
RESULTS: All patient samples initially negative for MPXV were confirmed as negative. All 247 initial inhouse PCR-positive results were reported positive with both tests. The correlation between results was very high (R2: 0.95) but with an intercept of about 4 Ct. The mean difference in Ct values determined by Bland-Altman analysis was 4.74 cycles, with the lower Ct values in the NeuMoDx MPXV Test strips. The probit analyses gave 95% hit-rates of 445cps/mL for the LDT and for the NeuMoDx MPXV Test a value below 32cps/mL, the last dilution step in our analysis.
CONCLUSIONS: In this comparative analysis the NeuMoDx MPXV Test assay showed a high specificity and very good sensitivity with a lower detection limit below 32 copies/mL. The random access capabilities of the NeuMoDx system allowed for improving turn-around-time of results in comparison to the batch-procedure-based LDT assay.
SOURCES OF FUNDING: QIAGEN GmbH
Keywords: MPXV, random-access

PP-159
Diagnostic stewardship & Diagnostic test accuracy

Diagnosing viral gastro-enteritis using the fully automated sample-in, result-out STARlet All in One system (AIOS)

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AIM: To evaluate the Seegene AllplexTM GI-virus assay by testing on the fully automated STARlet All-In-One system (AIOS) in comparison to LDT, using fecal specimens obtained from patients with suspected viral gastro-enteritis in two diagnostic laboratories in the Netherlands.
METHODS: The AllplexTM GI-virus assay is able to detect adenovirus (40/41), astrovirus, norovirus GI and GII, rotavirus and sapovirus. The assay was evaluated with a retrospective cohort of 72 positive specimens including all pathogens detected by the assay. In addition, a prospective evaluation was performed, including a total of 661 specimens.
RESULTS: The retrospective part of the study showed concordant results in 69 samples (96%). Three discordant results were observed, one norovirus GII positive with an invalid result in the AIOS and two specimens testing negative in the AIOS; an adenovirus eventually genotyped as genotype 2 virus and a sapovirus (CT 31). In the prospective evaluation, four had an invalid result in the AIOS. A total of 61 positive specimens were detected in LDT, of which 60 were also detected by the AIOS. One norovirus GII positive sample (CT 35.2) tested negative in the AIOS. AIOS detected two additional sapovirus positive specimens, CT 37 and 38, but could not be confirmed by the LDT.
CONCLUSIONS: Application of the Seegene AllplexTM GI-virus assay for diagnosing viral gastro-enteritis showed excellent concordance to the current diagnostic LDTs. In a prospective comparison, only three discordant results were observed, all with CT values over 35 and therefore unlikely of clinical relevance.
SOURCES OF FUNDING: AllplexTM GI-virus kits have been provided free-of-charge by Accuramed.
Keywords: gastro-intestinal, PCR, fully-automated platform, molecular diagnostics, LDT

PP-160
Diagnostic stewardship & Diagnostic test accuracy

Lack of amplification of SARS-CoV-2 E-gene in a widely used real-time PCR assay

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AIM: To describe a SARS-CoV-2 variant lacking amplification in a widely used real-time PCR assay targeting the E-gene, in a specimen obtained from a patient chronically infected with SARS-CoV-2 that had previously tested positive without failures.
METHODS: Two BAL specimens were obtained from an immunocompromised patient within a time-period of five months. Real-time PCR assays targeting E-gene and RdRP-gene were used (with minor modifications for the RdRP-gene) as published.* The first specimen was positive for both SARS-CoV-2 targets, while the later sample was E-gene negative/RdRP-gene positive. Both specimens were presented for amplicon-based sequencing to identify potential mismatches in the primer/probe regions resulting in PCR amplification failure.
RESULTS: Ct-values as determined by real-time PCR of E-gene and RdRP-gene were 27.0/28.7 and negative/28.9, respectively. Both SARS-CoV-2 viruses belonged to lineage HV.1 and the sequences had a perfect match with the probe and reverse primer of the E-gene assay.* Compared to the specimen testing positive for E-gene, the E-gene PCR-negative SARS-CoV-2 variant had a deletion of 3 nucleotides in the forward primer.
CONCLUSIONS: Here we report a deletion of 3 nucleotides in the E-gene target region of the forward primer leading to failure in amplification of this widely used real-time PCR assay which occurred during virus evolution in a five month time period in an immunocompromised individual. Our findings stress the continued need for monitoring of SARS-CoV-2 sequences that could potentially impact routine diagnostics tests.
SOURCES OF FUNDING: *Corman V.M., Landt O., Kaiser M., et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance 2020
Keywords: immunocompromised, mutation, failed detection

PP-161
Diagnostic stewardship & Diagnostic test accuracy

Rational use of meningitis/encephalitis (M/E) panel combined with clinical diagnostic criteria

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AIM: The objective of this study is to assess the effectiveness of using the meningitis/encephalitis(M/E) panel for diagnostic purposes.
METHODS: CSF samples from patients with suspected central nervous system (CNS) infection who came to our virology laboratory in January 2023-April 2024 were included in the study. One hundred and fifty two CSF samples were analysed with the multiplex PCR meningitis/encephalitis panel. Demographic data, CSF protein levels, CSF cell counts, blood samples (inflammatory parameters, cell count, serologic test results), radiologic results, and clinical data of the patients with suspicions of CNS infection were collected manually from medical records.
RESULTS: At least one pathogen was detected in 9 (6%) of the 152 samples evaluated. The distribution of the detected pathogens was as follows:three HSV-1,one ''E.coli'' K1,two HHV-6,one ''Haemophilus influenzae'',one ''Streptococcus agalactiae'' and CMV combined positive and one CMV positive. Since the clinical, radiological and other laboratory results of three positive patients (2 HHV-6,''H.influenza'') were not compatible with the diagnosis of meningoencephalitis, they were evaluated as false positivity. In the others, which were evaluated as true positivity, the same pathogen was detected in singleplex PCR or CSF culture. In addition, when samples of true cases were evaluated with the Modified Reller criteria, it was observed that these cases could be detected without being missed.
CONCLUSIONS: Diagnostic stewardship and developing a diagnostic decision rule approach may improve the appropriate use of the ME Panel. Additionally, it shows that cost savings will be achieved by applying modified Reller criteria to patients with a preliminary diagnosis of CNS infection.
SOURCES OF FUNDING:
Keywords: meningitis/encephalitis PCR panel, central nervous system infection, diagnostic stewardship

PP-162
Diagnostic stewardship & Diagnostic test accuracy

Diagnosing acute Epstein-Barr Virus infections: This is the Way

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AIM: A recent survey of Belgian laboratories revealed great variability in initial serological testing approaches for Epstein-Barr virus (EBV). Two strategies were most common: Strategy A, starting with EBV viral capsid antigen (VCA) IgG and IgM, followed by EBV Epstein-Barr nuclear antigen-1 (EBNA-1) IgG if reactive; and Strategy B, starting with EBNA-1 IgG, followed by VCA IgG and IgM if non-reactive. Our study aimed to evaluate the effectiveness of these strategies and provide guidelines for standardization.
METHODS: We retrospectively reviewed all valid EBV serological results in 2022 from patients older than 18 months. Every patient was tested with a combination of EBNA-1 IgG, VCA IgG and VCA IgM on Roche Cobas®. A total of 1635 samples (4905 tests) were evaluated.
RESULTS: Strategy A potentially missed 5 EBNA-positive patients (0.31%). Two of them (0.12%) had EBNA-1 IgG around cutoff, possibly due to recent blood transfusion contamination. In contrast, Strategy B would have missed 163 VCA IgM-positive patients (10.0%), but only one (0.06%) was considered to be suffering from recent EBV primo-infection. Using strategy A, 4681 tests would have been performed, compared with 2305 tests using strategy B.
CONCLUSIONS: Both test strategies are easy to comprehend and lead to excellent results with minimal misdiagnosis. Adopting an EBNA-1 IgG-based approach can streamline testing and reduce costs, potentially reducing tests by nearly 50%. When one chooses to walk the way of EBNA-1 IgG, be aware of EBNA-1 IgG values near the cutoff value as these should raise caution about nonspecific test reactions or contamination.
SOURCES OF FUNDING: None
Keywords: Epstein-Barr Virus, serology, diagnostic algorithms

PP-163
Diagnostic stewardship & Diagnostic test accuracy

Comparative evaluation of six commercialized RT-PCR Kits for measles virus detection
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AIM: In January 2024, the WHO issued an alert on the resurgence of measles worldwide. A rapid measles diagnosis enables early identification of contact cases to stem the chain of transmission and to start the administration of Ig in vulnerable people. The aim of this study was to evaluate the performances of the available kits for measles detection.
METHODS: For the evaluation were used the cycle threshold, the internal control detection, the amplification length, the sensitivity and specificity, the ease of use, the costs. The WHO amplification method was used as reference. Our study also compares two extraction methods from different samples types and two different automated amplifiers. The genotypes currently circulating and different distinct sequence ID were included.
RESULTS: A total of 40 samples were included. The sensitivity was evaluated using 5 dilutions for 2 different samples (until 10-4). There were no differences according to sample. The six B3 DSID and seven D8 DSID tested were consistent. The run duration varied between 40 minutes and 3 hours. One of the kit does not appear to be suitable for use in the tested conditions.
CONCLUSIONS: Five of the six evaluated kits show consistent results with the WHO reference method. In the context of the resurgence of measles, knowledge of the performance of available kits is essential for the implementation of techniques in laboratories.
SOURCES OF FUNDING: NRC for measles mumps and rubella viruses
Keywords: Measles detection, RT-PCR, comparaison, kits

PP-164
Diagnostic stewardship & Diagnostic test accuracy

Does specific IgM immunoblot testing improve diagnosis of acute viral infection?

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AIM: Serological IgM testing remains the cornerstone of many acute viral infection diagnoses, particularly in case of non-suggestive symptoms. Unfortunately, the non-specific reactivity of IgM antibodies between cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Parvovirus-B19 (B19) often impairs the accuracy of diagnosis and complementary analyses are then required. The aim of the study was to assess the added value of IgM specific immunoblots to validate the diagnosis.
METHODS: Twenty sera from patients with a B19, EBV or CMV acute infection, as documented by both the clinic and molecular biology, were selected based on the reactivity of IgM antibodies against different viruses, as detected by routine serological screening using MEIA (IgM and IgG). IgM (B19, EBV, CMV) specific immunoblots were performed on all sera.
RESULTS: For 11 cases of acute B19 infections, EBV immunoblots were negative despite reactivity of VCA-IgM by MEIA; CMV immunoblots were positive in 5/11 (45%) cases; B19 immunoblots were positive in all cases. For 7 acute EBV infections, 5 EBV-immunoblots had a profile in agreement with an acute infection but 2 gave an incorrect interpretation; all CMV immunoblots were positive and 5/7 (71%) B19 immunoblots were positive. For the unique acute CMV infection, both CMV and B19 immunoblots were positive.
CONCLUSIONS: This study demonstrates that IgM immunoblots do not provide any useful information to correctly diagnose acute viral infection when several IgM are detected. Polyclonal IgM production likely explains the cross-reactivity observed on different assay formats. IgM screening assays should be thoroughly selected to avoid misdiagnosis.
SOURCES OF FUNDING: None
Keywords: Specificity, misdiagnosis, avidity, accuracy, molecular biology

PP-165
Enteric viruses & Neurovirology

Retrospective Evaluation of Five-Year Test Results of Meningitis/Encephalitis Panel
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AIM: Molecular test results used for the detection of viral agents in the cerebrospinal fluid (CSF) of patients suspected of central nervous system (CNS) infection were retrospectively evaluated.
METHODS: The FTD Viral Meningitis Kit (Fast Track Diagnostics Ltd, Luxembourg) was used for identifying viral meningitis agents from January 2019 to February 2023; and the Meningitis/Encephalitis RT-qPCR Kit (Bio-Speedy, Bioeksen, Turkey) was used from March 2023 to April 2024.
RESULTS: A total of 105 out of 3169 CSF samples (3.4%) were found to have at least one viral agent. Of the patients, 1667 (52.6%) were female with a mean age of 37.4±25.1 years (range 2-105). There were 835 pediatric patients and 2334 adult patients. CNS viral infections were most commonly caused by EV (24.2%) and HSV-1 (21.2%) in the pediatric age group, whereas in adult patients, HSV-1 (44.6%) and VZV (25.6%) were detected most frequently. Among the 1946 samples tested during the period including the COVID-19 pandemic, HSV-1 was the most frequently detected viral agent in 26 samples, followed by VZV in 19 samples. Among the 1223 samples tested after the pandemic, HHV-6 was the most frequently detected viral agent in 17 samples, followed by HSV-1 in 12 samples and EV in 9 samples.
CONCLUSIONS: Tests should be evaluated in conjunction with clinical and laboratory findings to prevent uncertain results and unnecessary treatments. HHV-6 positivity should be carefully interpreted, and diagnostic investigations in blood and hair follicles should be conducted to determine viral integration, latency, or CNS involvement.
SOURCES OF FUNDING: No funding used
Keywords: Cerebrospinal fluid, CNS infection, multiplex PCR

PP-166
Enteric viruses & Neurovirology

Etiology and diagnostic methods in viral acute CNS infections - 2 years in a Romanian tertiary hospital

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AIM: Underlining the most common viral etiology of CNS infections in a tertiary hospital in Romania and highlighting the burden of making an etiological diagnosis even with access to modern diagnostic methods.
METHODS: A retrospective, descriptive study was conducted on a group of patients admitted to "Dr. Victor Babes" Clinical Hospital for Infectious and Tropical Diseases in Bucharest between 1st of April 2022 and 1st of April 2024 with the diagnosis of acute viral CNS infection. Data was collected from the informatics system and a consent for data processing was obtained upon patient admission. The diagnosis of acute CNS infections was made epidemiological, clinically and laboratory by cerebrospinal fluid (CSF).
RESULTS: A total of 145 patients were enrolled, median age 53 years. The majority were originated from urban areas (112;73,7%). CSF PCR testing was performed for 138 patients, out of which 52 yielded positive RESULTS: West Nile virus(2), enterovirus(18), VZV(16), HSV1(6), HSV2(2), HHV6(3), mumps virus(1), influenza virus(1), human metapneumovirus(1), measles virus(2) and 66 were negative. WNV IgM tests in plasma and CSF were performed for 68 patients, with 27 positive results. For 52 cases were not found an etiology. Median hospitalization time was 11.5 days with no significant differences based on age or sex, longer for meningo-encephalitis (p=0.038). 131(86,2%) patients had a favorable outcome and 20(13,2%) died.
CONCLUSIONS: Most frequent etiology was WNV, followed by herpesviruses and enteroviruses. A notable number of cases (35.8%) are not found an etiological diagnosis thus the range of available molecular tests should be extended.
SOURCES OF FUNDING: No funding.
Keywords: meningoencephalitis, West Nile virus, herpesviruses, enteroviruses, molecular diagnosis

PP-167
Enteric viruses & Neurovirology

Exploring non-A rotavirus infections in Spain: Are there age-specific trends?

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AIM: Four different rotavirus species (RVA, RVB, RVC and RVH) are able to infect humans, who are mainly affected by RVA. Our aim has been on studying antibody prevalence against RVA, RVB, RVC and RVH in human serum samples from the population of Valencia (Spain).
METHODS: Recombinant baculoviruses were constructed to produce VP6 proteins from RVA, RVB, RVC and RVH strains in insect cells. VP6 proteins were tested by EIA for IgG antibody in 420 sera collected from six age groups (<5, 5-10, 11-20, 21-40, 41-60, >60 years).
RESULTS: We detected IgG antibody prevalence rates of 79.3%, 17.9%, 18.8%, and 14.5% for RVA, RVB, RVC, and RVH, respectively. Higher prevalence of IgG antibodies against RVA, RVC and RVH was observed in the over-60's group (90.5%, 32.4%, and 44.6%, respectively), while for RVB, it was highest in children under 5 years old (29.2%). IgG antibody titers against RVA VP6 protein remained consistent across age groups (up to 26,600), whereas RVB showed lower titers except in younger individuals (around 2,000). Notably, RVC-specific antibodies peaked in the 5 to 10-year-old age group, and RVH exhibited the highest titers in those over 60 (up to 15,000), suggesting varying age-related infectivity.
CONCLUSIONS: Human IgG serum antibody against RVB, RVC and RVH have been detected in Spain. Indirect evidence of RVB and RVH infections are reported for the first time, highlighting the need to survey non-A rotavirus infections.
SOURCES OF FUNDING: Grants PI20/00801 from the Carlos III Health Institute and ACIF/2020/076 from the Generalitat Valenciana
Keywords: rotavirus, antibodies, seroepidemiology, VP6

PP-168
Enteric viruses & Neurovirology

Enterovirus 71 Infection: Five Years in Review (2019-2023)
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AIM: Evaluation of EV-71 infection cases identified at INSA in 2019-2023.
METHODS: 690 stool samples from suspected Enterovirus infections in children were analysed by real time RT-PCR and viral isolation. Enterovirus serotype was determinate by neutralization assay or genomic sequencing.
RESULTS: Of the 690 stool analysed, 221 (32.0%) were Enterovirus infection. EV71 was identified in 11 (5%) cases with the following temporal distribution:
• 2019: 4 cases (1.8%)
• 2020: 1 case (0.5%)
• 2021: 0 cases (0%)
• 2022: 4 cases (1.8%)
• 2023: 2 cases (0.9%)
Distribution by age showed 9(81.8%) cases in children under 5 yo. CNS infection was the main diagnosis with 3 (27.3%) cases of rhombencephalitis, 3 (27.3%) of meningitis and 2 (18.2%) of encephalitis. All cases of rhombencephalitis, encephalitis and one meningitis occurred in children under 5yo but without fatalities. In 2019 and 2023, EV 71 infection occurred between April and August, in 2020 in March and in 2022 in October.
CONCLUSIONS: Despite the low number of positive cases, this study showed that EV-71 infection in Portugal seems to be rare compared to other Enteroviruses, but is associated with serious neurological cases, especially in young children. Infection appears to occur in the spring/summer period, with the exception of the first post-pandemic year. This study shows the importance of the early identification of EV71 circulation, which should be considered in the evaluation of severe cases of CNS in childhood, leading to an improvement in the clinical management of these cases.
References: Lee KY.Enterovirus 71 infection and neurological complications.Korean JPediatr 2016;59(10):395-401.
SOURCES OF FUNDING:
Keywords: Enterovirus 71 infection

PP-169
Enteric viruses & Neurovirology

Infection study of human rotavirus on human intestinal enteroids

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AIM: Group A rotaviruses (RVA) are a leading cause of acute gastroenteritis worldwide, particularly in children. The characterization of RVA is primarily based on molecular knowledge. The propagation of human rotaviruses from patient samples using monolayer of immortalized cell lines is difficult and not feasible for all circulating genotypes so far. Therefore, an intestinal enteroid system was implemented as an innovative platform for propagation and characterization of RVA.
METHODS: Two different intestinal enteroid cell lines (duodenum and ileum) were infected with RVA from human samples. Differentiated enteroid cells were plated onto transwell membranes and were infected with filtrated RVA stool suspension under trypsin treatment for one hour or three days to determine virus replication. Medium of the apical and basal compartments as well as cells were harvested separately. RNA was isolated and a RT-qPCR was used for the quantification of virus copies.
RESULTS: Of the seven RVA samples belonging to genotypes: G8P [8]; G3eqP [8]; G4P [8]; G9P [8]; G21P [29] used for the infection, five samples could be cultivated on both cell lines (3x G3eq [P8], 1x G3P [8],1x G8P [8]). These samples showed an increase in virus titer in the apical medium after 3 days post infection. Lower virus copies were detectable in the cells and in the basal medium than in the apical cell culture supernatant. Samples, which infected human intestinal enteroids successfully, predominantly contained DS-like RVA strains.
CONCLUSIONS: We present an enteroid cell culture system capable of propagating different RVA genotypes from human samples.
SOURCES OF FUNDING: not applicable
Keywords: Group A rotaviruses, stool samples, enteroid cell culture system

PP-170
Enteric viruses & Neurovirology

Influence of COVID-19 Lockdown on Rotavirus Genotype Dynamics in Slovenia
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AIM: Rotavirus is a significant cause of acute diarrhoea, particularly in children aged 0-5 years. During the COVID-19 pandemic, preventive measures led to a significant reduction in rotavirus-positive stool samples from patients with diarrhoea, tested in our laboratory, from 9% in the 2018/2019 season to 2% in the 2020/2021 season. However, post-lockdown in the 2021/2022 season, 13.5% of stool samples were rotavirus-positive. Our aim was to analyse the dynamics of rotavirus genotypes following the COVID-19 pandemic compared to previous seasons.
METHODS: This study included four rotavirus seasons, from 2018/2019 to 2021/2022. A total of 521 rotavirus-positive stool samples were analysed to determine genotypes of VP7 and VP4 rotavirus genes according to EuroRotaNet protocol. Mixed or undefined genotypes, determined by gel electrophoresis, were confirmed using Sanger sequencing.
RESULTS: In the 2021/2022 season, the first season after COVID-19 preventive measures were lifted, a significant increase in the G9P [8] genotype was observed (59.5% compared to 12.5% in the previous season). The G3P [8] genotype, the most prevalent genotype in the 2019/2020 season, decreased season by season during the pandemic. A similar decline was observed for the G1P [8] genotype. Additionally, G2P [4] and G4P [8] genotypes completely disappeared between 2020-2022. Two new rotavirus genotypes, G8P [8] and G3P [9], emerged during these seasons.
CONCLUSIONS: The COVID-19 preventive measures impacted the occurrence of various infectious diseases, including rotavirosis. Our study observed a shift in rotavirus genotypes during the pandemic and post-lockdown. Monitoring the status of rotavirus genotypes in future seasons will be of great interest.
SOURCES OF FUNDING: The work was financially supported by the EuroRotaNet project.
Keywords: rotavirus, genotype, lockdown, shift of rotavirus genotype prevalence

PP-171
Enteric viruses & Neurovirology

Human parechovirus detection in a tertiary hospital over seven years: epidemiology and clinical features
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AIM: Review of cases of human parechovirus (PeV-A) detection in clinical samples collected from patients attended in a tertiary hospital during seven years; additionally, to describe the clinical characteristics of these patients.
METHODS: Retrospective study including PeV-A PCR-positive samples from patients attending at the tertiary hospital in Barcelona, between June 2017 and March 2024. PeV-A was detected by PCR using the RealCycler Progenie® reagent. The data analyzed included PeV-A specific genotype (determined in the National Centre of Microbiology, ISCIII), type of clinical sample, demographics data, comorbidities, clinical characteristics, antibiotic administration at admission.
RESULTS: The presence of PeV-A was studied in a total of 1787 samples, of which 53 were positive (2.97%), corresponding to 34 patients. Twenty-one samples (39.6%) were feces, 19 (35.9%) were cerebrospinal fluid, 12 (22.7%) nasopharyngeal aspirate, and 1 (1.8%) pericardial fluid. The most commonly detected genotype was PeV-A3, accounting for 28 (52.8%) samples. Twenty-two (64.7%) were male, 22 (64.7%) had ≤1 month, and 3 (0.8%) had prematurity as a comorbidity. The most observed symptom on admission was fever, in 27 (79.4%) cases. The most frequently observed discharge diagnosis was meningitis 18 (52.9%) and fever syndrome 6 (17.6%). Seventeen (50%) patients received antibiotic treatment, of which 13 (76.4%) were discontinued upon PeV-A detected.
CONCLUSIONS: Parechovirus is an emerging pathogen that can cause moderate-severe disease, particularly in children under 1 month of age. It's important to consider this virus in patients with compatible symptoms. Early detection, especially through fecal samples, is essential to avoid unnecessary antibiotic treatment and manage the illness effectively.
SOURCES OF FUNDING: N/A
Keywords: Parechovirus, PeV-A, PCR

PP-172
Enteric viruses & Neurovirology

Viral aetiology in acute gastroenteritis across all age groups in Ireland: an eight-year observational study

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AIM: The viral aetiology of acute gastroenteritis (AGE) in paediatrics is well described, as is the burden of norovirus in elderly cohorts. In contrast, data on AGE viral diversity in non-paediatric age groups are sparse. Here, we aim to describe the viral aetiology of AGE in Ireland across all age groups over an eight-year period, including data before, and after, introduction of rotavirus vaccination (Rotarix) in 2016.
METHODS: This observational retrospective study examined stool samples or rectal swabs investigated for viral gastroenteritis at the National Virus Reference Laboratory, Dublin, from January 2015 to December 2022. All samples underwent (RT-)qPCR for norovirus, rotavirus (wild-type and vaccine strain), sapovirus, astrovirus, and enteric adenovirus.
RESULTS: Samples (n=61,823) were tested for five target viruses, with 9,512 (15%) having ≥1 virus detected. Norovirus was most frequently detected (5400/10230; 53%), followed by wild-type rotavirus (n=2171; 21%), sapovirus (n=1037; 10%), astrovirus (n=895; 9%), and enteric adenovirus (n=727; 7%). Rotarix was detected in an additional 334 samples. Following Rotarix introduction, rotavirus positivity in under-fives decreased significantly. Norovirus was most frequently detected in non-paediatric age groups each year. In the 6-64-year-old cohort, over one-third of all detections were of viruses other than norovirus; rotavirus (16%), sapovirus (9%), astrovirus (8%) and adenovirus (3%).
CONCLUSIONS: Viral diversity is evident in the 6-64 years AGE cohort, yet testing in this group is often restricted to norovirus. These additional pathogens are likely underdiagnosed, with important infection control and management implications. Expanded testing should be considered in norovirus negative outbreaks of diarrhoeal illness.
SOURCES OF FUNDING: None
Keywords: Rotavirus, Rotarix, Norovirus, Sapovirus, Astrovirus, Adenovirus

PP-173
General virology

Differential distribution of varicella-zoster virus (VZV) clades among patients: A retrospective study from two French tertiary care hospitals

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AIM: VZV nomenclature identifies 7 different clades (1-6, 9): clades 1, 3, and 6 are prevalent in Europe and America, clade 2 in Japan and East Asia, and clades 4 and 5 in Asia and Africa. This distribution may be influenced by immigration, travels, and chickenpox vaccine implementation. Effective surveillance is essential for VZV epidemiology understanding. The aim of this study was to investigate the VZV clade distribution among patients from two French university hospitals.
METHODS: We included 339 VZV-infected patients (179 males, 160 females, median age 50 years), most of European (58.1%) or African (33.1%) origin. Patients with chickenpox and herpes zoster represented 19.5% and 66.1%, respectively. Clade determination relied on single nucleotide polymorphism (SNP) identification in open reading frames (ORFs)21, 22, 38, and 50.
RESULTS: VZV clade distribution was as follows: clade 1, 27.1%; clade 2, 3.2%; clade 3, 32.7%; clade 4, 2.4%; clade 5, 31.9%; clade 6, 2.7%. Patients infected with clade 5 were significantly more likely to be males (p=0.0036) and younger (p=0.0006) compared with patients infected with other clades. Moreover, European-born patients infected with clade 5 were younger than those infected with other clades, whereas this difference wasn't observed among Africa-born patients.
CONCLUSIONS: This study revealed similar proportions of VZV clades 1, 3, and 5 among patients. Moreover, clade 5 seemed to infect European men earlier than other clades. This result may suggest that the African clade 5 is able to spread more efficiently in the young European population than clades 1 and 3.
SOURCES OF FUNDING: National Reference Center for Herpesviruses
Keywords: VZV; Clade; Epidemiology; Age; Origin

PP-174
General virology

Abortive infection fate of lumpy skin disease virus in white blood cells

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AIM: LSD is an emerging, severe transboundary disease of cattle caused by a Capri pox DNA virus. Live attenuated vaccine is used to control the disease, yet the mechanism of pathogenesis and attenuation largely unknown. Hence, we characterized LSDV tropism and replication in susceptible bovine cells. Presence of LSDV in buffy coats preparation, as well as its suggested mechanical mode of transmission by biting arthropods between animals prompted us to investigate whether white blood cells support productive LSDV replication and dissemination.
METHODS: We constructed reporter LSDV recombinant versions of WT and Vaccine LSDV expressing GFP under a synthetic viral promoter. LSDV-GFP viruses were monitored for their infection, replication and dissemination properties by flow cytometry, fluorescence microscopy, quantitative PCR (qPCR)/RT-qPCR, and infectious progeny release titration.
RESULTS: Replication kinetics of both GFP viruses in kidney cell line (MDBK) were indistinguishable from that of parental viruses. WT-GFP and vaccine-GFP LSDV portrayed no differences in infection and replication in MDBK, in a macrophage cell line, and in primary foreskin cells. PBMCs infected ex-vivo with both strains resulted in early and late viral gene expression; however, these cells failed to support viral DNA replication and release of infectious progeny, suggesting an abortive infection. Nonetheless, infected PBMCs, co-cultured with MDBK cells, transmitted the virus to recipient neighboring cells, suggesting their potential role in viral dissemination.
CONCLUSIONS: While PBMCs restrict LSDV replication by a yet unknown mechanism, these cells may also act as carriers and disseminators of LSDV.
SOURCES OF FUNDING: Chief scientist fund, Ministry of Agriculture and Rural Development, Israel
Keywords: lsd, lsdv, pbmcs, vaccine, gfp

PP-175
General virology

Virological patterns in different parvovirus B19 related clinical situations
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AIM: The aim of this study was to describe PVB19 virological markers according to clinical presentation.
METHODS: PVB19 results from 2012 to April 2024 were retrieved from the Virology laboratory database of Necker hospital. The medical file of patients with both a positive PVB19 PCR in blood (B19RGene, BioMérieux) and a serological result (IgG and IgM Liaison XL, Diasorin) were reviewed and patients with 4 clinical presentations were selected:
Group 1: Immunocompetent patients with *Erythema infectiosum*.
Group 2: Acute red cell aplasia in patients with chronic hemolytic anemia.
Group 3: Myocarditis and other post viral complications.
Group 4: Anemia in immunocompromised patients.
RESULTS: 148 patients were included, blood viral load in log copies/ml and serology in index ratio were:
Group 1 (N= 24): moderate viral load (median = 5.66), high IgM ratio (median = 36), moderate IgG ratio (median = 14).
Group 2 (N=76): high viral load (median = 8.36), high IgM ratio (median = 48), low IgG ratio (median = 8.8).
Group 3 (N=25): moderate viral load (median = 4.69), low or negative IgM (median = 2.3), high IgG ratio (median = 31).
Group 4 (N=23): high viral load (median = 7.81), low IgM and IgG ratio (median = 10).
CONCLUSIONS: Describing these different virological profiles should help virologists and clinicians to attribute patient's clinical symptoms to recent PVB19 infection. Moreover, in group 3 with post-viral complications, nearly half of the patients were IgM negative. In this clinical situation, PCR appears essential for diagnosis.
SOURCES OF FUNDING: No
Keywords: Parvovirus B19, serology, PCR, clinical, biomarkers

PP-176
General virology

Molecular detection and identification of human enteroviruses and parechoviruses in children and adults admitted to a university hospital

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AIM: The present study attempted the prospective molecular detection and identification of human enteroviruses and parechoviruses in children and adults admitted to a university hospital. Clinical presentation included febrile illness, upper respiratory system infection, viral meningitis, exanthematous disease and HFMD.
METHODS: A Real-Time RT-PCR protocol targetting the 5'-UTR was used for enterovirus RNA detection in 529 samples from 520 different patients. Genotypic characterisation of the serotypes was performed by sequencing of the VP1-coding genomic region.
RESULTS: Twenty-seven enterovirus strains and one parechovirus strain (HPeV4) were detected and identified in 14 stool samples, 7 CSF, 8 rhinopharyngeal swabs and 1 vesicular fluid sample from 20 out of the 520 examined patients (5.2%). Ten E30 strains were identified in 7 CSF and 6 stool samples from a respective number of viral meningitis cases within a period of 6 months (April – September 2018). The rest of the identified serotypes included CV-B3 (1), E-18 (1), CV-B5 (2), E-6 (2), CV-A4 (1), CV-A6 (1), CV-A16 (1) and one EV-D68 strain from a respiratory swab sample. Seven strains detected in respiratory swab samples remain to be identified.
CONCLUSIONS: The present study showed the circulation of different enterovirus serotypes responsible for either sporadic cases, or outbreaks of disease. Both the epidemic prevalence of E-30 meningitis cases during a period of 6 months and detection of the emerging EV-D68 for the first time in Greece were particularly alarming, setting the ground for continuous epidemiological investigation for outbreaks, emerging enteroviruses and large-scale epidemiological studies.
SOURCES OF FUNDING: There are no sources of funding.
Keywords: Enterovirus,Parechovirus, EV-D68, E30

PP-177
General virology

Oral Combination vaccine against Hepatitis B & Influenza: Development and Characterization
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AIM: Vaccination has not only become vital but a lot of revolutionary changes are being observable in the field of vaccine delivery. Vaccine antigens administered by oral route are often degraded during gastrointestinal transit. Bile salt stabilized vesicles i.e. bilosomes are found to be effective in preventing antigen degradation and enhance mucosal penetration. The aim of the present work was to prepare a combination vaccine system against hepatitis-B(HBsAg) and influenza(r-H1N1Ags).
METHODS: Bilosomes containing HBsAg and r-H1N1Ags were prepared by a lipid cast film method. Antigen loaded bilosomes were characterized in-vitro for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was carried out to confirm uptake of bilosomes. The in-vivo study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.
RESULTS: Bilosomes formed were multilamellar and were stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by gut associated lymphoid tissues. In-vivo data demonstrates that bilosomes produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.
CONCLUSIONS: Thus, HBsAg potentiates the production anti-r-H1N1 antibody. Also measurable sIgA in mucosal secretions were observed. Thus, bilosomes are a promising carrier for oral combination vaccines. This approach could be adapted for human use because mucosal surfaces are the initial sites of infection and it therefore seems logical to attempt to develop vaccination strategies that evoke appropriate localized responses to counteract early events of pathogenesis.
SOURCES OF FUNDING: NA
Keywords: Bilosomes, Influenza, HepatitisB

PP-178
General virology

Early Life Dynamics of Torque-teno Virus Infection: Insights from the PreventADALL Mother-Child Cohort Study

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AIM: Torque-teno virus (TTV), a small, circular DNA virus, constitutes a significant part of the human virome, with unclear health impacts. The aims of this study were to determine the prevalence and timing of TTV infection and viral loads in infants.
METHODS: We included 846 infants from the general population-based PreventADALL antenatally recruited birth cohort study, with available blood samples at birth using umbilical cord blood, at 3, and/or 12 months. Quantification of TTV DNA was performed using the ARGENE® TTV R-gene real-time PCR kit. Possible maternal blood contamination of cord blood was assessed by IgA using ThermoFisher® Scientific human IgA ELISA kit and compared between TTV positive and random sample TTV negative samples with Chi-squared-test.
RESULTS: At birth TTV DNA was detected in 4,1% (35/842), with TTV identified in 61,3% (314/512) at 3 months and in 98.2% (532/542) at 12 months of age. The viral load (log10 copies/mL) in the TTV-positive samples increased during the observation period from a mean of 2.3 (range 0.3-4.4) at birth to 3,7 (range 1.6-6.3) at 3 months and 4.6 (range 1.6-8.8) at 12 months of age. Umbilical cord blood IgA was detected in 71% (25/35) of TTV-positive samples compared to 26% (9/35) ($p<0.00$).
CONCLUSIONS: Among unselected population-based children, 98% were infected by TTV by 1 year of age. While the higher rate of IgA-positivity in TTV positive samples may reflect maternal contamination, early intrauterine infection remains possible.
SOURCES OF FUNDING: Østfold Hospital Trust, Oslo University Hospital and Karolinska Institute Stockholm.
Keywords: Torque-teno virus (TTV), Infants, Viral load, Umbilical cord blood, Real-time PCR, Intrauterine infection

PP-179
General virology

Distribution of HPV Genotypes in Turkey and Factors Affecting Epidemiological Variation

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AIM: Cervix cancer can be protected with the HPV vaccine, and this cancer can be prevented by detecting aggressive genotypes at the earliest stage by diagnosing them with very specific and sensitive molecular methods. The aim of this study is to compile the genotypes of HPV detected in Turkey, which ranks first in the etiology of Cervix cancer, to discuss the factors that may be related to its epidemiological distribution, and thus to draw attention to strategies that can contribute to ending this silent pandemic.
METHODS: All available studies on HPV diagnosis and epidemiology published in our country were scanned from nine relevant search engines using pre-selected keywords. To compile the results of studies containing data on HPV genotype distribution in Turkey, the Prisma 2020 guide, was used. The researchers first found 380 full texts and 27 abstracts. Eventually, 2 different researchers re-examined these publications in detail according to the eligibility criteria and eliminated the unsuitable ones.Fixed-effect model used to synthesize data of the last 24 studies on HPV genotypes identified by different commercial molecular diagnostic platforms in a total of 15273 patients. Subgroup analysis used to explore possible causes of statistical heterogeneity.
RESULTS: Figure 1.Prevalence of human papillomavirus (HPV) infection
CONCLUSIONS: In this way, Turkey's HPV subtype profile and public health risk map were created. In addition, the advantages and limitations of the molecular diagnostic systems used were examined.
SOURCES OF FUNDING:
Keywords: HPV, genotype, systematic review

PP-180
Hepatitis, HIV and other blood borne viruses

Retrospective evaluation of HIV diagnostic algorithm as a single center
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AIM: This study aimed to evaluate the effectiveness of the current HIV diagnostic algorithm.
METHODS: In accordance with CDC algorithm, our laboratory uses Architect® HIVAg/Ab for screening, Geenius™ HIV1/2 and Artus® HIVirus-1 QS-RGQ for confirmation. The Geenius™ test was used as a reflex and the HIV-1-RNA required clinician order.
RESULTS: HIVAg/Ab test was performed in 82,882 sera and found to be reactive in 262 (0.3%). HIV-antibody confirmatory testing was performed on 79% of samples with reactive screening test, and the presence of HIV-1 antibodies was confirmed in 51% (105/206). Half of the samples with positive-screening but negative-antibody confirmatory result were tested for HIV1-RNA and viremia was detected in 5, confirming acute HIV-1 infection. HIV1-RNA was not ordered for 49 samples with positive-screening and negative antibody-confirmation test, 16 of these were considered as false-reactive by the clinician. The Geenius assay result was indeterminate in 1.45% (3/206) of the samples. In the algorithm, the number of Geenius tests would have been reduced by 25% if HIV1-RNA had been applied as a reflex test to HIV-Ag/Ab positive samples and Geenius testing had been performed on RNA negative samples
CONCLUSIONS: A retrospective analysis showed that the HIV diagnostic algorithm was not fully implemented. An important factor was that clinicians did not order HIV1-RNA-PCR from ELISA reactive and Geenius test negative patients. It is predicted that the use of HIV1-RNA-PCR as a reflex test in HIV-Ag/Ab positive samples will provide earlier diagnosis and the use of fewer Geenius test will reduce uncertain results and diagnostic cost.
SOURCES OF FUNDING: none....
Keywords: HIV; diagnosis; algorithm

PP-181
Hepatitis, HIV and other blood borne viruses

HCV screening in vulnerable population

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AIM: The prevalence of undiagnosed active hepatitis C virus (HCV) infection in Spain is 0.065% for the general population 20-80 years old. Vulnerable populations have a higher prevalence and barriers to access antivirals. An HCV microelimination opportunistic program focused on the vulnerable population was carried out in Vigo, Spain.
METHODS: Four drug addiction care units participated from October 2020 to October 2023. Whole blood samples obtained from finger pricks were stored in cobas plasma separation cards (PSC, Roche Diagnostics, Mannheim, Germany). The Internal Medicine specialist took responsibility at the Complexo Hospitalario Universitario de Vigo for reporting the positive results, the prescription and the remote follow-up of the treatment. Screening and post treatment samples were studied.
The cobas 6800/4800 system (cobas HCV test, HCV genotyping test, Roche Diagnostics) were used for HCV viral load detection and genotyping.
RESULTS: A cohort of 2574 people were assisted at the drug addiction care units. During the program, 1111 cards were analyzed from patients 21-92 years old (mean 50.4). A total of 241 active HCV infections were successfully treated (179 detected by PSC). According to PSC screening the genotype prevalence was: 1a (40.8%), 1b (8.3%), 3 (27.5%), 3a (3.3%), 4 (15%), coinfections (5%).
CONCLUSIONS: This microelimination program allowed to detect and treat a population with a high rate of active HCV (9%).
Plasma separation cards were suitable for the diagnosis outside the hospital setting.
The multidisciplinary approach allowed the successful treatment of 241 patients
SOURCES OF FUNDING: Consellería de Sanidade, Galicia and Gilead
Keywords: HCV, Screening, Genotyping, Microelimination

PP-182
Hepatitis, HIV and other blood borne viruses

Characteristics of recent HIV infections and associated risk factors among newly diagnosed individuals in Korea (2016-2022)

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AIM: It is important to acknowledge the proportion of newly diagnosed individuals each year who are recently infected (within 130 days of HIV) so that HIV prevention strategies can be tailored to this specific group. Our goal is to assess high-risk groups for HIV infection and identify those who has been under delayed detection by analyzing recent HIV infection rates among newly diagnosed infections.
METHODS: HIV antibody avidity assay was performed using Sedia-HIV-1- LAg-Avidity to differentiate recent infections from longstanding infections among newly diagnosed cases in the last 7 years (2016~2022). Risk factors were analyzed using logistic regression analysis based on subject information such as age, nationality, region, infection route, marital status, test purpose, and occupation.
RESULTS: The study group was 83.8% (6,599) of all the newly HIV-positive diagnosed individuals (7,873) during the study period, and the seven-year average recent infection proportion was 25.6%. Recent infection proportion of men (26.5%, $p<0.05$), 30 and younger aged groups (31.5%, $p<0.001$) and Koreans (26.9%, $p<0.001$) were higher than women (16.1%), 40 and older aged group (15.2%) and foreigners (19.0%), respectively.
CONCLUSIONS: Regression analysis of risk factors for HIV infection showed that gender (male), age (under 30), and nationality (Korean) were statistically significant. Men, including MSM, under 30 years old showed a higher proportion of recent HIV infection, highlighting the need for targeted prevention activities to curb HIV transmission. Women, individuals aged 40 and older, and foreigners, all with lower recent infection rates, might experience delays in recognizing HIV infection and require support to promote HIV testing.
SOURCES OF FUNDING: Unrelated.
Keywords: HIV, recent infection, Avidity assay

PP-183
Hepatitis, HIV and other blood borne viruses

IVDR-compliant establishment and validation of a laboratory-developed RT-qPCR for detection of Rocahepevirus ratti (ratHEV) using a fully automated workflow

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AIM: Recently, symptomatic infections with Rocahepevirus ratti (ratHEV) in humans have been reported, highlighting the need for a reliable diagnostic tool. Here, we aimed to develop an RT-qPCR assay for the detection of ratHEV in human plasma and stool on a fully automated system.
METHODS: Published primers (pan-ratHEV; Sridhar et al. 2018) were adapted for use on a fully automated system (cobasX800). Standards to assess analytical sensitivity and inclusivity were quantified using dPCR from cell culture lysates representing different subtypes of ratHEV (RatHEV/R63/GU345042, RatHEV/pt2/MN450851.1). For analytical performance evaluation (limit of detection (LoD), linearity, precision), negative plasma and stool suspension spiked with cell-culture ratHEV were used. To assess clinical performance, a set of plasma samples from patients post liver transplantation was tested.
RESULTS: LoDs (21 replicates) were 98.9 cop/ml (71.3 - 162) and 60.3 cop/ml (39.8 - 188) for plasma and stool suspension, respectively. Linearity was confirmed within the tested range of 21.8ct - 38.1ct in plasma and 24.5ct - 37.4ct in stool matrix. Intra- and inter-run variability was below 0.18ct, demonstrating high reproducibility of test results. Exclusivity was confirmed using samples positive for various human-pathogenic viruses (n=39). All patient samples (n=200) used to assess clinical performance yielded a negative test result, whereas cell culture-derived virus from ratHEV strains R63 and pt2 was positive.
CONCLUSIONS: The established assay demonstrated good analytical performance for the detection of ratHEV in human plasma and stool samples. However, clinical relevance has to be further assessed using a broader set of clinical samples.
SOURCES OF FUNDING: BMBF (zoonosis platform) and internal funding.
Keywords: ratHEV, viral hepatitis, molecular diagnostics

PP-184
Hepatitis, HIV and other blood borne viruses

HBV cryptic activity is frequently revealed in anti-HBc positive/HBs negative HIV patients despite HBV-active therapy and can threaten HIV suppression

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AIM: We investigate cryptic HBV activity and factors correlated with its detection in anti-HBc+/HBs- HIV patients (patients) by droplet-digital-PCR (ddPCR) assays for serum HBV-DNA and HBV-RNA quantification.
METHODS: 74patients negative for HBV-DNA by commercial assays are included. All treated for >12months with HBV-active antiretrovitral-therapy(ART): TAF/FTC(48.4%), TDF/FTC(37.1%) or LMV(14.5%). Serum HBV-DNA and HBV-RNA are quantified by ddPCR assays during ART at baseline(T0) and after 6months(T1) for all patients, and for 44/74 after 3years(T2).
RESULTS: At T0, despite ART(median [IQR] months: 54 [25-101]), 72.6%patients has cryptic HBV-activity: 24.3%HBV-DNA+ (median [IQR] 19 [13-34]copies/ml) and 47.3%HBV-RNA+ (median [IQR] 21 [13-28]copies/ml). Only 27.4% are HBV-DNA and RNA negative, indicating inactive HBV-reservoir. Notably, despite similar positivity rates of cryptic HBV-DNA across different ARTs, lower HBV-DNA levels are observed in TAF-experienced respect to TDF-experienced (median [IQR] HBV-DNA: 17 [12-24] vs 43 [25-61]copies/ml,P=0.01), suggesting a higher effectiveness of TAF in this setting. Focusing on HBV-markers' kinetics across all time-points, 40.9% patients have persistent HBV-activity, 50.0% an intermittent positivity, while 9.1% a persistent negativity to both HBV-DNA and RNA, suggesting a silent HBV-reservoir. Noteworthy, persistent HBV-activity correlates with detectable HIV-viremia (HIV-RNA detectable in 50% with persistent HBV-DNA-activity vs 19.2% with intermittent/no HBV-activity, P=0.05), supporting a worse HIV control in the setting of an underlying persistently-active HBV-reservoir.
CONCLUSIONS: In the setting of antiHBc+/HBs-/HIV+ patients, persistent cryptic HBV-DNA is frequently revealed, can threaten HIV suppression during ART and can be modulated by different HBV-active drugs. Overall, this highlights the importance to properly monitor patients with occult infection also by using highly-sensitive assays capable to reflect the activity of HBV reservoir.
SOURCES OF FUNDING: No founding
Keywords: HBV, HIV, Cryptic HBV, ddPCR

PP-185
Hepatitis, HIV and other blood borne viruses

Molecular diagnosis of hepatitis delta virus infections in Marseille university hospitals

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AIM: Hepatitis Delta virus (HDV) infection is ubiquitous. It aggravates the clinical progression of hepatitis of patients chronically-infected with hepatitis B virus (HBV). Its only treatment until now was pegylated interferon but other drugs are now available. In our center, we systematically perform a molecular diagnosis of HDV infection as part of a new diagnosis of HBV infection. Here we aimed to describe the data from the molecular diagnosis of HDV infection in our center over one year.
METHODS: Our study was performed between October 2022 and September 2023. Real-time PCR (qPCR) targeting HDV RNA and sequencing by Sanger or next generation technologies (NGS) were performed from blood samples, using in-house protocols. AP-HM_Ethics/Scientific Committee, No_CSE23-39.
RESULTS: We performed 268 qPCRs for HDV for 215 patients. 38 qPCRs were positive for 22 different patients whose mean age was 44+/-15 years. Mean HDV RNA load was 7.4 log10copies/mL (range, 3.8-8.5 log10copies/mL). HDV genotype was obtained for 18 patients: it was 1 and 5 in 16 and 2 cases, respectively. For 11 patients, HBV infection was newly-diagnosed; 11 patients were followed-up for their HDV infection. Fifteen patients had an undetectable serum HBV DNA load; for the 7 other patients, mean HBV load was 2.6 log10IU/mL. One patient was HCV/HIV-co-infected; six were HCV-seropositive but HCV RNA negative; and two patients were co-infected with HIV.
CONCLUSIONS: HDV infection is not uncommon in our region. Genotype is mostly 1. HDV molecular diagnosis and monitoring are essential for good virological, clinical and therapeutic management.
SOURCES OF FUNDING: National Agency for Research (ANR) (Méditerranée-Infection 10-IAHU-03).
Keywords: Hepatitis D virus; Molecular diagnostic; Genotype; Sequencing

PP-186
Hepatitis, HIV and other blood borne viruses

An evaluation of emerging Rat Hepatitis E Virus's risk to blood safety in England

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AIM: Evidence of rat hepatitis E virus (rHEV) infection has been identified in rats in Northern England, suggesting its presence in the UK. We investigated its circulation among blood donors and potential sources for human infection to inform blood safety.
METHODS: We developed a quantitative RT-PCR assay for the detection of rHEV RNA in wastewater samples and pork products, and an in-house ELISA based on recombinant core protein to assess its seroprevalence amongst blood donors in England. A total of 33 pooled wastewater samples were collected in London, 2021-22. Cured meat and sausages (n=25) produced in the UK or Europe were obtained in Oxford, 2024. Residual pooled blood donor samples were obtained from the NHS Blood and Transplant, 2023-24.
RESULTS: Molecular screening identified one positive wastewater sample. However, no rHEV RNA was detected in the 25 pork-based products. Serological investigations did not find evidence of antibodies to rHEV in 4800 blood donors in England.
CONCLUSIONS: Human cases of acute hepatitis linked to rHEV have been reported in Hong Kong, Spain and France and rHEV RNA has been detected in wastewater in Italy, Spain and Sweden. The absence of anti-rHEV and RNA in this study suggests it is not circulating in the blood donor population. However, the virus was found in London's wastewater likely reflecting its presence in rodent population. The potential of rHEV to infect humans and affect the blood supply should be considered in the future.
SOURCES OF FUNDING: NIHR203338 grant
Keywords: blood safety, emerging infections, blood-borne viruses

PP-187
Hepatitis, HIV and other blood borne viruses

Single-center evaluation of HDV-RNA quantification assays in a large cohort of Bulevertide-treated and untreated Chronic Hepatitis Delta patients

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AIM: The aim of this study was compared three HDV-RNA assays in untreated and Bulevirtide (BLV)-treated Chronic Hepatitis Delta (CHD) patients
METHODS: Plasma samples from consecutive untreated and BLV-treated CHD patients were analyzed by 3 different assays: Robogene HDV-RNA Quantification Kit 2.0 (LOD 6 IU/mL) on 7500 Fast Real-Time PCR System, EurobioPlex HDV PCR quantitative (LOD 100 IU/mL) and AltoStar HDV RT-PCR RUO Kit 1.5 (LOD <10 IU/mL) on CFX96™ real-time PCR detection system. HDV genotype was assessed by Sanger sequencing
RESULTS: 431 samples from 130 CHD (69 untreated and 61 BLV-treated) patients were analyzed. Most of the patients were middle-aged Caucasian males with compensated cirrhosis under nucleos(t)ide analoghe (NUC) therapy for HBV and genotype 1 HDV. Compared to Robogene, EurobioPlex reported higher HDV RNA levels [3.78 (0.70-7.99) vs. 4.69 (2.00-8.19) Log IU/mL, p<0.0001], with viral load higher than >0.5 Log in 160 (69%). Likewise, HDV RNA levels were higher with Altostar than with Robogene 2.0 [3.32 (0.70-7.37) vs. 3.91 (0.19-7.54) Log IU/mL, p<0.0001], with AltoStar reporting HDV-RNA levels >0.5 Log in 127 (52%). Although virological response rates (≥2 log decline vs. baseline) at week 24 (Robogene 2.0 vs. EurobioPlex and AltoStar) and 48 (Robogene 2.0 vs. AltoStar) were similar across assays, rates of HDV RNA undetectability significantly differed between the three assays at week 24 and 72 (p=0.003 and p=0.02)
CONCLUSIONS: This study demonstrated that quantification of HDV RNA levels in untreated and BLV-treated CHD patients is significantly influenced by the quantification method used.
SOURCES OF FUNDING: The present research did not receive any fiinancial support
Keywords: HDV RNA; Chronic Hepatitis Delta; Bulevirtide; HDV-RNA quantification assays

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Hepatitis, HIV and other blood borne viruses

First molecular characterization and phylogenetic analysis of goat hepatitis E virus in Portugal

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AIM: Hepatitis E virus, an enteric RNA virus classified within the family Hepeviridae, comprises of eight genotypes, with five capable of infecting humans. In industrialized nations, HEV transmission is commonly associated with swine, where they act as primary reservoirs for human infection. The recent discovery of HEV in sheep feces not only raises alarms regarding potential virus transmission from these animals to humans but also expands the spectrum of potential hosts. This study aimed to examine the occurrence of HEV among small ruminants in Portugal, aiming to assess the extent of HEV circulation and the potential for infection within these species.
METHODS: A total of 100 stool samples were collected from sheep and goats at a slaughterhouse in the center region of Portugal, in March 2023. First screening of HEV RNA in stool samples was performed using a broad-spectrum RT-qPCR assay that targets the ORF3 region, followed by next generation sequencing on the positives.
RESULTS: A partial HEV genome was obtained in one stool sample from a male kid, classified as HEV-3c.
CONCLUSIONS: This study presents the first molecular characterization of HEV in a farmed small ruminant in Portugal. Showing evidence of HEV infection through the prevalence of HEV RNA in these small ruminant species. Further research could provide additional insights into the factors influencing HEV transmission dynamics in small ruminants in Portugal and its potential implications for public health.
SOURCES OF FUNDING: This work was financed by Portuguese National Funds from the FCT-Fundação para a Ciência e a Tecnologia with the scholarship reference 2021.09461.BD.
Keywords: One health, Small Ruminants, HEV, zoonosis, NGS

PP-189
Hepatitis, HIV and other blood borne viruses

Contribution of HHV-6 Real-Time PCR in Hair Follicles and Nail Clippings for the Diagnosis of Chromosomally Integrated HHV-6 (ciHHV-6)

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AIM: To determine the impact of ciHHV-6 detection in hair follicles and nails, by real-time PCR, on the diagnosis and treatment of HHV-6 positive cases.
METHODS: Study cases: 29th and 36th-week-old newborns with respiratory distress syndrome when admitted to the neonatal intensive care unit, evidencing signs of ventriculitis and mild brain edema, respectively, upon transfontanellar ultrasound. 58th and 76th-year-old adults with suspected CNS infection when admitted to the hospital.
CSF were first screened for neurotropic viruses with a multiplex real-time PCR kit. A specific HHV-6 real-time PCR kit was used for DNA quantification in CSF/blood and ciHHV-6 diagnosis in hair follicles/nails.
RESULTS: HHV-6 was detected in CSF in the four cases. After treatment, high HHV-6 viral load was quantified in blood, 109766 to 516119 cop/ml. HHV-6 detection in patients' nail and hair follicles confirmed the presence of ciHHV6. DNA detection in one parent of each newborn, suggests congenital infection. Despite the persistency of high viral loads, antiviral therapy could be ceased when clinical improvement is observed.
CONCLUSIONS: Neglecting the possibility of ciHHV-6 can lead to misdiagnosis of HHV6 infection. Identifying individuals with ciHHV-6 is important because the knowledge of this patient's infection status would influence therapeutical decisions. In an individual with ciHHV-6, the results of HHV-6 DNA PCR tests must be interpreted in the context of tissue-and-sample-specific considerations. Our study reinforces the evidence that ciHHV-6 diagnosis in hair follicles/nails explains the high levels of HHV-6 DNA in blood/CSF even upon clinical improvement, guiding therapeutics on the course of disease associated with HHV-6.
SOURCES OF FUNDING: No
Keywords: ciHHV-6, Central Nervous System, Chromosomally integrated, nails & follicles, Real-time PCR

PP-190
Hepatitis, HIV and other blood borne viruses

HTLV seroprevalence in blood donors from Romania – a 15 years longitudinal study

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AIM: Romania is considered an area with a high prevalence of HTLV-1 infection, but the available data are limited and date back more than a decade ago. We conducted a 15-year survey (2008-2022) in first-time blood donors to provide an updated overview of HTLV-1 seroprevalence in this country.
METHODS: First-time blood donors aged between 18 - 65 years were initially screened for anti-HTLV 1/2 antibodies, using an immunoenzymatic assay, in 41 regional blood transfusion centers within the national program for transfusion safety. The reactive tests were retested in the National Institute of Blood Transfusion "Prof. Dr. C. T. Nicolau" from Bucharest, using a chemiluminescence assay, positive samples were confirmed using a line immunoassay.
RESULTS: Of the total number of 1.597.586 first-time blood donors during 2008-2022 (mean 106.505,712.090,8/ year), there were 1397 initially reactive samples and 437 were confirmed as anti-HTLV-1 positive. There were no anti HTLV-2 positive samples. A constant decline in the HTLV-1 seroprevalence was detected, with mean values of 3.5/10,000 donors between 2008-2012, 2.6/10,000 donors between 2013-2017 and 2/10,000 donors between 2018-2022. There were no gender related differences in the seroprevalence, but several small familial clusters were detected.
CONCLUSIONS: Romania remains a country with high prevalence of HTLV-1 infection, according to the ECDC definition (seroprevalence of more than 1/10,000 in first-time blood donors). Still, our study indicates a decreasing trend in the HTLV seroprevalence in the last 15 years, possibly reflecting lower viral transmission rates, associated to efficient hemovigilance programs and sex education.
SOURCES OF FUNDING: None
Keywords: HTLV-1 seroprevalence, first-time donors

PP-191
Hepatitis, HIV and other blood borne viruses

HBs isoforms can play a role in predicting virological response in patients with chronic hepatitis delta under bulevirtide monotherapy

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AIM: To investigate the still unknown kinetics of HBs-isoforms under bulevirtide-treatment (BLV).
METHODS: 36 patients with HDV-related compensated-cirrhosis starting BLV 2mg/day monotherapy were enrolled. HBs-isoforms were quantified by ad-hoc designed ELISAs (Beacle) at baseline and at week 48 (W48) for all patients and at week 96 (W96) for a subset of 16 patients. Virological (VR) and combined responses (CR) were defined as >2log HDV-RNA decay alone or with ALT normalization during BLV, respectively.
RESULTS: At baseline, median(IQR) HDV-RNA was 5.1(4.3-5.7)logIU/ml while S-HBs, M-HBs and L-HBs were 3681(1240-7184), 813(260-2262) and 5(1-12)ng/ml, respectively. At W48, VR and CR were observed in 69% and 67% of patients, while 50% of patients achieved HDV-RNA<100IU/ml. A >10% decline of S-HBs, M-HBs and L-HBs levels was observed in 53%, 53% and 31% of patients. HDV-RNA decrease during BLV was more profound in patients with baseline M-HBs>500ng/ml (3.5 [2.6-4.1] vs. 1.4 [0.5-3.1]logIU/ml, P=0.002). M-HBs>500ng/ml significantly correlated with achieving VR and CR at W48 (VR in 91% of patients with vs. 36% without M-HBs>500ng/ml, P=0.01; CR in 68% vs. 29%, P=0.04). Patients with baseline L-HBs<9ng/ml were more likely to achieve HDV-RNA<100IU/ml at W48 (63% vs. 17%, P=0.01). The combination of pre-treatment L-HBs<9ng/ml plus HDV-RNA<5logIU/ml was the best predictor for achieving HDV-RNA<100IU/ml at W48 (77% vs. 35%, P=0.04). Datum confirmed also at W96 (80% vs. 18%, P=0.04).
CONCLUSIONS: Quantification of L-HBs and M-HBs along with HDV-RNA may better reflect the burden of circulating infectious virions in HBV/HDV co-infection, providing a promising tool to identify patients more likely to respond to BLV.
SOURCES OF FUNDING: PRIN2022 #2022X2KWRK; PRIN2022PNRRR #P2022WEXP2
Keywords: Hepatitis Delta Virus, HBsAg isoforms, Non-invasive biomarkers, Bulevirtide-treatment, Virological response

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Hepatitis, HIV and other blood borne viruses

Evaluation of the ACCESS anti-HBs Assay on the DxI 9000 ACCESS Immunoassay Analyzer, as an aid in the diagnosis of and determination of susceptibility to hepatitis B virus (HBV) infection

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AIM: Beckman Coulter has developed a fully automated assay for the detection of antibodies to HBV surface antigen (Anti-HBs) on a new immunoassay platform. The objective of this European study was to evaluate the performance of the ACCESS anti-HBs assay* on the CE-marked DxI 9000 ACCESS Immunoassay Analyzer as an aid in the diagnosis of HBV infection and determination of susceptibility to HBV infection in individuals prior to or following HBV vaccination.
METHODS: The clinical specificity of the ACCESS anti-HBs assay was determined using anti-HBs negative samples from blood donors and hospitalized patients and potentially interfering (cross-reacting) blood specimens. The clinical sensitivity was determined using presumed anti-HBs positive samples confirmed positive by reference assays testing, and anti-HBs positive samples from blood donors and hospitalized patients. Sample anti-HBs status was determined by the consensus of three (3) CE-marked anti-HBs assays: Abbott ARCHITECT Anti-HBs assay, Beckman Coulter ACCESS HBs Ab assay (on the DxI 800 immunoassay analyzer) and VITROS – Immunodiagnostic Products Anti-HBs assay.
RESULTS: Clinical specificity was 99.13% (95% CI, 98.36–99.54%) on a total of 1,036 anti-HBs negative samples combining 485 non-selected blood donors, 314 hospitalized patients, and 237 potentially interfering blood specimens. Clinical sensitivity on a total of 1,089 anti-HBs positive samples was 98.90% (95% CI, 98.08 – 99.37%) combining 406 presumed anti-HBs positive samples, 539 anti-HBs positive samples from blood donors, and 144 samples from hospitalized patients.
CONCLUSIONS: The newly developed ACCESS anti-HBs assay from Beckman Coulter has demonstrated high clinical sensitivity and specificity, equivalent to currently marketed anti-HBs assays.
SOURCES OF FUNDING: beckman coulter
Keywords: HBs, hepatitis B, Immunoassay, automate, analyzer, DxI 9000

PP-193
Hepatitis, HIV and other blood borne viruses

Low rate of hepatitis Delta virus (HDV) coinfection among Dutch candidate blood donors with chronic hepatitis B virus (HBV) infection

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AIM: Hepatitis Delta virus (HDV) requires the presence of hepatitis B virus (HBV) for virion assembly and secretion and increases progression to advanced liver disease and liver-related mortality. HBV/HDV-coinfected patients will likely benefit from new HDV antiviral drugs, but even in high-income countries reliable estimates on HDV/HBV coinfection are lacking due to severe underdiagnosis of HDV.
METHODS: Candidate blood donors in the Netherlands (period 2006-2023) with a newly diagnosed chronic HBV infection were retrospectively tested for HDV antibodies, and for HDV RNA if tested anti-HDV positive. Posttest counselling, HBV genotyping and HBV phylogenetic analysis were performed to determine the most likely route of HBV transmission.
RESULTS: Anti-HDV testing was performed for 200 candidate donors with chronic (HBsAg-positive) HBV infection. Donors had a median age of 43 years (IQR: 33-51), were predominantly male (67%) and non-Dutch (74%): Middle East (n=63), Asia (n=34), Africa (n=21), Eastern Europe (n=15) or Latin America (n=15). The predominant HBV genotype was D (62%) followed by A (22%), B (9%), E (4%) and C (3%) and was strongly associated with country of birth. HDV-antibodies were detected in 5/200 (2.5%) donors; HDV RNA was detected in 1/200 (0.5%) donors, a female donor from Mongolia. None of the 17 candidate donors with occult (HBsAg-negative) chronic HBV infection had evidence of past HDV infection.
CONCLUSIONS: HBV/HDV coinfection rates in donors with newly diagnosed (non-clinical) chronic HBV infection (0.5%) in the Netherlands were lower than the estimated 2-5% for low-endemic countries, even when 74% of donors originated from HDV moderate to high endemic countries.
SOURCES OF FUNDING: None
Keywords: Hepatitis D virus, Hepatitis B virus, Epidemiology

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Hepatitis, HIV and other blood borne viruses

Blood-based biomarkers for screening of Herpes Simplex Virus and HIV co-infections in outbreak-associated cases of Human Hepatitis Viruses

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AIM: HBV is a short-term disease and is one of the major causative agents of chronic liver illness. For others, it can become a long-term, chronic infection like liver disease or liver cancer. The aim of this study was to evaluate the detection rates of these viruses that induce liver damage and can progress to cancer depending on the associated epigenetic factors throught blood-based serum biomarkers for screening and monitoring of Brazilian patients. So, it addressed an analysis of viral hepatitis A/B/C, Herpervirus Simplex (HSV) type 1/2 and the human immunodeficiency virus (HIV) as topics related to comprehensive care for people with sexually transmitted infections (STIs).
METHODS: This is a descriptive epidemiological study, which evaluated outbreak-associated cases of Human Hepatitis Viruses (HAV/HBV/HCV), correlated with Herpesvirus and HIV. For screening assays were carried out using the Chemiluminescence method – CLIA. The presence (reactive) or absence of anti-HBV and HCV antibodies (non-reactive) was also investigated by the Electrochemiluminescence method - ECLIA. Molecular assays (RT-PCR and Western Blotting) must be performed to confirm positive results.
RESULTS: A total of 2.750 samples were collected from 2.713 patients, of which 38,43% were for HCV; 31,20% for HIV research; 30,25% for HAV and 0,10% for HSV. In all, eight biomarkers of HBV were investigated, of which the HBsAg marker was non-reactive in 44,01%. About 31,41% were reactive for anti-HBs.
CONCLUSIONS: The expression of simultaneous positive results indicating the presence of IgM antibodies for different etiological agents does not rule out the possibility of cross-reaction among them.
SOURCES OF FUNDING: AllLab
Keywords: Outbreak-associated, blood serum, biomarkers, hepatitis viruses, clinical virology

PP-195
Hepatitis, HIV and other blood borne viruses

Verification of a commercial anti-hepatitis E virus IgG and IgM immunoassay
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AIM: The aim was to verify the Roche Elecsys anti-HEV IgG and IgM immunoassays (double antigen sandwich chemiluminescent assays) on Cobas® 8000 for routine use.
METHODS: The precision of the assay was evaluated by ten repeated measurements of quality control (QC) material (PreciControl Anti-HEV IgG and IgM) at two levels, one positive and one negative. Reproducibility was assessed by measuring the same QC materials twice a day for ten consecutive days. Results were compared with CV's from the QC material inserts (between brackets). Qualitative correlation between the Elecsys assay and the assay used by the Belgian National Reference Centre (NRC) for hepatitis (ELISA) was studied by comparing results of ten IgM or IgG positive and ten negative patient samples.
RESULTS: The IgG assay had a precision of 1.0% (1%) and 0.8% (0.8%) for the negative (level 1) and positive (level 2) QC respectively. Reproducibility was 1.7% (1.2%) at level 1 and 1.4% (1.2%) at level 2. The IgM assay had a precision of 1.8% (1.9%) and 2.1% (2.6%) for level 1 and 2 respectively. Reproducibility was 1.5% (5.5%) for level 1 and 2.4% (5.7%) for level 2. Correlation of the Elecsys assays with the NRC method was perfect (κ 1).
CONCLUSIONS: All criteria for verification of the HEV IgG and IgM immunoassay are met, except for the reproducibility of the IgG assay. However, CV's for reproducibility at level 1 and 2 were lower than 5%, an international acceptable CV for serological assays. Therefore, the assays can be used for routine analysis.
SOURCES OF FUNDING: Not applicable
Keywords: Hepatitis E serology

PP-196
Immune response and vaccines

Novel candidate peptide vaccine against HCV infection

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AIM: Hepatitis C Virus (HCV) is a worldwide public health. Neutralizing monoclonal antibodies that target HCV epitopes encompassing amino acids (aa) of the E2 protein. This study was designed to assess the immunogenic properties of genetically conserved peptides derived from the C-terminal region of the hypervariable region-1 (HVR-1) and to detect the presence of antibodies reacting with this peptide sequence in natural infection.
METHODS: One candidate peptide was selected based on sequence conservation among E2 sequences recorded on the HCV data base. Twenty-five serum samples from chronic HCV patients testing anti-HCV positive and 25 samples from healthy individuals who tested negative for anti-HCV antibodies were used to establish the reactivity of these blood serum with the synthetic peptide.
RESULTS: Several samples of patients reacted with the peptide BSA in different dilutions. So E2-peptides were able to recognize specific immunoglobulins some in chronic HCV patients. This epitope was able to induce humoral immune responses during the infection of the studied patients and that E2 conserved peptide used represent essential components of a candidate peptide vaccine against HCV infection.
CONCLUSIONS: Genetic variations particularly within the hypervariable region-1 and low titers of anti E2 or interference of non-neutralizing antibodies with the function of neutralizing antibodies may be associated with not protection against HCV. These findings suggest the detection of anti E2-peptide immunoglobulin in chronic HCV patients as potential therapeutic and/or prophylactic vaccines against HCV infection since most antiviral therapies fail and anti HCV vaccine is not currently available.
SOURCES OF FUNDING: National Council for Scientific and Technological Development
Keywords: Hepatitis C Virus, conserved peptides, vaccine, humoral immune responses

PP-197
Immune response and vaccines

Production of rabbit specific antibodies against conserved and potentially immunogenic peptides of the HCV envelope glycoprotein E2

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AIM: Hepatitis C virus (HCV) is a chronic infection that cause of hepatocellular carcinoma and end-stage liver diseases. Envelope (E1 /E2) proteins of HCV may generate neutralizing antibodies. At the end N-terminus of the E2 protein is a region characterized by a high degree of variability called hypervariable region 1 (HVR1), which corresponds to the 27 amino acids of the E2 protein, very important in neutralizing HCV. This study was designed to assess the immunogenic properties of conserved peptides derived from E2 HVR1 regions. Nucleotide sequences of all available HCV E2 region in the databases were aligned and four amino acid sequences with immunogenic potential were selected.
METHODS: We selected four neutralizing peptides from the sequence AJ238799 Japanese HVRI in different positions of aminoacids: peptide 1 (aa412-149), peptide 2 (517-531), peptide 3 (aa 523-535) and peptide 4 (aa 373-416). These peptides were synthesized conjugated with KLH and ressuspended in PBS (2µg/µL). These synthetic peptides located downstream of the hypervariable region I within the HCV E2 protein were inoculated into the five rabbits being one peptide for each animal.
RESULTS: All peptides shown to be highly immunogenic and neutralizing with detectable levels of specific antibodies appeared at the first determination 15 days post immunization increasing after 30 days showing better response to the peptide 4(aa 373-416). The serum at high concentrations showed high level of cross-reactivity.
CONCLUSIONS: All the designed peptides were able to generate anti-E2 specific antibodies in rabbits at relatively high titles.
SOURCES OF FUNDING: National Council for Scientific and Technological Development
Keywords: neutralizing antibodies, immunogenic peptides, nucleotide sequences, vaccines, Hepatitis C virus

PP-198
Immune response and vaccines

The investigation of immunogenic activity of the adenovirus vector
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AIM: Human adenovirus type 5 (Ad5) is often used as a vector in the development of new vaccines against SARS-CoV-2. The presence of specific neutralizing antibodies (nAb) to Ad5 can potentially lead to a decrease in the effectiveness of revaccination with such vaccines. The focus of this research is to investigate the changes of the anti-Ad5 nAb titers following vaccination against COVID-19.
METHODS: Blood sera from 302 individuals (median age of 40.5, 175 males) received one dose of the Ad5-nCoV vaccine were collected on the day of vaccination (D0), 28 days after vaccination (D28). A standard in vitro neutralization assay with 10-fold diluted sera was conducted utilizing A549 cells and Ad5 (ATCC, VR-5). To detect Ad5 in the A549 cells the ELISA with monoclonal antibodies to the Ad hexon was utilized. If Ad5 was detected at a dilution of 1:10, the nAbs titer in the sera was assigned as 1:5.
RESULTS: At D0 anti-Ad5 nAbs were detected in 40% (121/302) of the sera. The majority of sera had anti-Ad5 nAbs titers ≤1:160, geometric mean titer (GMT) 4,3.
At D28 the number of sera with anti-Ad5 nAbs increased, reaching 63,9% (193/302). Among these, 42,7% (129/302) had titers ≤1:160 and 21,2% (64/302) had anti-Ad5 nAbs titers >160, GMT 20,3.
CONCLUSIONS: The administration of the Ad5-vector vaccine leads to the development of nAb against the vector. These findings highlight the importance of considering the titers of nAb to Ad in the development and utilization of new vaccines or reusing existing ones based on Ad vectors.
SOURCES OF FUNDING: None.
Keywords: Human adenovirus 5, Adenovirus vector, Neutralizing antibodies, Vaccination, COVID-19, Immunogenicity

PP-199
Immune response and vaccines

Anti-Dengue Virus-Specific IgM and IgG Antibodies in Acute and Convalescent Serum Samples from Patients with Confirmed Dengue disease, Jazan region, The South West of Saudi Arabia

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AIM: Dengue virus (DENV) is a growing public health problem, with over 4 billion people at risk in 128 countries, including Saudi Arabia. Since 2005, Jazan region has affected by many DENV outbreaks with the highest number of cases was reported in 2019 (1,654 cases) followed by (876 cases) in 2020. Hence, measuring of DENV antibodies levels in infected patient serum samples during acute and convalescent phases may help to understand the kinetics of antibody response and their role in protection against infection and/or severe disease. This study aims to measure anti-DENV IgM and IgG antibodies in acute and convalescent serum samples from confirmed infected patients.
METHODS: An ELISA technique was used to measure anti-DENV antibodies in serum samples of infected patients, during acute (n=155) and convalescent (n=155) phases.
RESULTS: Through measuring anti-DENV antibody levels in patients with confirmed DENV infection, high level of anti-DENV IgM and IgG antibodies in serum samples were detected. A positive correlation between the anti-DENV IgM and IgG antibody levels and age were observed.
CONCLUSIONS: High levels of anti-DENV IgM and IgG antibody levels suggest the development of antibody-mediated immunity in individuals following infection which may protect against future new infections. The positive correlation between anti-DENV IgM and IgG antibody levels and patient's age were observed and that suggests an age-associated increase in anti-DENV antibody responses. These findings confirm that antibodies are induced in most patients during early exposure (IgM) and convalescent phase (IgG) are likely to play important roles in fighting the DENV infection.
SOURCES OF FUNDING: No sources of funding.
Keywords: Dengue virus, DENV, ELISA, IgM and IgG antibodies, Acute phase, convalescent phase.

PP-200
Immune response and vaccines

Is the high-dose influenza vaccine better for everyone over 60 years of age?

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AIM: Elderly population is more susceptible to seasonal influenza, with increasing mortality and decreasing seroprotection with age. Some guidelines exclusively recommend HD-IV for elderly individuals. A recent meta-analysis showed increased rVE of HD-IV vs SD-IV with age, with people ≥75 years old better protected than people 65-74 years old. We analysed real-world evidence comparing the effectiveness of HD-IV and SD-IV, focusing on age subgroups data.
METHODS: Qualitative literature search was performed to identify the articles describing the rVE of HD-IV vs SD-IV against hospitalization and lab-confirmed influenza cases within the elderly population, specifically, within age sub-groups (65-74; 75-84; >85).
RESULTS: Of 39 articles retrieved, 5 matched our criteria (Izurieta, Thadani et al. 2015, Richardson, Medvedeva et al. 2015, Young-Xu, Van Aalst et al. 2018, Lu, Chillarige et al. 2020, Doyle, Beacham et al. 2021).
RESULTS show rVE of HD-IV in preventing influenza or pneumonia-associated hospitalizations, reducing hospital admissions and ER visits, consistently increased with age, and was more effective than the SD-IV for people ≥ 85. However, subgroup analysis from one study did not show any difference between age groups 65-74 and ≥75 (Doyle, Beacham et al. 2021).
CONCLUSIONS: HD-IV showed the most benefit in ≥75 years and older, so it would be appropriate to recommend it for this age sub-group, allowing the use of SD-IVs in 60-74 age group. With such prioritization, the existing resources could be used optimally to increase vaccination coverage in the elderly group without sacrificing the protective effect of the population.
SOURCES OF FUNDING: Viatris
Keywords: Influenza Vaccines, hospitalization, Elderly, Comparative Effectiveness Research, high-dose influenza vaccine, standard-dose influenza vaccine

PP-201
Immune response and vaccines

Economic benefits of a workplace vaccination program against influenza: a case study series for local public transportation, air- and railways companies across Europe

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AIM: Effectiveness of influenza vaccines is established. Although there is a significant emphasis on vaccinating at-risk individuals, working-age adults receive less attention.
We modelled the economic impact for employers of workplace vaccination programmes in the public transport sector in Europe for the upcoming flu season 2024/25.
METHODS: A machine learning enabled SIR model, trained on historic flu data, was run for different vaccination and population scenarios, and combined with a country-specific ARIMA influenza forecast, to predict wave timing and magnitude for the upcoming flu season
Publicly available company reports provided workforce descriptors (age, banding by contact risk, mean salary). Available literature provided country-specific data on absenteeism, sick days, productivity loss. Historic data were used for vaccine effectiveness
We run six workplace scenarios in large local, railways, airways business (>10,000 employees) in four European countries.
RESULTS: Across the different companies, more than 60% of the employees were 18-49 years of age, and more than 65% were frontline workers.
In all scenarios, increasing vaccination rates led to a progressive reduction in the number and proportion of influenza infections, as reflected by the decrease in the number of sick days due to influenza in each company. Costs for sick days decreased accordingly. The balance between vaccine cost and savings from sickness prevention always supported the vaccination programme. The point estimate varies depending on vaccine coverage, mean company salary, and mean number of days off from sickness.
CONCLUSIONS: Vaccinating healthy adult workforce against influenza showed drastic medical productivity and economic benefits from an employer's perspective.
SOURCES OF FUNDING: Viatris
Keywords: Influenza; vaccine; workplace vaccination; economic benefits

PP-202
Immune response and vaccines

Antigen specific VZV reactive T-cells –Effect of the antigen type depending on the clinical question
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AIM: Infections caused by varicella-zoster virus (VZV) are typically pediatric diseases (Chickenpox). After primary infection, VZV is remaining dormant in human ganglia until reactivation decades after the initial infection. The resulting Herpes zoster is characterized by painful skin rash but also can affect other organs and may cause lethal complications. Due to this complex disease pattern, a broad-spectrum immune-monitoring beyond serological standard analysis is needed.
METHODS: With this proof-of-concept study we show various pattern of T-cell immune reactions against different types of VZV-antigens depending on the age and vaccination status of healthy donors (HD). An EliSpot assay has been used for detection of interferon-gamma-releasing-T-cells after stimulation with VZV lysate, native glycoproteins, recombinant glycoprotein E (gE) and synthetic peptide pools specific for lytic and latent immune response.
RESULTS: HD <50y are showing a strong reaction to all the tested antigens expect the synthetic lytic peptides. After vaccination, a strong immune-reaction against recombinant gE and lytic peptides is detectable. In samples from unvaccinated donors older than 60y the number of VZV reactive T-cells decreases dramatically. In this samples mainly weak reactions against the lysate could be observed.
CONCLUSIONS: Notwithstanding the small sample size of this study, clear age-related differences can be observed. We conclude that VZV lysate and native glycoproteins are unsuitable for the measurement of immune-response during or after reactivation in adolescent patients. For the determination of pathological changes in immune state, synthetic peptides are mandatory. Regardless, VZV lysate may be used as indicator to find the accurate moment for vaccination in adults.
SOURCES OF FUNDING: not available
Keywords: T-cells, VZV, vaccination, cytokine, EliSpot, IGRA

PP-203
Immune response and vaccines

Adapting LIBRA-seq/BEAM to whole BK polyomavirus particles to identify broadly-specific human monoclonal antibodies

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AIM: Data on B-cell repertoires against naked viruses, including polyomaviruses (PyV) is relatively sparse. Our objective was therefore to develop and validate a double fluorescence and oligonucleotide labeling of virus like particles (VLPs), in order to apply the LIBRA-seq/BEAM approach to these viruses. The aim of this strategy is to discover broadly neutralizing human monoclonal antibodies against BK polyomavirus (BKPyV), which could represent candidates for clinical development.
METHODS: Fluorescence- and oligonucleotide-labeled VLPs were produced in mammalian cells, using a streptavidin-biotin binding system based on 10X genomics BEAM reagents. VLPs were characterised by SDS-PAGE and electron microscopy, and their antigenic and receptor-binding properties were validated by ELISA and flow cytometry. A 10X Genomics scRNAseq experiment on PBMCs from four kidney transplants recipients, stained with double-labeled BKPyV (genotypes gIb2, gII, gIII, gIV) and murine PyV VLPs was performed.
RESULTS: The antigenic properties of BKPyV VLP-streptavidin-oligonucleotide complexes were conserved, as were their binding properties to viral receptors on 293TT cells. Paired light and heavy chain BKPyV-specific BCR sequences were obtained for 588 cells. Overall, 82,3% of B-cells bound only BKPyV gIb2, 4,8% bound gIb2 and gII, 4,3% bound gIb2 and gIV, 1,7% bound gIb2, gIII and gIV, and 3,4% bound all four BKPyV genotypes.
CONCLUSIONS: To conclude, we validated that complete VLPs, and not just individual virus proteins, can be double-labeled with fluorophores and oligonucleotides and used in LIBRA-seq/BEAM. This technique can be used to directly identify broadly binding antibodies within repertoires, and to compare functional repertoire breadth between individuals.
SOURCES OF FUNDING: ANR-22-CE15-0043 and Région Pays de la Loire
Keywords: Humoral immunity, monoclonal antibody, scRNA-seq, BK polyomavirus, VLPs

PP-204
Immune response and vaccines

Baseline gene expression differentiates the T cell response kinetics to BNT162b2 vaccination

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AIM: SARS-CoV-2 specific T-cells have been shown as an important correlate of protection against COVID-19. Detectable levels of these cells as early as day 10 after COVID-19 mRNA vaccination has been shown to coincide with the onset of vaccine efficacy. However, the kinetics of the T-cell response to vaccination is heterogeneous and the reason for this heterogeneity is not understood.
METHODS: Baseline and post-vaccination blood samples were collected at pre-determined time points from 27 healthcare workers who received the BNT162b2 vaccine. Whole blood bulk RNA sequencing was carried out to determine gene expression before and after vaccination. Spike-specific T cells were measured using IFN-gamma ELISpot.
RESULTS: We identified two subsets of subjects: fast responders who displayed high Spike-specific T-cell counts and slow responders who showed low T-cell counts on day 10. RNA sequencing of baseline blood samples revealed higher expression of genes involved in type I interferon regulation and viral defence pathways in fast compared to slow responders. Furthermore, many of these genes were highly expressed in myeloid cells, indicating that individuals with high interferon and inflammation endotypes may influence T-cell kinetics to mRNA vaccination.
CONCLUSIONS: The kinetics of T-cell response to vaccination and possibly also infection may be influenced by baseline endotypes. Cytokine and immune cell subset analyses are underway that may reveal further insights into baseline endotypes on cytokine responses to mRNA vaccines.
SOURCES OF FUNDING: Finnish Cultural Foundation, Finnish Foundation for Veterinary Research, NMRC Singapore OF-IRG grant [MOH-000610-00]
Keywords: t-cell response, sars-cov-2 vaccination, transcriptomics

PP-205
Pandemic preparedness and health services research

Exploration of the SARS-CoV-2 pandemic from the perspective of nursing home staff in Hesse, Germany
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AIM: This study aimed to explore the perception of the pandemic after its conclusion, the current situation in nursing homes and recommendations for future challenges from the perspective of nursing home staff.
METHODS: We used a purposive sampling strategy to conduct semi-structured interviews with 26 members of staff of different professions at two nursing homes in Hesse, Germany. Data collection at a third nursing home is currently ongoing. Interviews were analysed using reflexive thematic analysis.
RESULTS: Three main themes were developed by the research team. (1) “I remember exactly how it all began” sheds light on the specific details of how the pandemic started to change every aspect of daily life in the nursing homes. The significance is emphasised by most staff remembering the exact day and moment, especially of the first outbreak at their site. (2) “Us against the world” refers to the strong sense of team spirit, mutual understanding and of working towards a common purpose among staff. Often, this was contrasted against the outside world of relative normality. (3) “We’re experts now” depicts the development over time from a situation when everything was new and unknown to a strong sense of expertise and preparedness for future challenges among staff.
CONCLUSIONS: While results are still preliminary, they show common themes across nursing homes and professions. They stress the significance and all-encompassing nature of the pandemic from the nursing home perspective, and point towards potential priority areas for future pandemic preparedness.
SOURCES OF FUNDING: No external funding
Keywords: SARS-CoV-2, pandemic, nursing homes, evaluation

PP-206
Pandemic preparedness and health services research

How Venezuela faced the identification of variants and genomic surveillance of SARS-CoV-2

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AIM: Despite effective vaccines against SARS-CoV-2, responsible for the COVID-19 pandemic, variants emerged with increased ability to transmit and evade immunity. Five Variants of Concern (VOCs), and several Variants of Interest (VOIs) have been identified. All the VOCs circulated in Latin America (LA), and the Gamma VOC emerged in Brazil. Two VOIs also emerged in the region (Lambda and Mu), in Peru and Colombia, respectively. The World Health Organization (WHO) recommended genomic surveillance of these variants through whole-genome sequencing.
METHODS: Venezuela adopted a combined strategy for the identification of variants, based on alternative partial and complete viral genome sequencing, allowing to processing of a higher number of sequences (more than 10.000 partial sequences and more than 1400 complete genome sequences).
RESULTS: This strategy allowed to identify the introduction in 2021 of the Gamma VOC (Eastern region of the country), followed by the introduction of the Mu VOI in the Western States. Then the Delta VOI displaced the previous variants. In 2022, the partial genome sequence allowed the differentiation of the major sub-lineages BA.1 to BA.5 of the Omicron VOC. However, the increasing diversity of these isolates reduced the usefulness of partial genome sequencing, and the identification of sub-lineages is now based exclusively on complete genome sequencing. The year 2023 was characterized by the predominance of XBB.1.5 and its sub-lineages. In 2024, the main circulating variant is JN.1 and its sub-lineages.
CONCLUSIONS: The rational combined strategy proved to be very useful for monitoring the emergence and evolution of SARS-CoV-2 variants in Venezuela.
SOURCES OF FUNDING: MINCYT Venezuela
Keywords: SARS-CoV-2, Variants, genomic-surveillance, Venezuela, combined-strategy, sub-lineages

PP-207
Pandemic preparedness and health services research

Monkeypox virus detection in Bulgaria

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AIM: The study aimed to determine the pathogenesis of the monkeypox virus (MPXV), its genetics, route of transmission, methods, and clinical material for laboratory detection.
METHODS: A total of 181 clinical materials from 49 patients with possible monkeypox (mpox) were investigated. The tested clinical materials were: samples of vesicle contents, pustules, nasal swabs, urine, and ejaculate and were taken in the different periods after onset of clinical symptoms. Molecular genetic methods were used to detect the G2R (TNF receptor gene) region of the MPXV genome and the rpo18 gene of the orthopoxvirus genome.
RESULTS: From the total 181 tested in 20 clinical materials were both proven MPXV and OPXV DNA - 6 vesicle contents, 4 pustules, 6 nasal swabs, 2 urine, and 2 ejaculates, respectively. The MPXV and OPXV DNA were demonstrated in at least two different clinical specimens from each patient. The infected were six men between 29 and 44 years of age. The study showed MPXV DNA retention in clinical samples up to two weeks after the onset of clinical symptoms.
CONCLUSIONS: Despite the known epidemiology and clinic of MPOX, the human-to-human spread of the virus after May 2022 prompted the WHO to further investigate the virus transmission, genetics, and mutagenicity. The laboratory and epidemiological surveillance of cases is critical to contain the outbreak and to know the new fast transmission of the virus.
SOURCES OF FUNDING: The study was financed by the National Science Fund, research project: "Monkeypox in Bulgaria – clinical manifestation, transmission, virological and molecular genetic analysis" (Contract No. КП-06-H63/13 dated 03.07.2023).
Keywords: MPOX, orthopoxvirus, RT-PCR

PP-208
Respiratory viruses

Common respiratory viruses dynamics during the last 5 years period: How has it changed?

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AIM: The aim of this study is to investigate the annual changes in respiratory viral pathogens other than SARS-CoV-2 detected by multiplex PCR testing over the past five years based on our laboratory.
METHODS: Samples collected from patients who admitted to our hospital with RVI between December 2018-December 2023 were tested using respiratory viral panel (RVP). The distrubition of Influenza A(H1N1,subtypes H3,untyped),Influenza B,Adenovirus,Bocavirus, Seasonal Coronaviruses(NL63-229E-OC43-HKU1),Enterovirus,Parechovirus,Rhinovirus, Human respiratory syncytial virus A/B, and Parainfluenza virüs (PIV1-4) were retrospectively evaluated.
RESULTS: The overall positivity rate in RVP was 41.5% (4016/9664). Rhinovirus was the predominant pathogen, detected in 36% of cases, followed by RSV at 18%, Influenza A at 17%, and Adenovirus at 8% and multiple viral agents were detected in %22 of the samples.When analyzed by years, Rhinovirus was detected most commonly in all years.
CONCLUSIONS: Consistent with many studies reporting data for the same time period, our study identified Rhinovirus and RSV as the most common respiratory viral pathogens. Non-pharmacological interventions during the early pandemic period led to a decrease in the prevalence of all respiratory viruses, particularly Influenza viruses, in 2020-2021. In the following years (2022-2023), due to the decrease in exposure to these pathogens, the abolition of pandemic measures and the rapid change in social behaviors, a significant increase in positivity and co-circulation of respiratory viruses was observed, although their seasonal incidence characteristics were preserved. These temporal changes in the epidemiology of respiratory viruses need to be closely monitored to guide the prevention and treatment of RVI.
SOURCES OF FUNDING: None
Keywords: Respiratory virus, annual changes

PP-209
Respiratory viruses

Phylogenetic analysis of influenza B virus strains circulating in northern Greece in the 2023-2024 season
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AIM: Influenza B viruses, according to global annual epidemiological data, circulate mainly at the beginning and at the end of each influenza season. In Northern Greece 2023-2024, shortly after the middle of the season an increase in positive cases of type B was noticed, compared to European data. The present study aims at the phylogenetic analysis of B strains detected during the 2023-24 season, thus attempting the detection of probable genetic variations that might have led to the unexpected B virus circulation pattern.
METHODS: Sanger method was used to analyze 22 influenza B strains from nasopharyngeal swabs, amplifying the HA-1 gene. The analysed samples represented 10% of the total B positive samples of the National Influenza Centre for Northern Greece. Data were processed with the BioEdit tool, using the current vaccine strain as reference. A phylogenetic tree was created with the MEGA software, and also included strains that circulated according to latest epidemiological reports of the ECDC.
RESULTS: All the analysed viruses were classified into already existing phylogenetic branches. Among the 22 samples, 2 belonged to the V1a.3a.2(C.5), and 3 to the V1a.3a.2(C.5.7) clade. The remaining (17) viruses belonged to the V1a.3a.2(C.5.6) clade, and had been detected in various periods of the influenza season.
CONCLUSIONS: The strains that circulated in Northern Greece were similar to those of the rest of Europe. The observed uncommon circulation pattern was probably due to the first meta-pandemic season, for which various epidemiological particularities were already foreseen globally.
SOURCES OF FUNDING: Hellenic National Public Health Organisation
Keywords: Influenza B virus, surveillance, variations, sequencing, epidemiology, respiratory viruses

PP-210
Respiratory viruses

RSVTyper: A multiplex tiling approach for amplification, sequencing and downstream analysis of Respiratory Syncytial Virus

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AIM: Respiratory Syncytial Virus (RSV) can cause severe respiratory infections, especially in infants and the elderly. There are two RSV subtypes and multiple genetically distinct strains that spread through the population and multiple infections during lifetime are common. A vaccine is available since 2023, however, it is not yet known if vaccine escape viruses can occur and what effect the vaccine will have on strain distribution. The aim of this work was to establish a whole genome RSV sequencing and data analysis pipeline for enhanced RSV surveillance.
METHODS: RSV-A and RSV-B specific, degenerated PCR primers were designed for multiplex tiling PCR and tested in 222 RSV isolates from 2008-2023. PCR products were Nanopore sequenced and analyzed with the RSVTyper. The RSVTyper pipeline (https://anaconda.org/bioconda/rsv-typer) is available as a bioconda package. It implements (i) subtype detection, (ii) duplication detection (iii), within-subtype reference selection, (iv) variant calling and generation of the consensus sequence using the ARTIC pipeline and (v) clade assignment using nextclade.
RESULTS: Amplification and whole genome sequencing (>90% genome coverage) was successful in 190 of 222 samples (86%), including samples from before 2010. 80 isolates were RSV-A and 110 isolates were RSV-B. The subtype distribution corresponded to the previously published distribution for the years 2008-2022.
CONCLUSIONS: We successfully developed a routine sequencing and analysis pipeline for pan-subtype sequencing of RSV. This pipeline can be used for high throughput RSV sequencing for enhancing RSV surveillance.
SOURCES OF FUNDING: The project was funded by internal funding and the Robert-Koch Institute (Projekt#910003)
Keywords: Respiratory Syncytial Virus (RSV), whole genome sequencing, surveillance

PP-211
Respiratory viruses

Genomic biodiversity of SARS-CoV-2 strains identified during COVID-19 pandemic: single-center study, Bosnia and Herzegovina

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AIM: The study aimed to characterize the complete genomes of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) detected in the Clinical Center of the University of Sarajevo, OU Clinical Microbiology, Sarajevo, Bosnia and Herzegovina.
METHODS: The whole genome sequencing was carried out using the “sequencing by synthesis” platform on 69 SARS-CoV-2 highly positive respiratory samples collected from February to December 2021.
RESULTS: Among the obtained complete genome sequences, 7 clades/lineages of SARS-CoV-2 were identified and presented in GISAID/Pango/WHO nomenclature in chronological order: G (3/69, 4.35%; 1/3 B.1.258 and 2/3 B.1.258.17), GH (1/69, 1.45%; B.1.160), GRY (23/69, 33.33%; B.1.1.7; Alpha), GV (1/69, 1.45%; B.1.177), GR (4/69, 5.80%; 3/4 B.1.1.318 and 1/4 C.36.3), GK (26/69, 37.68; 22/26 AY.4, 2/26 B.1.617.2, 1/26 AY.27 and 1/26 AY.43; Delta), GR (1/69, 1.45%; C.37; Lambda), and GRA (10/69, 14.49%; 5/10 BA.1, 3/10 BA.1.1, and 2/10 BA.1.17.2; Omicron). Nucleotide sequence alignment revealed a total of 9 mutations in the nucleocapsid (N) gene and 53 mutations in the spike (S) gene across the genomes. The presence of the S_D614G mutation (known to increase the infectivity of the virus) in each sequence confirmed its fitness advantage. The most common substitutions detected in the clades G, GK (Delta), and GRA (Omicron) were S_G142D, reported to be related to antigenic drift, and S_T478K, known to have mild potential to increase the surface expression of the receptor binding domain.
CONCLUSIONS: Genomic analysis put into the time and place frame could provide information to understand the biodiversity of the SARS-CoV-2 virus.
SOURCES OF FUNDING: None.
Keywords: SARS-CoV-2, genomics, mutation, biodiversity, COVID-19

PP-212
Respiratory viruses

Surveillance insights of human Parechovirus (HPeV) respiratory infections among infants in Barcelona (2021-2022)

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AIM: Human Parechovirus (HPeV) is a member of the Picornaviridae family, the prevalence of which is not often studied, especially when its diagnosis is not routinely performed. The goal of this study is to report the single HPeV infection in <1-year-old children during 2021-2022 in respiratory samples and describe their epidemiological features.
METHODS: Respiratory specimens collected at the Hospital Universitari Vall d''Hebron (Barcelona) from week 36/2021 to week 39/2022 and negative for the routine diagnosis of all respiratory viruses were selected. Detection of HPeV was performed using a commercial real-time RT-PCR assay (Allplex™ Meningitis-V2 Assay, Seegene). Positive cases were further characterised by a phylogenetic analysis of the partial VP3/VP1 coding region sequences.
RESULTS: Out of a total of 1,200 negative samples, 1,017 (84.75%) were finally available and tested for HPeV laboratory-confirmation, of which 5 (0.5%) samples were positive and further characterised as HPeV-1 (2/5, 40%), HPeV-3 (1/5, 20%) and HPeV-5 (2/5, 40%). These hPeV-positive samples were collected at different times (October, November, June, and July) from infants aged 3 days to 11 months.
CONCLUSIONS: Herein, a low presence of HPeV within the most vulnerable population was observed in respiratory samples, correlating with the higher circulation of HPeV in uneven-numbered years previously reported in Spain (doi: 10.3201/eid3002.230647). The HPeV-positive cases detected corresponded to the months in which the virus has shown to be most prevalent (summer-autumn). However, HPeV-5 could be identified in our series, consistent with its circulation mainly in Spain (doi: 10.3201/eid3002.230647).
SOURCES OF FUNDING: No funding
Keywords: human parechovirus, pediatric population, respiratory viruses, molecular epidemiology

PP-213
Respiratory viruses

Monitoring the impact of SARS-CoV-2 variants on diagnostic cycle threshold values of target genes
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AIM: Cycle threshold (Ct), used as a surrogate marker of viral load, is helpful in decisions about patient isolation, disease severity and can provide information on the variant present in the sample as the accumulated genetic changes may cause diagnostic failure as was reported by S-gene target failure for at least one RT-PCR assay. We investigated the positivity rate in relation to gender, age and Ct values during the pandemic when different variants were in circulation.
METHODS: Data from samples collected for routine diagnostics in the NVRL was extracted from the LIMS system. Statistical analysis was performed using MedCalc statistical software version 19.0.5.
RESULTS: Samples were analysed with Ct values for 3 gene targets; Orf 1a/b, N and S genes. The positivity rates varied from 10.87% to 12.85% to 38.6% respectively. Although the number of samples received was higher in females at 56%, 53% and 67% respectively, the positivity rate was higher in males. Analysis of the proportion of samples with high viral loads (Ct<25) showed that the Orf1a/b gene was 79.5, 64.3 and 75.9%, N gene was 71.4, 60.5 and 74.7% and S gene Ct was 68.2, 69.7 and 72.3% during the 3 study periods. Chi-square analysis revealed significant differences between the alpha and delta waves for Orf1a/b ($p<0.002$) and N gene targets ($p<0.02$).
CONCLUSIONS: Our findings show an incremental increase in positivity rates corresponding to the emergence of different SARS-CoV-2 variants indicating upcoming surges despite the introduction of vaccination. The higher rates in males could inform targeted preventative measures.
SOURCES OF FUNDING: Pfizer (Grant 76774125).
Keywords: Cycle threshold (Ct), SARS-CoV-2, variants, age, gender

PP-214
Respiratory viruses

Environmental surveillance of elevator floors detects influenza clinical trends
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AIM: Our environmental public health surveillance program aimed to leverage elevator floor samples to quantitatively monitor temporal trends of influenza circulation in an urban New York City college campus during the prolonged 2023-2024 season. Migration corridors are a long-studied subject in ecology. These physical locations experience high animal traffic and density because of geographical or functional constraints. We sought to identify, on the scale of the human built environment, locations where traffic and density increased the sensitivity of environmental surveillance for viruses.
METHODS: Our team monitored both dormitories and community buildings multiple times a week for the length of an academic year. Surface swabs were performed on elevator floors in each location. Respiratory and gastrointestinal virus levels were measured using RT-qPCR assays.
RESULTS: Elevators floors indeed captured and chronicled levels of viral RNA shed by positive cases. Both weekly test positivity and cycle threshold values correlate with confirmed clinical tests in surrounding New York county. Comparisons may also be made with clinical testing performed at the university primary care health service. We were able to detect the start of the influenza season, determine trends of increasing or decreasing viral levels, and observe the extended circulation that persisted through 2024.
CONCLUSIONS: Surface monitoring provides strengths and weaknesses relative to other methods such as wastewater surveillance. Our data argue that similar migration corridors could be monitored at the local level without the need for significant organizational infrastructure. This feasibility could empower site-specific public health efforts.
SOURCES OF FUNDING: We thank Barnard College for supporting this project.
Keywords: influenza, surveillance, elevators

PP-215
Respiratory viruses

Human Respiratory Syncytial virus (hRSV) and codetected viruses: characterisation and clinical related data from infected children

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AIM: From a public health perspective, viral respiratory infections in children pose significant challenges. The diversification of vaccines, and monoclonal antibodies for the management of respiratory infections requires epidemiological monitoring of the responsible viruses. During the 2022-2023 hRSV epidemic, we analyzed clinico-virological information relating to children presented to the hospital for RSV infection.
METHODS: We randomly selected 50 patients with hRSV respiratory infection to cover the epidemic period. During the emergency room visit, the first virological results were obtained through the use of a multiplex respiratory panel, ePlex, and clinical data were recorded. The Illumina Respiratory Virus Enrichment Kit was then used to analyse the complete genome of hRSV and of the codected respiratory viruses
RESULTS: We obtained clinico-virological results for 47 out of the 50 included patients. The median age of the children was 1.98 months, ranging from 4 to 35 months. At the emergency room, clinical signs present included cough (44, 95.7%), tachypnea (33, 70.2%), rhinitis (43, 91.5%). RSVA was detected in 4 cases (8.5%) and RSVB in 43 patients (91.5%). The whole-genome NGS data allowed the characterization of RSV and the codetected viruses. Discrepancies between the eplex results and NGS multiplex were identified for the RSV type A or B. There was no palivizumab or nirsevimab mutations identified in hRSV analysed.
CONCLUSIONS: This approach enables rapid genomic surveillance, understanding how the pathogen evolves and the impact this could have on diagnostic or therapeutic efficacy. The method could be easily implemented in a routine laboratory if cost constraints are not considered.
SOURCES OF FUNDING: university
Keywords: hRSV, NGS sequencing, epidemiology

PP-216
Respiratory viruses

Genetic characterization of adenoviruses in pediatric population, Greece, 2022-2023

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AIM: Adenoviruses cause a wide range of human infections. They are highly contagious, and more common in children. Aim of the study was the detection and genetic characterization of the adenoviruses that caused a variety of infections in hospitalized children during 2022-2023.
METHODS: Blood, stool, and/or rhinopharyngeal samples from 43 children aged 9 months to 10.5 years (median 4 years) admitted to Ptolemaida hospital due to febrile illness, mainly purulent tonsilitis, and/or gastroenteritis were sent to our laboratory for adenovirus testing. A real-time PCR assay was initially applied for virus detection, followed by a nested-PCR combined with Sanger sequencing for typing of the viral strains. Next generation sequencing (NGS) was applied on representative positive samples using the Ion Torrent PGM platform.
RESULTS: Adenoviruses were detected in 25 of the 43 (58.1%) children [7/14 (50%) in 2022 and 18/30 (60%) in 2023]. The median age of the affected patients was 3.25 years. In mid-2023, human adenovirus 2 (mastadenovirus C) was replaced by adenovirus B3. A phylogenetic tree based on whole genome sequences of representative strains was constructed; the closest sequences for adenovirus 2 and B3 were MH121084 (99.78%) and OQ518517 (99.97%), respectively.
CONCLUSIONS: Adenovirus types 2 and B3 were the two virus types identified in the hospitalized children of the present study. NGS provided important information on the molecular epidemiology of adenoviral infections.
SOURCES OF FUNDING: The study was funded by Horizon 2020 VEO (874735).
Keywords: adenovirus, children, next generation sequencing, molecular epidemiology, Greece

PP-217
Respiratory viruses

Prevalence of respiratory viruses before and during the COVID pandemic both in primary care and in a hospital setting

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AIM: The introduction of non-pharmaceutical interventions (NPIs) during the COVID pandemic also coincided with a reduction in the detection of a range of respiratory pathogens. Here, the prevalence of respiratory viruses detected by molecular assays before and during the COVID-19 pandemic on samples collected in primary care (PC) (and in a hospital setting from patients presenting with an acute respiratory tract infection (ARI) is reported.
METHODS: Nucleic acids from nasopharyngeal swabs, or combined oropharyngeal and nasal swabs collected in the PC PREPARE (ALIC4E, n=3227,January 2016-April 2018) and RECOVER (SOS-COVID, n=858, April 2020-June 2021) studies and in the MERMAIDS-ARI studies (Mermaids ARI, n=1044, February 2016-May 2019) and RECOVER ARI studies, (n=826 (ARI1.0) and n=295 (ARI2.0), March 2020-December 2022), in hospital settings were retrospectively extracted by the NucliSens EasyMag (BioMérieux) and analysed by the Fast-Track Respiratory 21 kit (FTD, Fast-Track Diagnostics) and the Custom TaqMan® Array Cards and the Fast Advance Master Mix (ThermoFisher Scientific), respectively.
RESULTS: Viruses such as influenza A and B and RSV, causing the majority of the ARIs both in primary and hospital care before the pandemic, were hardly or not at all detected during the COVID pandemic; prevalence of influenza viruses before the pandemic was 52.1%, 27.1% in PC and 25.8% in hospital care whereas the prevalence decreased to 0% and 0.1% in hospital care during the pandemic ($p<0.0001$). If detected, it was at the end of the enrollment period. Prevalence of SARS-CoV-2 varied between 22.7% and 44.0% in PC and between 48.9% and 81.0% in hospital settings. Seasonal coronaviruses, other respiratory viruses, and viral co-infections were also detected less frequently during the pandemic. Remarkably the prevalence of rhinoviruses did not decrease during the pandemic in PC patients (7.1 and 14.7% versus 15.6 and 12.8%) although prevalence decreased in hospitalized patients (11.0% versus 4.4 and 0.3%).
CONCLUSIONS: Our findings are consistent with several reports about a worldwide reduction in various respiratory viruses circulating except for rhinoviruses during the COVID pandemic and confirm the absence of influenza viruses and RSV. Non-pharmaceutical interventions may have contributed to the decreased circulation of normally-circulating respiratory viruses.
SOURCES OF FUNDING: Sources of funding: PREPARE was funded by the European Commission's FP7 Programme under grant number 602525. ReCoVeR was funded by the European Union under the Horizon 2020 research and innovation programme under grant agreement No 101003589.
Keywords: respiratory viruses, prevalence, primary care, hospital, COVID pandemic

PP-218
Respiratory viruses

Rapid identification of Influenza and Respiratory Syncitial viruses with molecular Point-of-Care testing: impact on pediatric population management

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AIM: Rapid detection of respiratory viruses remain crucial in emergency and critical care settings, especially for pediatric population, when identification may be affected by direct sampling, clinician expertise and bacterial co-infections. We evaluated four molecular POCT platforms, in order to distinguish between Influenza (Flu) and Respiratory Syncitial(RSV) viruses, encouraging the most appropriate therapy adoption and the fastest patient care.
METHODS: Comparison of each molecular POCT platform was made in parallel with our multiplex real-time PCR method. Sensitivity, specificity, cross-reactivity and accuracy have been investigated on 50 processing swab sample residues that have already been tested with gold standard method for specific nucleic acid sequence detection. In addition, time of processing, user friendly interface enhabler and possibility to LIS connection have been evaluated.
RESULTS: All four molecular POCT platforms showed consistent results, even in cases where both Flu and RSV viruses have been detected. Sensitivity decreased at higher real-time PCR Ct (>38), suggesting better balancing specificity for screening application. Execution time ranged from 30 to 90min. Reactions were prepared individually, with minimal manual interventions, directly and from primary tube. Outcomes were immediate, colour code identified and LIS interfaced.
CONCLUSIONS: Despite multiplex real-time PCR has a broader range of pathogen detection, during epidemic season, rapid detection of etiological agents of bronchiolitis and influenza symptoms with a molecular POCT could be useful for appropriate diagnosis in children. Choice could be addressed in favour of easy-to-use systems for nursing, without forgetting the crucial role of laboratory in government of results, quality control and epidemiology surveillance purposes.
SOURCES OF FUNDING: none
Keywords: Respiratory viruses, children, molecular POCT, rapid identification, infection management

PP-219
Respiratory viruses

Genetic diversity of human Rhinovirus circulating in Portugal during 2023/2024 season

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AIM: The National Influenza and Respiratory Viruses Surveillance detects, monitors and characterize the respiratory viruses circulating in Portugal each season. Among these viruses, rhinovirus stands out as the most common agent of respiratory infections in humans. The study aims to genetically characterize the human Rhinovirus (hRV) detected, in Portugal during the 2023/2024 season.
METHODS: hRV diagnosis was performed by multiplex RT-PCR (Allplex™ Respiratory panel, Seegene). hRV positive nasopharyngeal swabs, from acute respiratory infection cases at primary healthcare units, with CT≤30 were selected for Sanger sequencing targeting the partial VP2/VP4 genetic regions. Genotype was based on the Picornaviridae Study Group reference strains using Mega 7 and serotype was determined using RVdb: rhinovirus database.
RESULTS: From 2023W40 to 2024W15, eighty-nine positive samples for hRV were detected. The peak of hRV detections occurred on 2023W45. The majority of hRV-positive cases were detected among women (57%; 51/89) and on 30-64 age group (62%; 55/89) of the study population.
Fifty-two hRV were sequenced, being 64% (33/52) from genotype hRV-A, 19% (10/52) from hRV-B and 17% (9/52) from hRV-C. All genotypes co-circulated during the whole season. High variability of serotypes (n=29) was identified within genotypes, with hRV-A31 and hRV-B104 predominating.
CONCLUSIONS: The predominance of hRV-A over hRV-B and hRV-C, along with significant genetic variability found in this study, is consistent with existing literature. Despite this variability, cross-protection among different hRV serotypes has been reported. Therefore, further research is warranted to deepen our understanding of hRV genetics and their role in respiratory infections and severe outcomes.
SOURCES OF FUNDING: None
Keywords: rhinovirus, genotype, serotype, portugal, sanger sequencing

PP-220
Respiratory viruses

Investigation of SARS-CoV-2 IgG binding capability to variants of the SARS-CoV-2 virus
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AIM: This study examines the ability of SARS-CoV-2 IgG in serum samples to bind Delta and Omicron spike protein, as well as the functional binding capabilities of the IgG to two different S-ACE2 complexes.
METHODS: Twenty SARS-CoV-2 positive samples from patients who had recovered from infection with original wildtype SARS-CoV-2 in the first wave of COVID-19, and 10 healthy control samples were studied using 4 quantitative ELISAs and 2 binding inhibition assays.
RESULTS: Patients showed significantly higher levels of IgG to SARS-CoV-2 S1/RBD ($p < 0.001$), N protein ($p < 0.001$), and Omicron spike variant ($p = 0.01$), but not to Delta spike variant ($p = 0.966$) when compared to controls. Furthermore, patient samples showed significantly greater inhibition of SARS-CoV-2 S1/RBD and E484K spike and ACE2 binding ($p < 0.001$ and $p = 0.015$ respectively). However, there was no correlation between the binding inhibition of S1/RBD and E484K spike to ACE2 receptor.
CONCLUSIONS: Antibodies to the SARS-CoV-2 wild-type strain do offer significant cross protection to the Omicron spike variant. The apparent presence of Delta-specific IgG generated by wild-type SARS-CoV-2 infection may be due to factors such as assay specificity and cross reactivity to other seasonal coronaviruses. The presence of IgG to SARS-CoV-2 does increase inhibition of S-ACE2 binding to the wild-type virus. Gender analysis showed that convalescent males exhibit less cross protection to the Omicron variant than females, but not the Delta.
SOURCES OF FUNDING: This project was funded by the National Virus Reference Laboratory.
Keywords: SARS-CoV-2, spike protein, ACE2 receptor, COVID-19, coronavirus

PP-221
Respiratory viruses

Genomic insights into respiratory syncytial virus during 2022-2024 in Catalonia's SIVIC program: focusing on neutralizing monoclonal antibodies

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AIM: To track the emergence and spread of mutations of human respiratory syncytial virus (HRSV) through whole-genome sequencing (WGS).
METHODS: A randomized selection of HRSV-positive samples from patients admitted at primary care centres (PCC) in Barcelona and all ICS hospitals from Catalonia was collected from October/2022 to April/2024, on which WGS was performed.
RESULTS: A total of 1027 specimens were collected, 670 in 2022-2023 and 357 in 2023-2024 seasons, if which 504 belonged to HRSV-A and 523 to HRSV-B. Overall, 837 whole genomes [405 (80.4%) HRSV-A and 432 (82.6%) HRSV-B] were successfully sequenced. Circulating linages changed in prevalence within these two seasons (A.D.2.1 and A.2.5.1-2 decreased while A.D.1, A.D.3, A.D.3.1 and A.D.5 increased for HRSV-A and B.D.E.1, B.D.E.4 and B.D.4.1 decreased in favour of B.D.4.1.1 for HRSV-B). Within the antigenic site II (palivizumab's epitope), one (S276N) and three (D263E, M264I and S276N) mutations in HRSV-A and -B genomes were observed, none of them reported to confer resistance to the mAb. Within the antigenic site 0, nirsevimab's epitope, three (N63S, K65R and K209E) and six (K65Q, E66G, K68N, I206M, Q209R and S211N) mutations in HRSV-A and -B genomes were observed. Three of these mutations are reported to affect nirsevimab's susceptibility when combined with other not-found mutations.
CONCLUSIONS: This work describes the HRSV variants circulation before and during the administration of nirsevimab to all <6-months-old infants in Catalonia. Though no concerning mutations were found, molecular surveillance of this virus is of utmost importance, especially in the context of monoclonal antibodies and future vaccines administration.
SOURCES OF FUNDING: No funding
Keywords: human respiratory syncytial virus, surveillance, mutations, evolution, monoclonal antibodies

PP-222
Respiratory viruses

Respiratory syncytial virus in adult patients at a tertiary care hospital in Germany: clinical features and molecular epidemiology of the fusion protein in the severe respiratory season of 2022/23

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AIM: Following an interseasonal rise in mainly pediatric respiratory syncytial virus (RSV) cases in Germany in 2021, an exceptionally high number of adult cases was observed in the subsequent respiratory season of 2022/2023. The aim of this study was to compare (1) the clinical significance of RSV infections to the three seasons before the SARS-CoV-2 pandemic and (2) the local molecular epidemiology of the RSV fusion protein.
METHODS: Clinical data were retrieved retrosectively from patient charts. The RSV fusion protein was analyzed on molecular genetic and amino acid level.
RESULTS: RSV detections peaked 8 weeks earlier than the earliest peak observed in the three pre-pandemic seasons. Although, the median age of the adult patients was not different (66.5 vs 65), subtle differences between both periods were noted. High rates of comorbidities prevailed, however a significantly lower amount of patients with a history of lung transplantation (p=0.009), chronic kidney failure (p=0.013) and immunosuppression (p=0.038) were observed in the season of 2022/2023. In contrast, significantly more lower respiratory tract infections (p<0.001), in particular in the form pneumonia (p=0.015) and exacerbation of obstructive lung diseases (p=0.008), were detected. Sequence analysis of the fusion protein gene revealed a close phylogenetic relatedness regardless of the season of origin. However and especially for RSV-B, an accumulation of amino acid exchanges was noted.
CONCLUSIONS: The SARS-CoV-2 pandemic had a tremendous impact on the seasonality of RSV and the introduction of new vaccination and immunization strategies against RSV warrant further epidemiologic studies of this important pathogen.
SOURCES OF FUNDING: This research received no external funding.
Keywords: RSV; molecular epidemiology; fusion protein; respiratory infections; respiratory viruses

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Respiratory viruses

Study of viral respiratory infections in an **in vitro** model of polarized primary human airway epithelial cells from children and adults

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AIM: In contrast to pre-pandemic respiratory viruses like influenza, respiratory syncytial and rhino- viruses, the clinical presentation related to SARS-CoV-2 infection is most of the time milder in paediatric, compared to adult patients. Several clinical studies recently suggested that this reduced severity likely involves immune response, especially at the level of the nasal mucosa. The goal of our study is to investigate the innate immune response of the nasal epithelium *per se* in children versus adults during acute infections by SARS-CoV-2, influenza A virus (IAV), rhinovirus (RV) and respiratory syncytial virus (RSV).
METHODS: We established an in-house *in vitro* model of polarized human airway epithelium (HAE) cultured in air-liquid interface from nasal swabs collected from child and adult donors. These reconstituted epithelia were first morphologically and functionally characterized. Then, we studied the intrinsic response of child and adult HAE at baseline or after infection by SARS-CoV-2, IAV, RV or RSV, firstly in a focused approach with RT-PCR, secondly in an unbiased approach with a comparative transcriptomic analysis.
RESULTS: No morphological difference was observed between tissues from young and adult donors. Our RT-PCR data showed that interferon (IFN) induction was comparable between the two groups in mock- and IAV-infected tissues; while higher induction of IFN-β and -λ was found in RV-infected HAE from children, compared to adults. The comparative transcriptomic analysis is ongoing.
CONCLUSIONS: It is aim is to unveil more mechanistic clues to better understand the pathogenesis of respiratory viruses in children versus adults.
SOURCES OF FUNDING: Cargiest SA
Keywords: Children, SARS-CoV-2, influenza virus, rhinovirus, respiratory syncytial virus, polarized human airway epithelium

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Respiratory viruses

Virological characteristics of the SARS-CoV-2 XBB variants

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AIM: The SARS-CoV-2 circulation leads to the emergence of novel XBB subvariants exhibiting significant diversification. A comparative analysis of their replication efficiency and elicited immune response may provide valuable insights into the dynamics of viral evolution and the efficacy of immune responses against emerging variants.
METHODS: XBB variants were obtained from nasopharyngeal swabs using Caco-2 cells. Human cell lines and primary cells were utilized to assess replication kinetics and immune response.
RESULTS: Most XBB variants exhibit accelerated replication rates in Calu-3 cells, resembling the efficiency observed in the Delta rather than the Omicron BA.1 variant. Infection of A549-ACE/TMPRSS2 cells revealed that XBB, XBB.1.5 and XBB.2.3.2 increased IRF promoter activity, whereas XBB.1.9.2 failed to elicit any activity. Investigation of dsRNA sensing pathways showed that MDA5, but not RIG-I was crucial for IRF activation during infection with XBB variants. Intriguingly, XBB.1.5 and 1.9.2 differ only in four cording mutations outside the spike protein but show opposite host IFN responses. Using primary cell models, monocytes and air-liquid interface cultures of bronchial epithelium, we validated the differences in IFN response between XBB.1.5 and 1.9.2. Crucially, super-infection assay with Influenza A virus revealed that XBB.1.5 but not XBB.1.9.2 induced functional antiviral state enabling control of viral infection.
CONCLUSIONS: Our findings demonstrate the differential induction of IFN signalling by XBB variants. Furthermore, the distinct host response to XBB.1.5 and 1.9.2 highlights the necessity of examining the complete genomic landscape to fully grasp the complexities of immune evasion and adaptation by SARS-CoV-2 variants.
SOURCES OF FUNDING: GRK2336 funded by DFG, Coropan funded by HMWK
Keywords: SARS-CoV-2, XBB, IFN

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Respiratory viruses

Low prevalence of nirsevimab resistance associated mutations (NRAMs) during acute RSV infection in children and immunocompromised patients in a tertiary hospital in Amsterdam, The Netherlands during the 2023/2024 respiratory season

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AIM: Investigate the genetic diversity and prevalence of nirsevimab resistance associated mutations (NRAMs) in respiratory syncytial virus infections in both children and immunocompromised patients during the 2023-2024 respiratory season.
METHODS: We selected >100 available stored RSV RT-PCR positive nasopharyngeal swab samples with ct-values below 28, from the 2023-2024 respiratory season after anonymization for age in years, week of sampling and underlying illness. We performed whole genome amplification combined with nanopore sequencing to fully characterize RSV-A and RSV-B strains directly from these clinical samples.
RESULTS: The majority of patients were infected with an RSV-A strain and we identified multiple different RSV A and B clades. Patients infected with an RSV-A strain showed that the majority belonged to the A.D.5.2-clade, followed by the A.D.1-clade. For patients infected with an RSV-B strain, the majority belonged to the B.D.E.1-clade followed by the B.D.4.1-clade. No known nirsevimab resistance associated mutations at F-gene positions 68, 201, 206 and 209 were detected for RSV-A strains. For RSV-B strains, the B.D.E.1-clade strains contained a F:S211N mutation, which was previously associated with a <10 fold change in IC50.
CONCLUSIONS: The availability and usage of the monoclonal antibody nirsevimab as well as multiple RSV vaccines containing the pre-fusion F-protein could potentially lead to the emergence of nirsevimab-resistance associated mutations in the community. In this study, we identified F:S211N as a prevalent mutation in circulating strains. Genomic surveillance of RSV remains crucial in order to guide future vaccination strategies.
SOURCES OF FUNDING: This work was funded by the Amsterdam Institute for Immunology and Infectious Diseases.
Keywords: RSV, nanopore, sequencing, viral evolution, nirsevimab

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Respiratory viruses

Antiviral Susceptibility in Influenza virus isolates: Genotypic and phenotypic assessment during the 2023-2024 season in Catalonia

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AIM: To detect influenza strains with a reduced susceptibility to antiviral drugs during the 2023-2024 influenza season in Catalonia (Spain) at hospital and Primary Care settings.
METHODS: Influenza-positive cases, randomly selected by sex, age and from different primary care and hospital settings, involved in the new surveillance system for respiratory infections in Catalonia (SIVIC, https://sivic.salut.gencat.cat/), were characterised through WGS using Illumina sequencing technology. Sequences were aligned with those from seasonal vaccine strains, and mutations within the neuraminidase (NA) and acid protein (PA) were evaluated according to the WHO guidelines in order to assess for their resistance profile on NA and PA inhibitors (NAIs and PAI). In parallel, a selection of strains were also isolated in MDCK-SIAT1 cell line and the susceptibility of these to NAIs (Oseltamivir carboxylate and Zanamivir) was assessed by an in house MUNANA assay.
RESULTS: 520 influenza positive samples have been genetically characterized. None of the sequenced strains contained mutations associated to lower susceptibility to NAI or PAI. In the phenotypic assay, 139 samples were inoculated in MDCK-SIAT1, and 91 have been successfully isolated. In the NAI phenotypic assay, 66 viral isolates have been assessed (26 H3N2, 30 H1N1 and 10 B FluB), and none met the WHO criteria to be considered resistant.
CONCLUSIONS: No antiviral resistance has been detected in the isolated influenza strains, nor in hospital setting where antivirals are usualy administered, Both genotypic and phenotypic antiviral susceptibility assessment strategies are important to strengthen the influenza surveillance, especially when novel resistance mutations can emerge.
SOURCES OF FUNDING: No external funding
Keywords: influenza, antiviral, neuraminidase inhibitors, MUNANA

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Respiratory viruses

The clinical burden of rhinoviruses compared to influenza and respiratory syncytial virus in an 8-year period at the National Influenza Centre in Slovenia

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AIM: Rhinoviruses (RV) are the most common cause of acute respiratory infections (ARIs), predominantly common colds. The introduction of syndromic diagnostics revealed their role in severe clinical manifestations, like bronchiolitis and pneumonia. We aimed to assess the clinical burden of RVs in comparison to influenza (INF) and respiratory syncytial virus (RSV) by analysis of the data collected at the National Influenza Centre, Slovenia.
METHODS: In an 8-year period nasal/throat swabs from 20.425 patients with ARI were collected from the surveillance sentinel of 50 primary healthcare clinics (PHC) and 2 hospitals (H), together with demographic and standardised clinical data. Respiratory viruses, including INF, RSV, RV, were detected using multiplex RT-rPCRs. Infection rates were calculated. Correlations between infections and standardized clinical data were determined using Chi-square and Fisher's exact test. A p-value<0.05 was considered statistically significant.
RESULTS: In patients from PHC rhinovirus infections correlated to cough, runny nose, sore throat and fever, while infections with INF and RSV additionally correlated to headache, myalgia and wheezing. In H settings rhinovirus infections correlated to fever, breathing difficulties, bronchiolitis in children and pneumonia in the elderly. In H settings INF infections correlated to fever, cough and pneumonia, while RSV infections correlated to fever, cough, breathing difficulties and bronchiolitis. Only INF infections correlated to acute respiratory distress syndrome.
CONCLUSIONS: Patients examined in PHC with RVs had milder symptoms than those with INF or RSV. However, our results confirm a significant clinical burden of RVs in hospitalized patients, which is similar to the burden of INF and RSV.
SOURCES OF FUNDING: National influenza programme.
Keywords: rhinovirus, influenza, RSV, clinical symptoms, correlation, clinical burden

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Respiratory viruses

Molecular Characterisation and Seasonal Circulation of Human Metapneumovirus from 2022-2024 in the North-Eastern Region of Slovenia

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AIM: Human metapneumovirus (HMPV) is one of the leading causes of respiratory illness. The virus can be categorized into sublineages (A1, A2a, A2b, A2c, B1 and B2). The objective was to determine the prevalence of HMPV within the samples tested and identify the specific sublineages currently circulating in our region.
METHODS: From 2022 to March 2024, 14949 nasopharyngeal swabs were tested for HMPV, of which 304 were positive. The partial F gene was amplified and the products sequenced. After comparing 94 sequences with known genotypes from GenBank, we classified them into sublineages using phylogenetic analysis.
RESULTS: In 2022, 2023 and up to March 2024 the sample positivity for hMPV was 2.3%, 2% and 1.5% respectively. HMPV circulates from December to May and is absent during the summer. Among the age groups, 33.9% were under 5 years old, 25.3% were in the 6-60 age group, and 40.8% were 60 and older. A2c was the predominant sublineage in 2022 and 2023 with 68.8% (22/32) and 100% (45), respectively. In 2022, B1 and B2 were present with 9.4% (3/32) and 21.9% (7/32), respectively, but were absent in 2023. By March 2024 the predominant sublineage was B2 with 47.1% (8/17), followed by A2c with 41.4% (7/17). In 2024, A2b was also present (11.8%).
CONCLUSIONS: HMPV circulates from early winter to late spring. Infections are particularly prevalent in the elderly. A2c is dominant in our region with indication of a circular pattern for B2. A1 was not detected. No correlation between genotype and age group was observed.
SOURCES OF FUNDING: No funding.
Keywords: Human metapneumovirus, genotyping, HMPV, sublineages

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Respiratory viruses

To assess the role of community respiratory viruses in patients with bacteria confirmed hospital pneumonia

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AIM: Bacterial lower respiratory tract infections (LRTIs) in hospital (HAP and VAP) are linked to: base-line health; reason for admission; staff pressures; hospital demographics. In older patients aspiration is a feature of Nursing and Health Care-Associated Pneumonia(1). Patients in hospital can be exposed to respiratory viruses before admission or on-going through interaction with staff and visitors. This study reviewed the role of respiratory viral infections in hospitalized patients with bacterial LRTIs.
METHODS: Ninety-two lower respiratory samples from 75 hospitalized patients were tested by culture and a new metric (EGS - combining Gram stain, 16S RTPCR and 16S WGS) (2) and co-tested for respiratory viruses by RTPCR. Community virus transmission for the period was available for review. Bacterial LRTIs by (a) culture and (b) EGS, with and without respiratory viral infections, were tested for significance by the Mann-Whitney test using two-sided stringency, with p-values < 0.05 as significant.
RESULTS: Bacterial LRTIs in patients with a confirmed viral infection were significantly increased using the EGS metric (z-score -2.19; p-value = 0.028) and approached significance for culture (z-score -1.70286; p-value = 0.089). Viruses mirrored those in the community while bacteria reflected strains seen in a hospital setting.
CONCLUSIONS: Community respiratory viruses are significantly associated with bacterial pneumonia in hospital patients.
1. Miyashita et al Functional outcomes in elderly patients with hospitalized COVID-19 pneumonia: A 1 year follow-up study. IORV 2022;16(6):1197-8.
2. Hamza et al A New Metric Combining Gram Stain and 16S RTPCR Was Validated for Early Confirmation of Hospital Pneumonia. JMM submitted.
SOURCES OF FUNDING: Hamad Medical Corporation MRC-01-19-316
Keywords: Hospital Pneumonia, Viral respiratory infections, EGS,

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Wastewater-based surveillance of respiratory viruses in Belgium: integration of RSV and influenza to SARS-CoV-2

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AIM: In Belgium, a national wastewater-based surveillance program was established in 2020 to monitor SARS-CoV-2. In September 2024, this program will be extended to other respiratory viruses: RSV and influenza. Challenges encountered to adapt the existing surveillance program will be discussed in this work.
METHODS: Over 5 million inhabitants representing 40% of the Belgian population are monitored throughout 30 wastewater treatment plants since September 2020. Analysis of RSVA, RSVB, influenza A, influenza B, SARS-CoV-2 viral concentrations are performed by RT-ddPCR. The weekly epidemiological assessments are conducted using wastewater-based indicators. Weekly assessments are published by the Belgian Public Health Institute, Sciensano.
RESULTS: Lower concentration were measured for RSV and influenza than for SARS-CoV-2 during the 2023/2024 winter. Therefore, two distinct ways for analysing the data were developed. The proportion of data above the limit of quantification being high for SARS-CoV-2, the epidemiological indicators were computed based on quantified measurements. And since 2020, the wastewater-based surveillance was able to detect resurgences and peaks of SARS-CoV-2. Regarding RSV and influenza, the low proportion of measurements above the limit of detection motivated the development of another reporting system. The number of treatment plants, with a detected target, were summed by date. The dates of maximal numbers reported for influenza and RSV correspond to the ones reported by the clinical-based surveillance.
CONCLUSIONS: Three respiratory viruses were effectively monitored through wastewater-based surveillance at a national level.
SOURCES OF FUNDING: This research was funded by the European Commission, Belgian Federal Government, AVIQ, Vivalis and Department Zorg Vlaanderen.
Keywords: wastewater-based epidemiology, SARS-CoV-2, RSV, influenza, surveillance, alerting indicator

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Respiratory viruses

Molecular epidemiology of respiratory syncytial virus in autumn-winter 2023-24 in Rome, Italy
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AIM: This study aimed to characterise the RSV strains circulating during the 2023-24 epidemic season.
METHODS: Children attending the Sapienza University Hospital (October-March 2023-24) for acute respiratory infections were tested for RSV by molecular methods and phylogenetic analyses of the G gene were performed.
RESULTS: Among the 520 children tested (median age 9 months), RSV was the most common viral infection (41%); co-infection with rhinovirus, the second most common virus, was detected in 15% of cases. Influenza A and B accounted for 15% and 8% of cases respectively. RSV cases increased from the second half of November with a peak in early January, but continued until March; RSV-A was dominant (83%) over RSV-B. Circulating RSV-A (genotype ON1) strains belonged to the same divergent clades as previously identified in Rome [1]. In 2023-24, RSV-B strains sequenced all belonged to lineage GB5.0.6a, but without the previously reported [1] amino acid change K209N, introducing a predicted N-glyc site.
CONCLUSIONS: The RSV season appeared to be intense and unusually coincided with the influenza A season in December/January. Monitoring the changing epidemiology and local evolution of RSV is essential to prevent severe outbreaks and to plan passive and active RSV prophylaxis.
Reference [1] Pierangeli A, Nenna R, Fracella M et al. Genetic diversity and its impact on disease severity in respiratory syncytial virus subtype-A and -B bronchiolitis before and after pandemic restrictions in Rome. J Infect. 2023;87:305.
SOURCES OF FUNDING: NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (project no. PE00000007, INF-ACT).
Keywords: respiratory syncytial virus, genetic diversity

PP-232
Respiratory viruses

Elucidation of the conformational dynamics of SARS-CoV-2 Spike-protein through MD simulation
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AIM: The study aims to elucidate how the Spike-protein shows conformational dynamics over time and whether one RBD (Receptor Binding Domain) 'up' conformation exclusively favors the receptor binding as previously reported. The study involves understanding the dynamics of RBD and NTD(N-terminal Domain) through MD simulation.
METHODS: In this study, we present findings from 200ns all-atom MD simulation of S-protein. We did the trajectory analysis of S-protein with GROMACS 2022 emphasizing on the Center of mass distance between the RBDs and NTDs from the stalk of the trimeric (S2) S-protein throughout 200ns. We also performed PDBePISA analysis of S-protein (PDB ID: 6VXX) before and after the simulation to study the necessary salt bridge interaction throughout the simulation.
RESULTS: The movement of the center of the mass distance of RBDs from S2 represents the movement of RBDs away from the core. We reported the subtle movement of all three RBDs along with NTDs, which leads to the breaking of H-bonds and salt bridge interactions. We observed few conserved intra-protomer H-bonds between RBD and NTD remained constant throughout the simulation while inter-protomer H-bonds changed through trajectory. Changes in inter-protomer salt bridge interactions were reported.
CONCLUSIONS: Our study suggests a dynamic behavior of RBDs and NTDs in Spike-protein, potentially leading to domain transitioning. We could not observe the entire domain transition of RBDs but, the subtle movements of RBDs, NTDs, and changing H-bond interaction network and salt bridges suggest the domain movements.
SOURCES OF FUNDING: The student was supported financially by the Council of Scientific and Industrial Research, (CSIR), India.
Keywords: S-protein, RBD, NTD, S2, MD simulation

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Respiratory viruses

Enterovirus D68 Detection in Respiratory Specimens: Retrospective Study Over A Five-Month Period from July to November 2023

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AIM: Enterovirus D68 (EV-D68) causes mild to severe respiratory illness, with some cases associated with acute flaccid paralysis or acute flaccid myelitis. The National Virus Reference Laboratory tests patient specimens for respiratory infections covering up to twenty viral targets in the respiratory screening test. A retrospective study was performed on rhinovirus/enterovirus positive specimens to determine what proportion, if any, were EV-D68.
METHODS: A total of 324 respiratory specimens which were positive for rhinovirus/enterovirus on the respiratory screening test were included in this retrospective study during the five-month time-frame from the beginning of July 2023 to the end of November 2023. EV-D68 testing was performed using a real-time one-step reverse transcriptase PCR (RT-PCR). A proportion of EV-D68 positive samples were then further characterised by sequencing.
RESULTS: EV-D68 was detected in 6 specimens (1.9%) of those tested by EV-D68 RT-PCR. Of the 3 positive specimens submitted for further genetic classification, one sample yielded EV-D68 Clade-B3 and two samples could not be classified further. The age of patients varied from <3 months to 84 years of age. No severe respiratory or neurological symptoms were noted in these EV-D68 positive patients.
CONCLUSIONS: This study highlights that EV-D68 may be under-reported when relying on some commercial CE-IVD certified assays, and there is a need for regular EV-D68 screening and characterisation to determine true incidence of this genotype. The EV-D68 sub-genogroup B3 identified in this study is the same subgenogroup as those which were circulating in Europe in 2021/2022 (Simoes MP, Hodcroft EB, Simmonds P, et al. 2024).
SOURCES OF FUNDING: None
Keywords: Enterovirus D68

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Respiratory viruses

Antibody avidity 10 weeks post BNT162b2 vaccination and risk of subsequent breakthrough infection: A nested case-control-study within the Shieldvacc vaccine trial

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AIM: The antibody avidity is known to correlate with the neutralizing or other effector functions. Using nested case-control data within the Shieldvacc phase IV open label vaccine trial we aimed to characterize the predictive value of avidity maturation of vaccine-induced antibodies against SARS-CoV-2 in determining breakthrough infection.
METHODS: We conducted a nested case-control study among 272 individuals (n=68 breakthrough infections and n=204 sex-and age-matched controls (1:3 case-control)) nested within the Shieldvacc- vaccine trial (N=2760). Patients who presented with a positive SARS-CoV-2 antigen test during the weekly self-test were considered cases. We determined binding antibody levels, neutralization assay and avidity of anti S antibody.
RESULTS: Cases had lower baseline binding and neutralizing antibody concentrations as compared to controls (GM (95% CI): 1385.04 (1164.30-1681.98) vs. 1826.13 (1622.07-2041.57), p=0.006 and 294.47 (242.28-366.61) vs. 385.42 (335.09-448.02), p=0.047). Mean antibody avidities were also lower among cases as compared to controls (14.2% vs. 16%), however not statistically significant (p=0.16). Although we observed an impressive superiority of the antibody avidity among subjects with a history of previous infection as compared to those with no history of infection (29.8% vs 12.6% for controls and 25.7% vs. 11.7% for cases), this was not significantly different between cases and controls irrespective of the pre-vaccine infection status.
CONCLUSIONS: We recommend that further studies with larger sample size examine if the small sample size of positive cases in this study obscured a potential a significant difference.
SOURCES OF FUNDING: Virology diagnostic
Keywords: breakthrough infection, avidity, nested case-control

PP-235
Viral infections in pregnancy

Increased incidence of Parvovirus B19 infections in pregnant women in Germany, 2023/2024

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AIM: To report an increase in Parvovirus B19 (B19V) detections in our laboratory in pregnant women in 2023/2024.
METHODS: The laboratory information system was used to retrospectively identify pregnant women tested for B19V DNA by PCR in serum or plasma between January 2016 and March 2024. We extracted information on PCR results and, if available, maternal age, gestational age, first digit of postal code and type of referring physician/medical facility. The infection status was arbitrarily defined according to the B19V loads (IU/mL) as acute (>10e5), recent (10e4-10e5) or past (<10e4).
RESULTS: During the observation period a total of 10,083 PCR tests were identified. Median (IQR) maternal age and median (IQR) gestational age at time of testing was 32.8 (29.6; 35.9) years and 13 (9; 22) gestational weeks, respectively. The number of tests per year varied between 746 and 1,736, with the highest number in 2024 (January to March, n=1,736). The positivity rate was 13-21% in the SARS-CoV2 pre-pandemic years, 0-7% between 2020 and 2022 and 42% in 2024. Analysis by the first digit of postal codes suggests that B19V activity increased in each zip code area.
CONCLUSIONS: Even though our data do not result from a systematic surveillance, they strongly suggest a nationwide B19V epidemic in 2023/2024. We assume that lockdowns and hygienic measures during the SARS-CoV2 pandemic effectively restricted B19V transmission resulting in a significantly larger population of susceptible young children, the most common source of B19V infection in pregnant women.
SOURCES OF FUNDING: Supported in part by the German Federal Ministry of Health
Keywords: Parvovirus B19, pregnancy, epidemic

PP-236
Viral infections in pregnancy

Congenital CMV diagnosis using saliva and urine: A study in Region Zealand, Denmark

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AIM: Congenital CMV (cCMV) is the most common non-genetic cause of sensorineural hearing loss in children. New-borns with symptomatic cCMV infection may benefit from antiviral treatment initiated within the first month of life, and early diagnosis is therefore important. In this study, we investigated the usefulness of CMV detection in saliva versus urine for the diagnosis of cCMV in Region Zealand.
METHODS: During April 2021 to March 2024, both saliva and urine were collected for detection of CMV in new-borns who failed the hearing screening test within 21 days of birth. All samples were tested using cobas® CMV quantitative assay for Cobas 6800® (Roche).
RESULTS: This study included 186 patients. Two patients (1.1 %) were found CMV positive in both saliva and urine; one showed high levels of CMV (3,000,000 IU/mL) in both saliva and urine and received antiviral treatment; the other had low levels of CMV in both saliva (266 IU/mL) and urine (<34.5 IU/mL). Eleven patients (5.9 %) were CMV positive only in saliva, though all showing low levels of CMV (< 34.5-1000 IU/mL). The remaining 173 patients (93 %) were CMV negative in both urine and saliva.
CONCLUSIONS: Compared with urine, no false negative results were observed in saliva specimens for CMV detection. This indicated that saliva could be an easy alternative to urine for diagnosing cCMV. The low levels of CMV detected only in saliva could be explained by transfer during breast-feeding. Therefore, we suggest that a urine specimen should confirm findings of low level CMV in saliva.
SOURCES OF FUNDING: None
Keywords: cCMV, saliva, urine

PP-237
Viral infections in pregnancy

Investigating host factors implicated in vertical transmission of SARS-CoV-2

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AIM: Identification of host factor expression due to trimester specific COVID exposure
METHODS: COVID exposure and infection history was studied from hospital-based survey of pregnant women by questionnaire method. This is in accordance to ethical clearance obtained from them. Relation between time of exposure and pregnancy outcome was drawn. Via in-silico interactome and molecular docking analysis, two placental interactors of Spike protein were identified- CD147 and LDLR. These proteins are involved in placental development [1]. Expression of these two proteins were checked and compared between control and exposed group of patients via RT-PCR.
RESULTS: From the hospital-based survey of pregnant women (n=60), it was found that COVID exposure was associated with more complications during delivery ($r=0.27$, $p<0.05$). Expression analysis of CD147and LDLR revealed, trimester-wise COVID exposure had altered CD147 expression compared to control. LDLR, expression was found to be higher in the second trimester exposure and the odds of higher LDLR expression was 1.4 (1.01-2.08) times higher when exposure was later.
CONCLUSIONS: We conclude that two placental interactors, CD147 and LDLR might play important role in vertical transmission of SARS-CoV-2 and the time of exposure is critical in the pregnancy outcome.
[1](Cheuk-Lun Lee, Kevin K.W. Lam et al. Identification of CD147 (basigin) as a mediator of trophoblast functions. Human Reproduction 2013; 28: 2920-2929)
SOURCES OF FUNDING: NFSC scheme under UGC, DBT-BUILDER
Keywords: COVID, Spike, CD147, LDLR, Trimester, Exposure

PP-238
Viruses, tumors and immunocompromised hosts

Surveillance of Cytomegalovirus viral load in Living Donor Liver Transplant (LDLT) recipients and its correlation with pre and post-transplant Immunological markers

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AIM: To find out the occurrence (time-line) of Cytomegalovirus (CMV) viral infection in Living Donor Liver Transplant recipients and its correlation with immunological markers.
METHODS: It was a Prospective observational study in which eligible patients for LDLT were enrolled. Quantitative Real-Time PCR for measuring CMV DNA. Cytokines measured using Sandwich-ELISA. Descriptive statistics used to analyse baseline characteristics and to analyse CMV viral loads and IFN-gamma responses. Mann–Whitney test used to determine differences in CMV occurrence among patient groups.
RESULTS: Among 26 patients, samples were collected for both the pre-transplant(pre-tx) and post-transplant(post-tx) points. Median age was 48 (IQR, 37.5-52.25) years, M:F being 4:1. Median pre-tx titres of CMV IgG was 83(IQR, 65.5-105) AU/ml. CMV DNA qPCR results indicated CMV infection in 9 (34.6%) patients (group 1) with no instance in 17 (65.4%) (group 2). Median time of occurrence of CMV infection was 36(IQR, 30-44) days. Median viral load was 3.24 log10(IQR, 2.64- 3.1) IU/ml. The median duration of the treatment was 20.5(IQR, 8.7-20.7) days in group1. Cytokine profiling among group 1 indicated decreased levels of IL-6 from the pre-transplant levels and increased levels during the resolution of CMV infection.
CONCLUSIONS: Our previous results found higher CMV IgG (>250 AU/ml) correlated with lower infection rates. Contrary, high CMV-positive antigenemia is linked to low CMV disease rates. Post-Tx CMV infection risk was 34%, slightly lower than prior studies. Median infection occurrence and clearance times differed from previous findings. Over 10 years, improved surgical techniques and post-surgical care prolonged infection duration (>30 days) despite stable prevalence.
SOURCES OF FUNDING: Self-funded
Keywords: CMV-CMI, post transplant, CMV infection, Cytokine profiling

PP-239
Viruses, tumors and immunocompromised hosts

High-risk human papillomavirus detection in cervical samples as a predictive factor for vaccine coverage

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AIM: An acceptable screening method for the detection of HPV should consider the tests that, in addition to their analytical performance, provide appropriate clinical values. The study aimed to analyze HPV-DNA detection with simultaneous genotyping of HPV16, HPV18, and other high-risk (hr) types in a group of women from Bosnia and Herzegovina (B&H) in order to assess the potential vaccine coverage of the target population.
METHODS: The study included 715 cervical swabs in a cell collection medium obtained from a group of women with abnormal cytology from May 2023 to April 2024. Testing was done by an automated real-time polymerase chain reaction (rtPCR) system for qualitative HPV detection, which provides individual results for HPV16, 18, and twelve other hrHPV types in one run.
RESULTS: The overall number of HPV DNA-positive results was 36.78% (263/715). Among the total positive results, HPV16 was present in 22.81% (60/263) of cases, HPV18 in 1.90% (5/263) of cases, and other hrHPVs were detected in 58.55% (154/263) of women. Mixed infections reported by the analysis software were HPV16 and other hrHPVs in 15.21% (40/263), HPV18 and other hrHPVs in 0.76% (2/263), and two cases of HPV16, HPV18, and other hrHPVs. Considering each infection as a single, the estimated potential vaccine coverage against HPV16 was 38.76% and HPV18 was 2.71%. Other hrHPVs in total were detected in 58.52%.
CONCLUSIONS: Determining the clinically important genotypes of HPV contributes to a better prognostic aspect of the infection and the epidemiological prediction of potential vaccine coverage against them.
SOURCES OF FUNDING: We had no sources of funding.
Keywords: HPV, High-risk, vaccines

PP-240
Viruses, tumors and immunocompromised hosts

SARS-CoV-2 intra-host evolution in immunocompromised patients
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AIM: Immunocompromised patients are at risk for prolonged infection with SARS-CoV-2. Here, we study SARS-CoV-2 intra-host evolution in prolonged infections in relation to antiviral treatment.
METHODS: Eleven immunocompromised patients treated for prolonged SARS-CoV-2 infections in the period 2020 to 2023 were selected for evolutionary analyses in relation to four immunocompetent controls. Treatment regimens included nirmatrelvir/ritonavir, casirivimab/imdevimab, remdesivir and convalescent plasma. Duration of infection ranged from 5 to 78 days. A median of 4 samples per patient were deep-sequenced in duplicate using the Illumina COVIDSeq Assay for the NovaSeq 6000 with a median of 2.8M reads per sample. Consensus between three minor variant callers was used to identify and quantify variants down to an allele frequency of 1%. In addition, novel methods were used to study the dynamics of the SARS-CoV-2 sequences, including viral population based phylogenetics and machine learning methods.
RESULTS: Several SARS-CoV-2 major and minor variants appeared during different treatment regimens of which some persisted over time. Both non-synonymous and silent mutations were detected and studied in relation to the drug and on- and off-target positions. Disappearance of mutants after discontinuation of treatment was suggestive for decreased fitness of the selected mutated viruses.
CONCLUSIONS: SARS-CoV-2 intra-host evolution in prolonged infection occurs at amino acid and nucleotide levels and is affected by the replication extent, the replication duration, and the treatment regime. Similar distribution of mutations to those previously described were found. The sequence dynamics display fractal organization for which machine learning methods can assist in analyses.
SOURCES OF FUNDING: Corona Accelerated R&D in Europe.
Keywords: SARS-CoV-2, intra-host evolution, minor variants, immunocompromised patients

PP-241
Viruses, tumors and immunocompromised hosts

The Genetic Variation of Human Papillomavirus Type 16 (HPV16) E6 and E7 gene and the Association with the Development of Cervical Cancer

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AIM: The current study aimed to investigate the distribution of HPV16 E6 and E7 gene variations in a group of HPV-16 positive women with and without abnormal cytology. Moreover, we investigated the association of HPV16 E6 and E7 gene variations with the development of cervical cancer.
METHODS: PCR and DNA sequencing were used to identify the sub-lineages of HPV 16 in a group of women with and without abnormal cytology, then the association between different sub-lineages and the clinical outcomes was investigated.
RESULTS: The most prevalent HPV16 sub-lineage in this study of Scottish samples was A1. No significant association between the sub-lineage and cervical cancer development 16 was observed..
CONCLUSIONS: No specific sub-lineage association with the enhanced risk of developing cervical cancer was detected. (p=0.315).
Some mutations have been shown to provide a protective effect against cervical cancer.
SOURCES OF FUNDING: University of Warwick
Keywords: Human Papillomavirus Type 16 (HPV16); E7 Gene; E6 gen, Cervical Cancer; Variants

PP-242
Viruses, tumors and immunocompromised hosts

Frequency and context of HPV DNA detection below the clinical threshold of the commercial kit Alinity m HR-HPV Assay (Abbott)

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AIM: Clinical thresholds of HPV tests are designed to provide an optimal balance of sensitivity and specificity for cervical cancer screening (CCS). However, analytical and clinical thresholds may differ slightly for some commercial kits, creating a "grey-zone" between these 2 thresholds. Here, we aimed to determine the frequency and context of results within this grey-zone in routine practice.
METHODS: Cervical specimens were collected for CCS over a 22-months period at our institution, and tested using the Alinity m HR-HPV Assay (Abbott). Clinical and biological data were collected retrospectively.
RESULTS: Of 6,101 specimens, 1.7% (n=102) had an HPV result within the grey-zone. The proportion of results in the grey-zone varied according to the HPV genotype (reaching 11.8% for samples containing HPV31/33/52/58). Reflex cytology was normal or showed ASC-US results in 74.6% and 17.9% of cases, respectively. Previous or subsequent detection of a possible identical HPV genotype was observed in 58% and 38% of cases, respectively. Two women with a known persistent HPV had a CIN2+ lesion 1 year after the grey-zone result.
CONCLUSIONS: HPV results in the grey-zone are common and vary by genotype. In the majority of cases, cytology is normal or has only minor abnormalities. Rare women with a history of persistent HPV infection should even be closely monitored in case of transient HPV result within the grey-zone.
SOURCES OF FUNDING: None
Keywords: HPV; analytical threshold; clinical threshold; cervical cancer screening

PP-243
Viruses, tumors and immunocompromised hosts

HHV-6 DNA is highly prevalent in the blood after haploidentical stem cell transplantation

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AIM: Human Herpesvirus 6 (HHV-6) has a major impact in allogeneic stem cell transplantation (SCT). The frequency of HHV-6 DNA detection in blood varies according to the type of allograft. Here, we aimed to compare the detection frequency of HHV-6 DNA between haplo-, pheno- and genoidentical SCT. The detection frequencies of CMV, EBV, and BKV between the 3 groups were also considered.
METHODS: 3 groups of 35 age- and sex-matched adult patients (≥18y) with a recent haploidentical, phenoidentical, or genoidentical SCT at our institution were included. For each group, HHV-6, Cytomegalovirus (CMV), BK virus (BKV), and Epstein-Barr virus (EBV) viral loads were monitored in whole blood over the next 100 days post-SCT (R-GENE kits, Biomerieux).
RESULTS: The cumulative proportion of patient with a positive HHV-6 result during the next 100 days post-SCT was higher after haploidentical (85.7%) than after phenoidentical (60%, $p<0.05$) and genoidentical (28.6%, $p<0.001$) SCT. One patient in the haploidentical group was diagnosed with a HHV-6 chromosomal integration. No difference was observed in the percentage of detection of EBV and BKV DNA between the 3 groups (54.3%, 51.4%, and 48.6%, respectively, for EBV in haplo-, pheno-, and genoindentical SCT; 48.6%, 31.4%, and 34.3%, respectively, for BKV). CMV DNA detection was higher after haplo- and phenoidentical SCT (45.7% and 54.3%, respectively) than after genoidentical SCT (20%, $p<0.05$).
CONCLUSIONS: Haploidentical SCT is associated with a higher risk of HHV-6 DNA detection compared to geno- and phenoidentical SCT. HHV-6 and its potential clinical manifestations should be monitored in this population.
SOURCES OF FUNDING: None
Keywords: HHV6; haploidentical stem cell transplantation

PP-244
Viruses, tumors and immunocompromised hosts

Review of Management of Herpes Simplex Virus (HSV) Viraemia Among Immunocompromised Patients in a London Hospital, United Kingdom

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AIM: HSV can cause life-threatening disease in immunocompromised patients. At Royal Free Hospital, whole-blood HSV PCR is performed for post-transplant monitoring of HSV-seronegative patients, and in immunocompromised patients with HSV-positive swabs/biopsies or if high clinical suspicion of HSV infection. Follow-up blood PCR is often performed to guide antiviral duration. Here we present a review of our practice.
METHODS: HSV-viraemia results from September 2021 to February 2024 were reviewed. 29 immunocompromised patients (18 solid organ transplants, 6 rheumatology, 3 oncology, 2 HIV-positive CD4<200) were included.
RESULTS: Of 29 immunocompromised patients (31–80 years) with HSV-viraemia, 20 had HSV-1, 8 HSV-2 and 1 HSV-1/HSV-2 co-infection. Testing indications were superficial lesions (12/29,41%), HSV-positive lesion-swabs (5/29,17%), sepsis (5/29,17%), transaminitis (4/29,14%), encephalopathy (1/29,3%) and post-transplant monitoring (2/29,7%). 90% (26/29) HSV-viraemic patients were treated with antivirals. 46% (12/26) were commenced on treatment on clinical suspicion or swab positivity, and 54% (14/26) were started on receipt of a positive-blood PCR result. 73% (19/26) received intravenous aciclovir, stepped up from or stepped down to oral (median total duration 15 days,ranges 3–69 days). 27% (7/26) patients received oral antivirals only (median duration 7 days,ranges 5–24 days). Of 23 patients completing antivirals, 43% (10/23) stopped after at least 2 consecutive negative-blood PCR results, 35% (8/23) after one negative-blood PCR result, and 22% (5/23) without re-testing.
CONCLUSIONS: Blood HSV PCR testing is useful for identifying primary infection in HSV-seronegative transplant patients and for managing systemic illness in immunocompromised patients. Its role in patients with exclusively superficial lesions needs to be further analysed.
SOURCES OF FUNDING: None
Keywords: HSV viraemia, herpesviruses, serum PCR, immunocompromised patients, transplant medicine, antivirals

PP-245
Viruses, tumors and immunocompromised hosts

Role of human herpes viruses in the development of head and neck squamous cell carcinoma in Turkish patients

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AIM: Head and neck cancer (HNC) is the seventh most common malignant tumour. Viruses are one of the most significant risk factors. Among them viruses most commonly associated with HNC are human herpes viruses (HHVs). The aim of this study was to investigate the role of HHVs in the development of head and neck squamous cell carcinoma (HN-SCC) in the turkish patients.
METHODS: Pathological archive tissue samples of 300 patients diagnosed with HN-SCC (patient group) and 200 patients with benign oral cavity/oropharyngeal disease (control group) between 2000 and 2016 were evaluated. The presence of HHVs in tissue samples was investigated by PCR.
RESULTS: HHV-1, -2, -7, and -8 were not detected. EBV-DNA was positive in one patient (0.3%) and three control samples (1.5%). VZV and CMV were only detected in one (0.5%) and five (2.5%) samples of the control group, respectively. HHV-6 DNA was found in three samples (0.9%) of the patient group and two samples (1%) of the control group.
CONCLUSIONS: Despite the high HHV seroprevalence in the Turkish population, our results indicate that risk factors other than HHVs might be more prominent in developing HN-SCC. On the other hand, using archive material might also have impacted the results of PCR analysis as studies show that fresh samples yield a higher positivity rate than archive ones.
SOURCES OF FUNDING: The study was supported by Ankara University Project Office (No: 21L0230022).
Keywords: Head and neck cancer, squamous cell carcinoma, human herpes viruses

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Genetic Signatures is a molecular diagnostics company, providing flexible molecular testing for infectious diseases. The expansive menu offers syndromic testing for clinically relevant respiratory, enteric, antimicrobial resistance, sexual health, tropical diseases, and atypical pathogens. Flexibility is enhanced by proprietary 3baseTM technology, providing uniform sample processing for all specimen types. The streamlined, automated workflow supports simplified, flexible multiplexed real-time PCR assays which can detect large numbers of pathogens in a single sample, whist preserving throughput. Genetic Signatures workflow supports laboratories in improving diagnostic capabilities and 'time to result'. Automated reporting supports clinicians in providing rapid and appropriate therapies, improving patient management.

MWE

Web : www.mwe.co.uk

Medical Wire & Equipment Co. (Bath) Ltd has been at the forefront of laboratory sciences and developing pre-analytical medical devices (swabs and media) since 1952. Our innovation ensures every sample is collected and transported in its optimum condition, enabling the most accurate and reliable results, because results matter. By maintaining the highest standards, we empower healthcare providers and researchers to make informed decisions that drive the right actions, ultimately improving outcomes and advancing the quality of care and scientific discovery.
Visit MWE's stand to discuss how we can help you or contact us on talk2us@mwe.co.uk

MIKROGEN GmbH

Address : Anna-Sigmund-Straße 10 Phone : +49 89 548010
Web : www.mikrogen.de

Since 1989 we are a pioneer in the field of in vitro diagnostics: our focus is early detection of infectious diseases. It is realized in our automated workflows keeping our customers competitive with a focus on top-tier LINE-Assays, ELISA, CLIA, and Real-Time PCR.

QIAGEN

Web : https://www.qiagen.com

QIAGEN pushes the boundaries of innovation to improve our understanding of DNA, RNA and proteins – the building blocks of life. We enable our customers to unlock valuable insights from any biological sample – be it blood, a throat swab or many other materials. Our products for molecular testing serve a wide range of applications from basic life sciences research to clinical healthcare. Our reputation is based on confidence in decisions, clarity of results and pride in the insights our customers achieve. More than 35 Nobel Prize winners among more than 500,000 customers worldwide are proof of that success. Together, we are making improvements in life possible.

Quidelortho

Web : https://www.quidelortho.com/

Randox Laboratories

Web : www.randox.com

Established in 1982, we at Randox are dedicated to improving healthcare worldwide with the use of innovative diagnostic technologies. Randox have a range of Molecular products that include The Vivalytic, Qnostics, QCMD and more. This offers a comprehensive insight into diagnosis to facilitate disease management and treatment. Randox have also been the heartbeat of the UK's National COVID Testing Programme by completing more than 23 million gold-standard PCR tests by 2022.
As a world leader in diagnostics, our expertise and product portfolio have assisted laboratories across the world in improving global health and saving lives.

Roche

Web : www.roche.com

Roche is a global pioneer in pharmaceuticals and diagnostics focused on advancing science to improve people's lives.
Roche is the world's largest biotech company, with truly differentiated medicines in oncology, immunology, infectious diseases, ophthalmology and diseases of the central nervous system. Roche is also the world leader in in vitro diagnostics and tissue-based cancer diagnostics, and a frontrunner in diabetes management. The combined strengths of pharmaceuticals and diagnostics under one roof have made Roche the leader in personalised healthcare – a strategy that aims to fit the right treatment to each patient in the best way possible.
For more information, please visit www.roche.com.

Seegene Inc.

Web : www.seegene.com

Seegene is the world's leading developer of multiplex molecular technologies and multiplex clinical molecular diagnostics (M-MDx). Seegene's core enabling technologies -- DPO™, TOCE™ and MuDT™ -- are the foundation for multiplex molecular diagnostics and companion diagnostic tests that can simultaneously detect, differentiate, detect minority species and quantitative multiple targets with high sensitivity, specificity and reproducibility. Seegene's products detect multi-pathogens with great reliability and throughput, providing the most economical basis for saving time, labor and cost. Seegene's mission is to maintain leadership in molecular diagnostics for infectious diseases, genetics, pharmacogenetics, and oncology using innovative proprietary technologies.

Thermo Fisher Scientific

Web : www.thermofisher.com/infectiousdisease

Thermo Fisher Scientific Inc. is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Applied Biosystems brand, we help clinical laboratories accelerate innovation, increase productivity in their laboratories and improve patient health through diagnostics or clinical research.
Thermo Fisher Scientific supplies innovative testing solutions for infectious diseases from sample collection through results – we provide a broad range of products and services including sample extraction, qPCR assays predesigned or customed, qPCR instruments and software.

ZeptoMetrix

Web : https://www.zeptometrix.com/

ZeptoMetrix, an antylia scientific company, is the industry leader and manufacturer for innovative solutions solving challenges in the evolving Diagnostic Microbiology Infectious Disease, Oncology market and Analytical Reference Materials for the Applied Markets. We focus on our customer's success by providing premium product quality, reliability, and expert technical knowledge, enabling our customers to develop and advance many applications across diagnostics, pharmaceutical, environmental, food and beverage industries. From in stock solutions to custom control and panel development through to launch, our scientific teams provide our customers with comprehensive, performance-oriented, and cost-effective products and services that positively impact the field of clinical diagnostics, analytical testing and contribute to a healthier world.

Zymo Research Europe

Web : https://www.zymoresearch.de/

From its birthplace in a small garage in Orange, California 30 years ago, to the industry leader it is today, Zymo Research has been led by a vision to have a positive impact in the biomedical field and to contribute to the greater good of humanity. That vision touches every aspect of Zymo Research and has guided the company's growth, culture and creation of the most innovative and valuable biomedical tools and services since 1994.
Now, Zymo Research is a globally established biotechnology company and industry leader in the fields of epigenetics, microbiomics and the emerging Next-Gen Sequencing space. With international facilities and a global distribution network, Zymo Research enables researchers in academia and the biomedical field to make cutting-edge discoveries all over the world. While the company provides some of the most technologically advanced products in the industry, everything is driven by the fundamental belief that "the beauty of science is to make things simple."

altona DIAGNOSTICS

Visit our booth #2

CHOOSE FROM A VARIETY OF AUTOMATED REAL-TIME PCR TESTS

Your experienced partner in transplant and immunocompromised testing

Broad assay portfolio of AltoStar® PCR tests for monitoring of common viruses in transplant and immunocompromised patients

- Quantitative single and multiplex assays
- Various specimen types
- CE-IVD marked assays for human adenovirus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6A and 6B, herpes simplex virus 1 and 2, varicella-zoster virus, BK virus, JC virus, parvovirus B19

Why choose our PCR kits?

Flexibility: manual or automated use with AltoStar® workflow

Ease of integration: designed for integration of existing lab workflows

High sensitivity & specificity: verified through independent EQA (QCMD)

Certification according to Regulation (EU) 2017/746 coming soon

VISIT OUR WEBSITE FOR FURTHER INFORMATION

GBD mbH

Multiplex samples

Customized control samples

Consulting & data evaluation

Our newest product on the market:
multiplex samples for gastrointestinal pathogens (viruses and bacteria).

Our work includes consulting and data evaluation.

Our portfolio includes the customized design and production of quality control samples.

NEWS

BENEFIT

Benefit from well-defined and standardised control samples that provide guidance within various testing and comparative analyses.

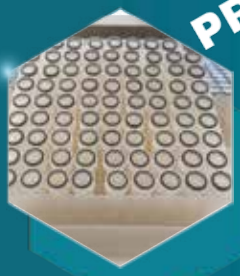
Benefit from our tailor-made solutions for your project requirements.

Benefit from our complete workflow control samples and from their commutability and cross-platform compatibility.

Benefit from our quality and service standards.

benefits:
- **standardised**
- **tailor-made**
- **commutable**
- **high quality**

PRODUCTS



Specializing in virus and bacterial control samples.

Our focus is on the production of control samples for virus and bacterial genome detection and virus immunology.

We produce run and screen controls and multiple analyte samples for our customers. Our portfolio contains more than 100 parameters.

Are you in search of specific samples that simplify your daily laboratory tasks and assist in addressing particular inquiries? Are you enhancing processes or training staff, validating tests when materials are rare? Reach out to us!

SERVICES

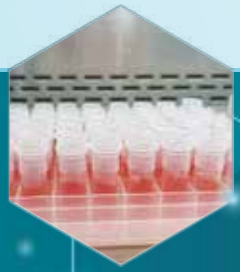
FOCUS

Enhancing lab diagnostics with quality and bespoke quality control samples.

The purpose of our work is to contribute to the continuous quality improvement and standardization of laboratory diagnostics.

We are a strong partner in the preparation of EQA samples. We have expanded this contribution to quality assurance and are increasingly manufacturing control samples for your individual quality needs and assurance.

PURPOSE



STAY INFORMED

newsletter

catalog

altona DIAGNOSTICS

CHOOSE RELIABLE PCR ASSAYS FOR VARIOUS RESPIRATORY PATHOGENS

Are you prepared for the respiratory season?

Broad assay portfolio as single or multiplex assay

Human adenovirus, enterovirus, human metapneumovirus (hMPV), influenza virus (type A, B and A (H1N1)pdm09), parainfluenza virus 1-4 (PIV), RSV, SARS-CoV-2, MERS-CoV, *Pneumocystis jirovecii*, Bordetella (*B. pertussis*, *B. parapertussis*, *B. holmesii*)

Why choose our PCR kits?

Flexibility: manual or automated use with AltoStar® workflow

Ease of integration: designed for seamless integration of existing lab workflows

High sensitivity & specificity: verified through independent EQA (QCMD)

Genetic Signatures

Transforming Molecular Diagnostics

Simplified syndromic detection for respiratory and gastrointestinal pathogens

One-step sample extraction & PCR set up, plus AI-driven result interpretation

Visit booth 7

geneticsignatures.com

Abbott

ID NOW™ PLATTFORM

FLEXIBLE ATEMWEGSTESTS **NOW**

WANN UND WO SIE DIESE BRAUCHEN

Die ID NOW Plattform liefert innerhalb von Minuten Ergebnisse molekularer Atemwegsschnelltests mit einer Genauigkeit, die der von RT-PCR-Tests entspricht.

COVID-19 | INFLUENZA A und B | RSV | STREP A

RSV = Respiratorisches Synzytialvirus

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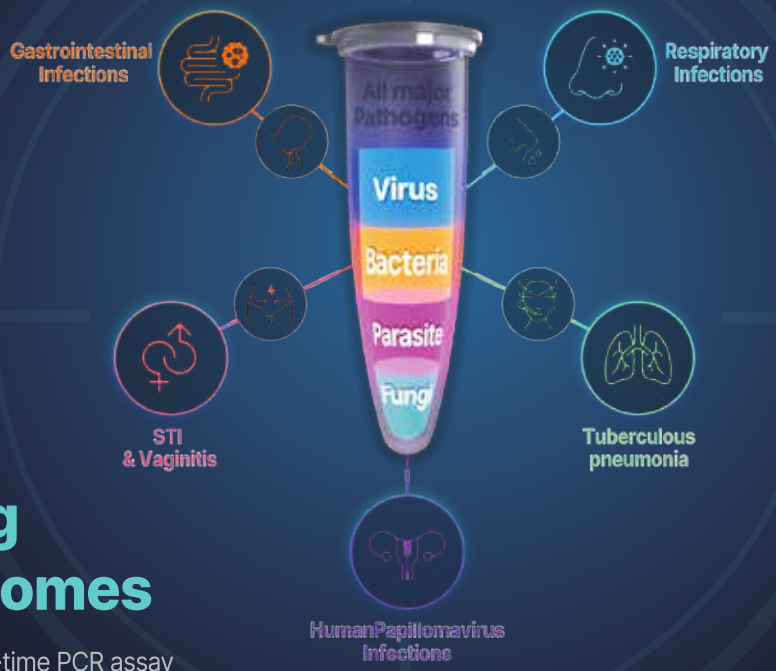
Seegene



Better Syndromic Testing for the Best Clinical Outcomes

Seegene's Syndromic Testing Solution is a high-multiplex real-time PCR assay designed to simultaneously detect and identify the causative pathogens of disease in patients based on their symptoms.

Visit Seegene Booth# 11

to find more about Seegene's Syndromic Testing Solution

seegene.com