SCIENTIFIC Conference

e-Book

International Alliance for Biological Standardization

Training Workshop &

4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animal

December, 3-5 2024

Frankfurt, Germany

www.iabs.org

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Sponsors

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PathoQuest

MilliporeSigma

Boehringer Ingelheim

About the Conference

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2024 is a key year for globally considering NGS implementation with the recent publication of the revised ICHQ5A(R2) guideline and the upcoming draft chapter on NGS from the EDQM/European Pharmacopoeia.
The 4th NGS meeting will continue to highlight current scientific data and knowledge with focus on NGS implementation for adventitious virus detection in biological products. Discussions will include capabilities of NGS for virus detection in different matrices and validation of the technical and bioinformatics steps involved in NGS for its applications in characterization and safety evaluation of biologics such as vaccines for human and veterinary use, gene and cell therapy products, and biotherapeutics.

The meeting will bring together representatives from industry, academia, technology providers, and international regulatory bodies for discussing the current applications of NGS as an alternative method for replacing or supplementing the current conventional assays for detection of adventitious viruses in biologics.

A one-day NGS training workshop (December 3rd) to be associated to this conference (December 4th & 5th) is in preparation and further details will be communicated soon.

Scientific and Organizing Committee

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Scientific Committee

Arifa S. Khan - Co-chair, FDA-CBER (FDA), U.S.A.
Laurent Mallet - Co-chair, EDQM, France
Pieter Neels - IABS Chair, Belgium
Johannes Blümel - PEI, Germany
Noémie Deneyer - GSK, Belgium
Sigrid De Keersmaecker - Sciensano, Belgium
Blandine De Saint-Vis - Boehringer- Ingelheim, France
Ivana Knezevic - WHO, Switzerland
Carine Logvinoff - Sanofi Pasteur, France
Marie Murphy - Eli Lilly, Ireland
Siemon Ng - Notch Therapeutics, Canada
Yoji Sato - National Institute of Health Sciences – Japan
Michael Wall - Health Canada, Canada

Organizing Committee

Arifa S. KHAN - Co-chair, FDA-CBER (FDA), U.S.A.
Laurent MALLET - Co-chair, EDQM, France
Pieter NEELS - IABS Chair, Belgium
Madinina COX - IABS Secretariat
Marlène LOUIS - IABS Secretariat

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Training Workshop on NGS
Tuesday, December 3, 2024

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Training Workshop on NGS

8:30 - 9:00	*Registration & Welcome Coffee*
9:00 - 9:10	Welcome Remarks: IABS & Chairs **Laurent MALLET**, European Directorate for the Quality of Medicines & HealthCare (EDQM) – France – Co-Chair
9:10 - 9:55	General Overview of the NGS **Carine LOGVINOFF,** Sanofi, France **Siemon NG,** Notch Therapeutics, Canada
9:55 - 10:10	Q&A Session
10:10 - 10:40	*Coffee Break*
10:40 - 11:05	Sequencing Instrument Presentations **Illumina** Felix KROMBHOLZ
11:05 - 11:30	**Nanopore** Veronica FOWLER Tim WALKER
11:30 - 11:55	**PacBio** Felix WOLTER Natalie KAHN

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11:55 - 12:15	Round Table & Q&A Illumina, Nanopore, PacBio
12:15 - 1:15	*Lunch Break*
1:15 - 2:00	Sample and Library Preparation (Challenges, Specific examples) **Siemon NG**, Notch Therapeutics, Canada
2:00 - 2:15	Q&A Session
2:15 - 3:15	Bioinformatics (different tools, targeted/non targeted, reference database) **Pei-Ju CHIN**, USFDA, U.S.A.
3:15 - 3:30	Q&A Session
3:30 - 4:00	*Coffee Break*
4:00 - 4:30	Follow-up investigations **Christophe LAMBERT**, GSK, Belgium

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4:30 - 4:45 Q&A Session

4:45 - 5:45 Panel Discussion – Technical Challenges for Using NGS for Adventitious Virus Detection
Perspectives from presenters and participants
Carine LOGVINOFF, Sanofi, France
Pei-Ju CHIN, USFDA, U.S.A.
Siemon NG, Notch Therapeutics, Canada Pei-Ju CHIN, USFDA, U.S.A.
Christophe LAMBERT, GSK, Belgium Sigrid DE KEERSMAECKER, Sciensano, Belgium
Blandine DE SAINT-VIS, Boehringer Ingelheim Animal Health, France
Sigrid DE KEERSMAECKER, Sciensano, Belgium
Johannes BLÜMEL – Paul-Ehrlich Institut (PEI) – Germany

5:45 - 6:45 Closing remarks
Arifa S. KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER (FDA) – U.S.A. – Co-Chair

6:45 **Meet & Greet: Advanced Virus Detection Technologies Working Group (AVDTWG)**
Open to all on-site attendees of the Training workshop and the 4th NGS Conference
Link to the Working Group presentation at the IABS Webinar (September 25-26, 2024)

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4th NGS Conference

8:30 - 9:00 *Registration & Welcome Coffee*

9:00 - 9:10 Welcome Remarks: IABS & Chairs
Rick Hill, IABS, USA
Arifa KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER (FDA) – U.S.A. – Co-Chair
Laurent MALLET, European Directorate for the Quality of Medicines & HealthCare (EDQM) – France – Co-Chair

Introduction

9:10 - 9:30 Introduction – Summary of Previous Meetings and Goal of the 4th meeting
Arifa KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER (FDA) – U.S.A. – Co-Chair
Laurent MALLET, European Directorate for the Quality of Medicines & HealthCare (EDQM) – France – Co-Chair

Session I: Current Perspectives on Using NGS for Adventitious Virus Testing

Moderators: Laurent MALLET & Arifa KHAN

9:30 - 10:00 Next Generation Sequencing and ICH Q5A(R2)
Johannes BLÜMEL, Paul-Ehrlich Institute (PEI), Germany

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10:00 - 10:30	EDQM/Ph. Eur. perspectives on NGS/HTS **Laurent MALLET**, Co-Chair & **Gwenael CIREFICE**, European Directorate for the Quality of Medicines & HealthCare (EDQM), France
10:30 - 11:00	FDA **Arifa KHAN**, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER (FDA), U.S.A. – Co-Chair
11:00 - 11:30	WHO perspectives on the use of HTS for detection of adventitious agents in biologicals **Ivana KNEZEVIC**, World Health Organization (WHO), Switzerland
11:30 - 12:00	*Coffee Break*
12:00 - 12:30	Perspectives from the CAACB on the Current Benefits and Challenges of NGS Adoption for Viral Safety Testing **Charles SWOFFORD**, MIT Center for Biomedical Innovation
12:30 - 1:00	EFPIA's Perspectives on the Validation and Implementation of Next Generation Sequencing for Virus Safety Testing of Biological Products **Noémie DENEYER**, GSK, Belgium, Member of the EFPIA WG
1:00 - 2:00	*Lunch Break*

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2:00 - 2:45

Panel Discussion
Johannes BLÜMEL, Paul-Ehrlich Institute (PEI), Germany
Laurent MALLET, Co-Chair, European Directorate for the Quality of Medicines & HealthCare (EDQM), France Gwenael CIREFICE, European Directorate for the Quality of Medicines & HealthCare (EDQM), France
Arifa KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER (FDA), U.S.A. – Co-Chair
Ivana KNEZEVIC, World Health Organization (WHO), Switzerland
Charles SWOFFORD, MIT Center for Biomedical Innovation
Noémie DENEYER, GSK, Belgium, Member of the EFPIA WG
Juliati DAHLAN, National Agency of Drugs and Food Control, Indonesia (Virtual)
Hadeer ABOSALEM, Egyptian Drug Authority EDA, Egypt (Virtual)
Hamida BEGUM, Bangladesh (Virtual)

Session II: Reference Materials, NGS Qualification and Validation

Moderators: Sigrid DE KEERSMAECKER, Johannes BLÜMEL

2:45 - 3:10

Transcriptomic NGS assay of cells as a substitute for conventional virus testing techniques
Marc ELOIT, Pathoquest, France

3:10 - 3:35

Matrix effects on Limit of Detection for NGS-based Adventitious Virus Detection Assays
Bradley HASSON, MilliporeSigma, U.S.A.

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3:35 - 4:00	AVDTWG Spiking Study 4 - Evaluation of Long-Read Sequencing for Adventitious Virus Detection in a Low Complexity Viral Background **Valeria ZANDA**, Merck, Italy
4:00 - 4:30	*Coffee Break*
4:30 - 4:55	Head-to-head comparison of NGS with in vivo animal assays and in vitro cell culture assays for adventitious virus detection **Alison ARMSTRONG**, Merck KgAG on behalf of NIIMBL PC3.1-305 project team
4:55 - 5:20	Refinement Efforts on CBER's Reference Virus Database (RVDB) to Enhance Accuracy and Specificity of Virus Detection **Pei-Ju CHIN**, US FDA, U.S.A.
5:20 - 5:50	Next Generation Sequencing: Validation, Regulatory Approval and Implementation of NGS as an alternative In Vivo Testing for Live Attenuated Influenza Vaccine **Alice ALSTON**, AstraZeneca (Virtual)
5:50 - 6:30	Panel Discussion
6:30	*End of Day 1*

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8:00 - 8:30 *Registration & Welcome Coffee*

Session III: NGS Applications

Moderators: Michael WALL, Blandine DE SAINT-VIS

8:30 - 8:55 Comparison of Non-Targeted and Broad-Spectrum Targeted NGS for Adventitious Virus Detection
Gibran Horemheb RUBIO QUINTANARES, Paul Ehrlich Institute, Germany

8:55 - 9:20 Comparison of Long- and Short-Read Sequencing Methods for Recombinant Adeno-Associated Virus Sequence and Adventitious Agent Identification in a GMP Environment
Megan GURA, Regeneron, U.S.A.

9:20 - 9:45 Selecting and developing approaches for adventitious virus detection by HTS, contrasting release testing with investigational follow-up
Carine LOGVINOFF, Sanofi, France
Song SUN, Sanofi, Canada

9:45 - 10:30 *Coffee Break - **POSTER SESSION***

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10:30 - 10:55 Current status for testing of adventitious agents by NGS at Serum Institute of India Pvt. Ltd. and way forward
Subhashis CHATTERJEE, Serum Institute of India, India

10:55 - 11:20 BLOODVIR – Surveillance system for novel viruses based on next generation sequencing and artificial intelligence
Martin MACHYNA, Paul Ehrlich Institute, Germany

11:20 - 12:20 Panel Discussion

12:20 - 1:30 *Lunch Break*

Session IV: Strategies for Optimization of NGS Virus Detection and Follow-up of NGS Signal

Moderators: Siemon NG, Marie MURPHY

1:30 - 1:55 Rapid alignment-free detection of adventitious agents using next-generation sequencing
Tom J.B. DE MAN, Millipore Sigma, U.S.A.

1:55 - 2:20 Viral metagenomic analysis to complement the viral risk assessment and adventitious agent testing of live virus vaccines
Vanessa V. SARATHY, Merck, U.S.A.

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2:20 - 2:45 Evolution of a bioinformatics pipeline for adventitious agent detection
Robert L. CHARLEBOIS, Sanofi, Canada

2:45 - 3:20 Panel Discussion

3:20 - 3:50 *Coffee Break*

Session V: Implementation of NGS in Biologics

Moderators: Ivana KNEZEVIC, Laurent MALLET, Arifa KHAN

3:50 - 5:20 Final Panel Discussion (Regulators, Industries and CROs)
Johannes BLÜMEL, Paul-Ehrlich Institut (PEI), Germany
Ken KONO, National Institute of Health Sciences, Japan
Marc ELOIT, Pathoquest, France
Alison ARMSTRONG, Merck KgAG
Carine LOGVINOFF, Sanofi, France
Blandine DE SAINT-VIS, Boehringer Ingelheim Health Animal, France
Marie MURPHY, Eli Lilly & Co, Ireland
Siemon NG, Notch Therapeutics, Canada
Christophe LAMBERT, GSK, Belgium
Gwenael CIREFICE, European Directorate for the Quality of Medicines & HealthCare (EDQM)
Pei-Ju CHIN, US FDA, U.S.A.
Ajmeer RAMKISHAN, Ministry of Health & Family Welfare, India (Virtual)
Koji ISHII, National Institute of Infectious Diseases, Japan (Virtual)
Igishe USHIE, National Agency For Food And Drug Administration And Control (NAFDAC, Nigeria (Virtual)

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5:20 - 5:30	Summary & Conclusion
5:30 - 5:40	Closing Remarks
5:40	*End of meeting*

Upcoming IABS Conferences and Workshops

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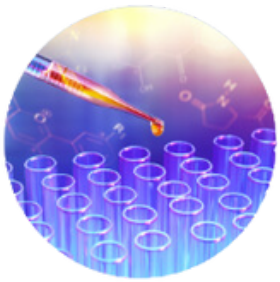
Advances in Analytical Technologies for Biopharmaceutical Products

Rockville, MD, USA
March 19-21, 2025

Leveraging Analytical and Bioprocess Platforms for Biological Product Development and Commercialization

Brussels, Belgium
May 14-15, 2025



AFSA – IABS Conference about Animal testing replacement for vaccines: A One Health View: global outlook and future strategy

Bangkok, Thailand
December 2-4, 2025

Biosketch

Hadeer Abosalem, MsC

Technical manager, Biotechnology unit
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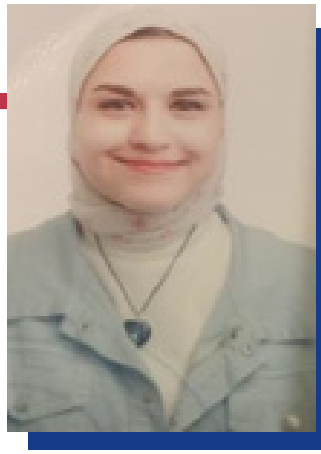
- M.Sc. holder in biochemistry and molecular biology with publication in immune cell therapies.
- 10 years of experience in the field of biological products, Gene therapy and ATMPs at Egyptian Drug Authority (EDA).
- Serving as technical manager of biotechnology unit at the Central Administration of Biological and Innovative Products and Clinical Trials, EDA.
- Member in IPRP cell and gene therapy working group.

Performing, supervision and training others on CTD's quality module assessment for recombinant product &ATMPs throughout the product lifecycle including the reliance pathways for registration, post market variation, IMP (investigational medicinal product)and evaluation of biosimilars .

Supervising different lab techniques performed for control the release of biological products including ELISA, tissue culture, clotting assay, gel diffusion, SDS-PAGE, Western blot and in-vivo techniques.

Biosketch



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GMP inspectors on local and overseas biological companies for recombinant products and biosimilars .

Participating in drafting, issuing, and reviewing different EDA guidelines for biological products, biosimilars and ATMPs registration. Participating as reviewer in global regulatory framework (IPRP) for cell and gene therapy products.

Participating as a speaker in the workshops held by EDA for biological companies training and for other assessors on the evaluation of CTD file and "Evaluation of comparability Exercise for biosimilars registration "

Participating in Regional Centre of Excellence for Vaccines, Immunization and Health Supply Chain Management (EAC RCE-VIHSCM) training workshop held in EDA.

Biosketch

Alice Alston

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Director of Global Quality Control at AstraZeneca, with direct responsibility for all safety testing of drug products within the biologics function. Held many roles within AstraZeneca over 21 years, all within the quality function, prior to that held roles within the quality function at Baxter Healthcare.
Passionate about 3R's and the strive to replace animal testing with newer more sensitive / robust technologies for lot release purposes.

Abstract

Alice Alston

Next Generation Sequencing: Validation, Regulatory Approval and Implementation of NGS as an alternative In Vivo Testing for Live Attenuated Influenza Vaccine

Next Generation Sequencing (NGS) has been proven to address some of the limitations of the current testing methods for adventitious agents testing in biologics. NGS is recognized as a powerful technology with capabilities for broad virus detection, this addresses the challenges through validation, contamination control approach in vaccine manufacturing and eventual approval for the use of NGS as an alternative to the traditional In Vivo assay for lot release testing of a flu vaccine. This will also include data from 12 months of utilising NGS as a lot release assay as a replacement of the In vivo assay

This presentation summarises the efforts taken to replace the in vivo adventitious agents lot release test for Live Attenuated Influenza Vaccine with the NGS adventitious agent detection assay.

Abstract

Alison Armstrong

Head-to-head comparison of NGS with in vivo animal assays and in vitro cell culture assays for adventitious virus detection

This study has evaluated next generation sequencing (NGS) as an alternative method for adventitious virus detection by conducting a head-to-head comparison with the compendial in vivo animal assays and in vitro cell culture assays. Two viruses were selected from the WHO reference virus reagents, with distinct physical, chemical, and genomic properties, such that at least one was expected to produce a positive result in each of the different test assays. Based on infectious titer and genome copy number, different virus levels were spiked in CHO unprocessed bulk material to determine LOD in a complex biological matrix. To ensure a direct comparison of the different assays using the identical sample materials, spiked and unspiked samples were generated, blinded, aliquoted, stored at -80°C and distributed to each of the participating labs. Six spiked samples were selected based on pre-study results from ddPCR and in vitro cell culture assays. Two labs conducted the in vitro assays and three labs performed NGS. To reduce the number of animals used in the study, only one lab conducted the in vivo assays. Each lab followed their own workflow and assay protocols. Study results will be presented supporting the ICH Q5A(R2) guideline for considering NGS as an alternative method for broad adventitious virus detection to replace or supplement the conventional in vivo and in vitro adventitious virus detection assays.

This project (PC3.1-305) was supported by The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) by the Bill & Melinda Gates Foundation through funding from the Bill & Melinda Gates Foundation.

Biosketch

Johannes Blümel

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Dr. Johannes Blümel is leading the virus safety section at the Paul-Ehrlich-Institut, Langen. He is dealing with assessment of virus safety and TSE safety of blood products, recombinant DNA products such as monoclonal antibodies, and advanced therapy medicinal products (ATMPs) for clinical trials and marketing authorization. He participates as expert in EMA-Biologics Working Party (BWP) and EDQM TSE-certification procedure and contributed in drafting of numerous Guidelines and Monographs on viral safety. Further, he is working in several research projects on virus inactivation and detection of Viruses by Next Generation Sequencing (NGS). Since 2023 he is chairing the group on blood-associated pathogens for the national advisory board "Arbeitskeis Blut" in Germany as well as the Ph. Eur. Working Party on High Throughput Sequencing (HTS), in charge of elaborating a general chapter on HTS for the detection of viral extraneous agents.

Prior to joining the Paul-Ehrlich-Institut in 1998, Dr. Blümel worked at the University Hospital, University of Bonn (1993-1998). He performed basic research on virus replication and received a five years training in medical virology and virus diagnostics (Fachvirologe). Dr. Blümel completed his Diploma Study in Biology (molecular genetics, microbiology, biophysics and physical chemistry) in 1991 at the University of Freiburg, Germany. He received his Ph.D. degree at the Department of Virology, University of Freiburg, Germany (1993). In 2010 he received teaching graduation (Habilitation) in Medical Virology from the University Frankfurt.

Abstract

Robert L. Charlebois

Evolution of a bioinformatics pipeline for adventitious agent detection

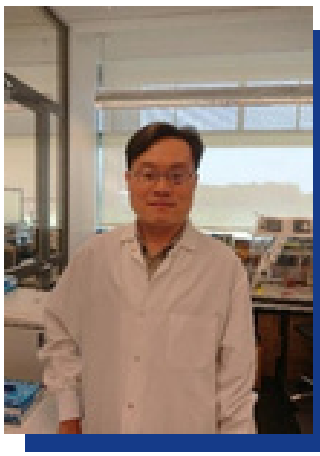
High-throughput sequencing offers an unbiased view into the biological composition of a sample, and has found important applications in characterizing the biosphere and its ecology. Such metagenomics approaches have also been leveraged to search for, and simultaneously identify, any potential contaminating viruses or microorganisms in vaccines, biologicals, or their intermediates. All of this depends on robust bioinformatics that can reliably assign nucleic acid sequences to their respective taxonomic origin.

A family of approaches can be envisioned for running such an adventitious agent detection pipeline, as outlined in Lambert et. al 2018 (Viruses 10: 528). In this talk, I will describe the evolution of Sanofi's PhyloID™ pipeline, as a concrete instance within this design space. Its architecture is modular, facilitating both development and validation.

Our pipeline development strategy needed to be sensitive to both aspects impacting public health—patient safety and product delivery—in spite of initial uncertainties in the technology. From the practical side, we first focused on utility, then performance, and then automation. Concurrently, we framed our strategy with initial caution and skepticism, then thoughtful risk taking, with the goal of eventual acceptance.

This work was funded by Sanofi. Robert Charlebois is a Sanofi employee and may hold shares and/or stock options in the company.

Biosketch



Pei-Ju Chin, M.S, Ph.D.

Staff Fellow
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Pei-Ju Chin is a Staff Fellow in Arifa Khan's lab at OVRR/CBER/FDA. His current research focus is to evaluate the platforms, protocols and bioinformatic strategies for adventitious virus detection by next-generation sequencing (NGS). In addition, Pei-Ju is the leader of the Advanced Virus Detection Working Group (AVDTWG) Subgroup C working on database refinement, and is responsible for the update, maintenance, and refinement of reference virus database (RVDB), which is currently hosting 5K users worldwide. His regulatory responsibilities include review of investigational viral vaccines including SARS-CoV-2 vaccines.

Pei-Ju received his Ph.D. in molecular genetics and biochemistry at Georgia State University, Master of Science degree in bioinformatics and human genetics at National Yang-Ming University, Taiwan, and Bachelor's degree in microbiology at Soochow University, Taiwan. Before joining the US FDA, he was an IRTA fellow at National Institute on Aging/NIH studying the role of homologous recombination and DNA repair mechanisms in telomere length maintenance.

Abstract

Pei-Ju Chin

Refinement Efforts on CBER's Reference Virus Database (RVDB) to Enhance Accuracy and Specificity of Virus Detection

Authors:
Pei-Ju Chin1, Jaysheel D. Bhavsar2, Trent J. Bosma1, Madolyn L. MacDonald2, Shawn W. Polson2 and Arifa S. Khan1

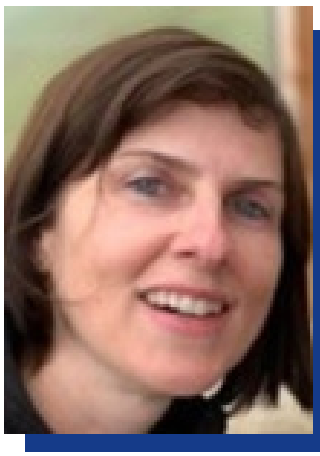
1 Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, USA.

2 Department of Computer & Information Sciences, Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE 19713, USA.

The potential of next generation sequencing (NGS) for broad adventitious virus detection in biological products was demonstrated by the unexpected detection of porcine circovirus type 1 (PCV1) in a licensed rotavirus vaccine (Victoria et al., J. Virol. 2010) and the discovery of a novel rhabdovirus in Sf9 insect cells, a commonly used cell substrate for baculovirus-expressed products (Ma et al., 2014). NGS is now being recognized by regulatory agencies and health authorities as an alternative method for adventitious virus detection to supplement or replace the currently recommended in vivo and in vitro assays. However, the qualification and validation of NGS methods relies on the public availability of reference materials.

Reference materials are needed to evaluate the entire NGS workflow, including the upstream wet-bench steps for sample and library preparation to the downstream bioinformatics analysis pipeline. CBER's non-redundant, reference viral database (RVDB; https://rvdb.dbi.udel.edu/) was developed to enhance bioinformatics analysis for detection of known and novel viruses. This included all viral, virus-related, and virus-like nucleotide sequences (excluding bacteriophage) with a reduced cellular sequence content to enhance broad virus detection with less computational burden (Goodacre et al., 2018). This talk will describe the improvements and ongoing work on RVDB for identifying and removing or annotating some of the non-viral sequences that are present in RVDB due to direct input of sequences from GenBank.

Biosketch



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Sigrid De Keersmaecker, PhD in Bioscience Engineering, heads the BIOTECH Platform unit within the Transversal Activities in Applied Genomics scientific service at Sciensano, the Belgian Institute for Health. Her expertise encompasses developing, implementing, and validating new methods for applied genomics, including NGS, for both routine analysis and innovative scientific and technological research across various health applications, including in the field of molecular detection/characterization of microorganisms and human (epi)genetic and microbiome biomarkers. She actively contributes to shaping genomics standards through participation in quality working groups. With a solid publication record and leadership in research projects, she is dedicated to advancing applied genomics for proactive public health policies, such as in the areas of pathogen detection, genomic surveillance, exposome, product quality and food safety, for the benefit of society.

Biosketch

Tom J.B. De Man

Head of Omics and Machine Learning R&D
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Tom de Man is head of the Omics and Machine Learning R&D department at Merck, supporting the BioReliance® services portfolio. His department develops and validates multi-omics bioinformatics algorithms for adventitious agent testing. Previously, Tom has worked at the US Centers for Disease Control and Prevention (CDC) and various academic institutions, which provided him with a broad set of professional skills. His expertise includes comparative genomics, software development, and (GxP) data analysis. Tom has authored over 20 peer-reviewed manuscripts on various molecular and bioinformatics topics, including pathogen detection and characterization. He holds a Bachelor (BSc) as well as Master of Science (MSc) in Bioinformatics.

Abstract

Tom J.B. De Man

Rapid alignment-free detection of adventitious agents using next-generation sequencing

Authors: Tom J.B. de Man, Brendan Redler, Amber Overgard, Afshin Sohrabi - Merck KGaA

Next-Generation Sequencing (NGS) has revolutionized adventitious agent testing (AAT), becoming integral to the ICH Q5A(R2) guidelines due to its sensitivity and breadth of sample analysis. NGS technology facilitates detection of both known and novel adventitious agents that contaminate biopharmaceutical products, offering comprehensive understanding of sample composition. As reference genome databases and whole-genome sequencing output continue to grow, the demand for scalable and accurate computational methods increases, with traditional full-read local alignment approaches lagging in efficiency.

Our newly designed workflow employs a k-mer based analysis algorithm that addresses these issues as it is more application-appropriate for AAT metagenomic datasets. The workflow enables the rapid, accurate detection of viruses, bacteria, and fungi, identifying k-mers unique to respective taxa. By comparing test sample k-mers against a streamlined query database, consisting of diagnostic k-mers derived from public nucleic acid sequences, the method significantly cuts down search space and computation time. The workflow can screen out cell-line and other manufacturing process data to improve the reliability of results.

This algorithm is not only suitablefor highly accurate, short Illumina sequencing reads (error rates
<0.5%, lengths 100–250 bp) but also compatible with long-read data from platforms like Pacific Biosciences and Oxford Nanopore.
Benchmarking indicates that the pipeline can reduce false positives compared to existing AAT analysis methods and displays a detection limit of 1x104 viral genome copies per mL across various backgrounds. Consistent output across multiple analysis runs affirm its GMP compliance as an AAT bioinformatics pipeline. These findings demonstrate performance on par with current bioinformatics methodologies, while offeringenhanced efficiency in the processing of NGS AAT data.

This algorithm significantly improves the scalability and reliability of AAT assays and ensures the ability to adapt to the next era of genomic data complexity.

Biosketch

Blandine de Saint-Vis, PhD

Global MSAT Sr Scientist
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Blandine de Saint-Vis, PhD in immunology, is Sr Scientist-expert at Boehringer Ingelheim Animal Health, in Global MSAT Bio-Analytics department in France.
Following several years in Immunology Research at Schering-Plough to identify and characterize new genes, she joined Boehringer-Ingelheim in R&D, and participated in the development of veterinary vaccines.
She has a strong expertise in molecular biology (real-time PCR, sequencing, DNA chips) and proposes innovative methods to help and accelerate the development of vaccines for all kind of microorganisms and all species. She proposed NGS approaches for vaccine seeds controls and actively contributed to the implementation of the method.
More recently, in Global MSAT Analytics department, she continues to propose and implement new analytical methods including NGS, and new technologies for Life Cycle products.

Biosketch

Noémie Deneyer, Ph.D.

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Noémie Deneyer is a Senior Manager and Associate GSK Fellow working as Molecular Biology Lead at GSK, with a focus on innovative analytical technologies including NGS. Noémie holds a PhD in Molecular and Cellular Biology from the UCLouvain, Belgium. In 2019, she started her journey at GSK where she was coordinating scientific projects aiming to deploy novel analytical methods for Quality Control testing. In January 2021, she joined the Global Quality Control department where Noémie and her team worked together to develop and implement Next Generation Sequencing in Quality Control to drive innovation and strengthen product safety. Since May 2024, Noémie joined the Manufacturing Sciences & Technology department where she is leading the strategical deployment of new analytical technologies and ensuring connections between Analytical R&D and Quality groups for molecular biology applications. Noémie is also involved in different external working and interest groups such as the AVDTIG where she had the chance to increase her engagement by becoming a co-chair in 2023.

Abstract

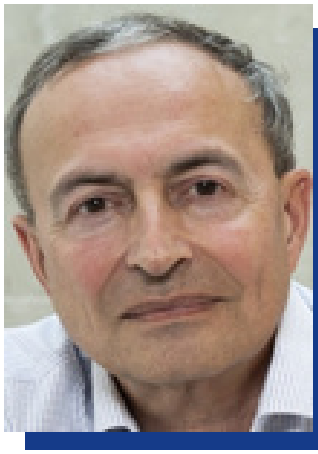
Noémie Deneyer

EFPIA's Perspective on the Use of Next Generation Sequencing for Virus Safety Testing

Viral safety testing of biotechnology products relies on extensive testing of the materials used in manufacturing, with a focus on cell banks of animal origin as a critical starting material. The ICH Q5A guideline, used worldwide as a reference for viral safety, has been recently revised to integrate the most up-to-date scientific knowledge and approaches developed over the last decades. Among them, Next Generation Sequencing (NGS) has emerged as a promising technology to detect a broad spectrum of viruses. Also known as high-throughput sequencing, NGS allows massive parallel generation of nucleic acid sequence data without prior sequence information, offering the potential to detect unknown or unexpected viruses.

In parallel with the development of Revision 2 of ICH Q5A, the EFPIA Working Subgroup for Clonality, Characterisation and Viral Safety of Cell Lines has been elaborating a position paper on the use of NGS for virus detection during manufacturing of biotechnology products, focusing mainly on recombinant proteins and vaccines. Envisaged as a practical guide, the paper aims at complementing ICH Q5A with more technical details and at providing a position from Industry for dialogue with Regulatory Authorities. Starting with an overview of the various types of tests used for virus safety testing, including molecular methods (PCR, NGS), the paper provides a detailed discussion on the technical and regulatory challenges that need to be addressed to successfully implement NGS for virus safety testing. The discussion is focused on three topics: recommended approaches for validation of the NGS-based analytical procedure; considerations related to the comparability of NGS-based methods with traditional virus safety tests in view of a replacement; and elements to elaborate a regulatory strategy for successful Health Authority approval of NGS for virus safety. The presentation will provide an overview of the key messages covered by the position paper.

Biosketch



Marc Eloit

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Marc ELOIT is a veterinarian specializing in infectious diseases and a former Professor of Virology at the National Veterinary School of Alfort (ENVA). He served as Director of the INRA-ANSES-ENVA Virology Unit before leading the Pathogen Discovery Laboratory at the Pasteur Institute. His academic research has focused on identifying new or unexpected viruses in patients and investigating viruses circulating at the human-wildlife interface. For 20 years, he was a member of the Virus Safety Committee of the French Medicines Agency. In 2010, he founded PathoQuest, a spin-off from the Institut Pasteur, dedicated to testing biologicals using next-generation sequencing (NGS).

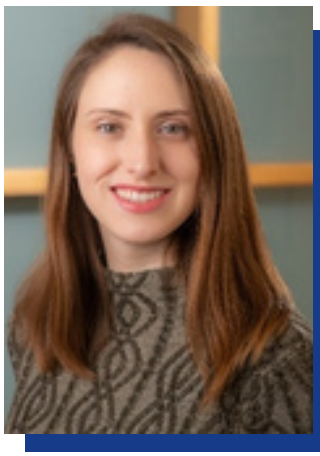
Abstract

Marc Eloit

Transcriptomic NGS assay of cells as a substitute for conventional virus testing techniques

Testing for viruses in cells employed in cell therapy and biomanufacturing is detailed in various monographs and guidelines. Traditionally, this involves the use of PCR techniques to detect human viruses, animal tests such as HAP, MAP, and RAP tests for rodent cells, in vitro assays in a variety of indicator cell lines, and immunofluorescence techniques (9CFR) for bovine or porcine cells or using raw materials from these species.
We will show how an NGS transcriptomic analysis of cells can effectively replace these conventional methods, supported by data showcasing its sensitivity, capacity to detect viral replication signatures, and broad detection range. We will also explore additional applications, such as bulk testing when cells are readily available, and in cell therapy, where its ability to distinguish between latent herpes viruses and replicating viruses provides a significant advantage.

Biosketch



Megane Gura

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Megan Gura is a Senior Quality Control Virology Scientist at Regeneron Pharmaceuticals, Inc. As a scientist on the QC Virology Development team, she has worked to create assays that will be used in Good Manufacturing Practices (GMP) virology testing, including PCR- and NGS-based approaches for adventitious agent detection and rAAV critical quality attribute testing. The QC Virology Development team has participated in AVDTWG Spiking Study #4. Megan has a Ph.D. in Molecular Biology, Cell Biology, and Biochemistry from Brown University.

Abstract

Megane Gura

Comparison of Long- and Short-Read Sequencing Methods for Recombinant Adeno-Associated Virus Sequence and Adventitious Agent Identification in a GMP Environment

Authors:
Megan Gura, Patrick Blatt, Sriramana Kanginakudru, Marissa Martinek, Morgan Hewes , Tyler Dion, Chris Remillard, Devika Torvi, Ping Li, Steven Davis, Jared Richardson, David VanHoute - Regeneron

Recombinant adeno-associated virus (rAAV) is an emerging class of treatment within the cell and gene therapy field that requires additional testing to ensure the delivery of safe and effective medicine to patients. Under Good Manufacturing Practices (GMP), the Critical Quality Attributes (CQAs) of an rAAV therapeutic must be assessed, including ensuring that the genomic sequence of the rAAV is complete and matches the reference sequence. High-throughput sequencing (HTS) approaches can address multiple CQAs in one experiment such as testing for exogenous contaminants introduced by the manufacturing process. We have developed a bioinformatics pipeline that can accept both long- and short-read HTS data to evaluate CQAs when sequencing rAAV. Using this pipeline, we evaluated both long- and short-read sequencing methods using the Illumina NextSeq 2000 (short-read) and Oxford Nanopore Technologies PromethION (long-read) systems, respectively, to evaluate their strengths and weaknesses. The main goal of these experiments was to determine the sequence identity of the rAAV target(s) tested and identify any sequence variants in our samples. Additionally, we tested if using adaptive sequencing on the PromethION, which can selectively enrich or deplete a chosen sequence, improved the results of long-read sequencing. Secondary goals of these experiments were to evaluate other CQAs such as determining ITR sequence identity and contaminant testing such that this pipeline could be applied to both CQA testing and adventitious agent detection within one HTS method. Multiple sample and library preparation approaches were also tested to gain an understanding of the optimal approach for sequencing rAAV for CQA and contaminant detection. This presentation will provide a comprehensive overview of our findings.

Biosketch

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Bradley Hasson is the Director of Lab Operations for Next Generation Sequencing Services at Merck (MilliporeSigma). Brad leads a global team of Scientists, laboratory technicians and bioinformaticians that perform regulated GMP NGS-based testing services in support of product characterization, adventitious agent detection and product release for biologically derived products worldwide. He has over 20 years of industry experience, including 16 years developing, validating and commercializing molecular-based methods for the purposes of biosafety testing and characterization in a variety of biologically derived products.

Abstract

Bradley Hasson

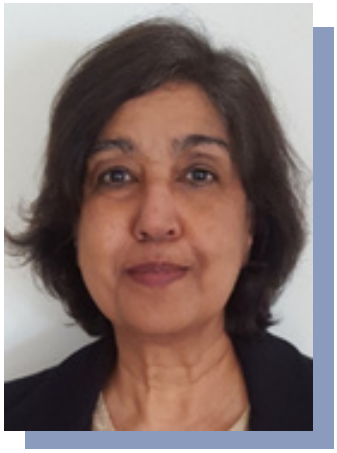
Matrix effects on Limit of Detection for NGS-based Adventitious Virus Detection Assays

A matrix specific evaluation of limit of detection for NGS-based Adventitious virus assays is outlined within the regulatory documents, most notable ICH Q5A and Ph Eur 2.6.41 DRAFT. This is typically accomplished by spiking well-characterized viruses into the sample matrix at varying concentrations to assess detectability of viruses with different physiochemical properties. The information gained from spiking studies allows the sponsor to model the specific limit of detection for the unique sample matrix and determine assay suitability through risk assessment.

Unlike other Molecular methods like PCR, a typical NGS assay does not employ the use of an exponential amplification step to boost low level signals to a detectable level above background. Instead, the NGS based approach generates the genomic profile of all nucleic acids present in the test sample, without distinguishing between background, product or adventitious agents. This means that sample matrices rich in extraneous nucleic acid composition (bulk harvest samples) may have challenges achieving a specific or suitable Limit of Detection (LOD) and LODs between different sample matrices may vary widely depending on the characteristics of the product and the matrix.

One advantage of a CRO is being able to assay many different products. In fact, limits of detection for NGS-based assays are relatively consistent across the industry but do vary between different matrix types. But to what extent and is there truly a universal representative matrix? This presentation will focus on the CRO experience in assessment of limits of detection in a wide variety of matrices. Data will be presented- demonstrating the need for LOD assessments for each different type of product. Along those lines, how can we apply the concept of "prior knowledge" when assessing suitability of a particular sample type and potentially apply this to platform-based technologies to expedite the use of NGS technology in testing.

Biosketch



Arifa Khan

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Dr. Arifa S. Khan is Supervisory Microbiologist and Head of the Molecular Retrovirology Unit in the Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration. She moved from NIAD/NIH to CBER in 1991, where she is currently leading efforts on high-throughput sequencing for detection of adventitious viruses and endogenous retroviruses for safety evaluation of cell substrates and biologics. Her primary regulatory responsibilities include viral vaccines, such as HIV-1, influenza virus, RSV and SARS-COV-2. Dr. Khan also provides expert consultation on viral safety and testing to OTAT/CBER and CDER. She has been involved in the licensure of several viral vaccines and contributed to the development of various guidances and guidelines from the FDA, ICH, PHS, USP, and EDQM. She is currently the FDA Deputy Topic Lead in the Implementation Working Group for the ICH Q5A(R2). Dr. Khan obtained her Ph.D. in Microbiology from the George Washington University, Washington, D.C. She has authored more than 100 publications.

Biosketch

Ivana Knezevic, MD, PhD

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Dr Knezevic is Specialist in Medical Microbiology and Parasitology. She received her MD from the University of Novi Sad, MSc in Medicine (Microbiology) and PhD in Medicine (Virology) from the University of Belgrade, Republic of Serbia. Dr Ivana Knezevic has 30 years of professional experience in standardization, scientific and regulatory overview of biologicals.

Dr Knezevic joined WHO in September 2000, and since then her activities have been devoted to the standardization and evaluation of biologicals at the global level. Since 2006, she has been leading the team for standardization of vaccines and biological therapeutics in WHO Headquarters. Dr Knezevic is the Secretary of the WHO Expert Committee on Biological Standardization. Main aspects of her work include development and establishment of WHO International Standards as well as the assistance to regulators, manufacturers, academia and other users of these standards.

Being responsible for 8 WHO Collaborating centers for standardization and evaluation of vaccines and other biologicals, Dr Knezevic initiated numerous scientific projects of importance for global public health. In 2012, she created a network of the Collaborating centers that are playing a leading scientific and regulatory role in their countries as well as at the global level (UK, Japan, Australia, USA, Republic of Korea, Canada, China and Germany).

Dr Knezevic is the author of many publications that made broad audience aware of WHO initiative in the development, establishment and implementation of standards for vaccines, biotherapeutics and cell & gene therapy products. She is also contributing to the peer-review scientific journals with the international reputation.

Abstract

Ivana Knezevic

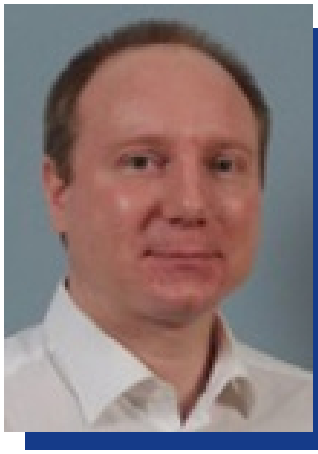
WHO perspectives on the use of HTS for detection of adventitious agents in biologicals

HTS technologies have the ability to detect both known and novel adventitious viruses in biological products. As an alternative to conventional adventitious virus detection, which relies on in vivo animal testing and in vitro cell culture assays or on polymerase chain reaction (PCR) assays, HTS has the potential to expand the breadth of virus detection while also significantly shortening the time required for the quality control testing of biological products. Importantly, the increasing implementation of HTS for this purpose aligns with, and provides support for, the shift towards reducing the use of animals in such testing. Encouraged by regulatory guidance worldwide, the need for international viral standards for HTS process qualification and validation to support the application of HTS to biological product virus safety testing has long been recognized.

The First WHO International Reference Panel for adventitious virus detection in biological products using high-throughput sequencing technologies established by WHO Expert Committee on Biological Standardization (ECBS) at its 79th meeting in March 2024 supports the wider use of such highly advanced and sensitive non-animal methods, with considerable benefits envisaged in accelerating testing timelines and thus expediting access to safe and affordable biological products. This reference panel supersedes WHO international reference reagents established by the ECBS in 2020.

WHO has initiated review of the current use of HTS in the regulatory evaluation of biological products with the aim to identify needs for technical support for its implementation into regulatory and manufacturing practices. Feedback from 16 participants who responded to the questions regarding the current practice in their countries will be presented at the IABS conference as a basis for discussion on the way forward.

Biosketch



Christophe Lambert, PhD

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Christophe Lambert is Head of Viral Computational Genomics at GSK Vaccines in Belgium. He graduated M.Sc. in Chemistry and was awarded PhD in bioinformatics from the University of Namur (Belgium) in 2003 for the automated prediction of protein 3D structures and for the automated bacterial genome annotation.

In 2003, Dr. Lambert co-founded a company providing expert bioinformatics services to pharmaceutical, biotech and agrofood companies. In 2006, he also co-founded BioWin, the Health competitiveness cluster of Wallonia (Belgium), bringing together stakeholders participating in innovative projects and/or education in the fields of Health biotechnology and medical technologies.

He joined GSK in 2014 to provide expertise in protein 3D structure, HTS data analysis and manage the development of an analysis pipeline for Adventitious Virus Detection.

Since 2016, Dr. Lambert is the co-leader of the AVDTWG Subgroup DE exploring the optimization of bioinformatics pipelines for adventitious virus detection and strategies for follow-up investigations of identified hits, and also reviewing best practices and perspectives about using HTS for virus detection.

Abstract

Christophe Lambert

Considerations for the Follow-Up of Putative Adventitious Virus Signals Detected by HTS

BACKGROUND – High-throughput sequencing (HTS) has demonstrated capabilities for the broad and sensitive detection of known and novel adventitious viruses that could be inadvertently introduced during manufacturing of a biological product from raw materials, handling, or facilities and equipment.

CHALLENGES – All nucleic acids present in the sample can be potentially sequenced including adventitious viruses but also sequences from host, vector, sequencing adapters, etc. Additionally, nucleic acids can be introduced in a sample from various reagents, water, kits, and even exposed skin during the various sample handling, processing, and preparation steps in the HTS workflow.
The taxonomy assignment of sequences resulting from HTS is based on a comparison to viral sequence databases. Therefore, using a high-quality and well-characterized database is important for accurate identification of a signal due to an adventitious virus. The presence of misannotated and nonviral sequences in a database may result in extensive follow-up to verify the identity of the signal as an adventitious virus or a nonviral sequence. Additionally, sequencing errors or natural variations in viral genomes may lead to the misidentification of multiple related or unrelated viruses.

PROPOSED APPROACH – The breadth and sensitivity of HTS necessitates thoughtful follow-up strategies for assuring the absence of an adventitious virus in biological products without delaying their release.
In this presentation, we will review potential sources of false-positive signals and sample variability and explore considerations for viral reference database selection, criteria for actionable signals, and bioinformatic and laboratory follow-up of identified hits.

Biosketch

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Carine Logvinoff received her PhD in Virology from University Claude Bernard (Lyon, FR). Following her postdoctoral research with C.M. Rice, she joined Sanofi R&D in 2004 and there contributed to the development of different viral vaccines from early phases to registration.
Since 2018, Carine is the Virology Global Analytical Expert in Analytical Sciences, sharing expertise and providing support on viral safety topics and analytics for vaccines on diverse subjects including adventitious virus testing package and viral vaccine characterization. She also promotes innovation and supports new approaches/technologies implementation; as so, she continues to actively contribute to the implementation High Throughput Sequencing for adventitious virus detection within vaccine testing profile and its regulatory acceptance.

Abstract

Carine Logvinoff & Song Sun

Selecting and developing approaches for adventitious virus detection by HTS, contrasting release testing with investigational follow-up

Multiple approaches for adventitious virus detection by HTS (e.g., viromics, genomics, transcriptomics) are described in Ph.Eur 2.6.41. Within this presentation, we will discuss Sanofi Vaccines' rationale for approach selection based on sample type, considering both validated release testing as well as investigational testing. We will also discuss how we designed and configured our HTS pipeline for testing under both scenarios.

This work was funded by Sanofi.

Carine Logvinoff and Song Sun are Sanofi employees and may hold shares and/or stock options in the company.

Abstract

Martin Machyna

BLOODVIR – Surveillance system for novel viruses based on next generation sequencing and artificial intelligence

Authors:
Machyna M.1,*, Braun M.1,*, Rubio Quintanares G.H.2,*, Brückmann J.2, Margiotta E.1, Schatzl J.1, Spekhardt D.1,3, Santos P.3, Miskey C.3,#, Childs L.1,#, Blümel J.2,#, König R.1,#
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Continuous population growth and intensity of long-distance travel contribute to emergence and spread of viruses. Many of these viruses are bloodborne and a pose an immediate risk to receivers of blood transfusions. Therefore, it has become vital to establish a reliable and high-throughput surveillance system for screening of blood and blood-derived products for novel and re-emerging viruses. Technologies such as next-generation sequencing and artificial intelligence possess the capability to analyze samples in a massively parallel fashion. The surveillance system will allow continuous screening for infection risks in a representative cross-section of the population to best prepare for future virus outbreaks.

Here we report a novel metagenomic pipeline based on next-generation sequencing and machine learning capable of detecting a broad range of known as well as novel viruses. Sequencing reads from blood samples undergo removal of host sequences and are cross-referenced to a curated virus database. Simulated reads and viral spike-in reads were used to evaluate several state-of-the art classification tools for detection of known viruses and identified MiCoP as the most accurate classifier. A high sensitivity (1.0) and precision (0.875) of sequencing reads classification result in low limit of detection of 10 viral genome copies per milliliter of sample.

Unclassified reads from the previous step could originate from previously unobserved viruses and are used to predict presence of novel virus species. Selected deep neural network models were evaluated on their ability to successfully predict viral origin of sequences using a leave-one-out benchmarking strategy. We identified VirHunter architecture trained on human, bacterial and viral genome data to have the highest prediction capabilities (precision = 0.97, recall = 0.99) of novel viral sequences.

BLOODVIR detections system thus holds a potential to significantly enhance and synergize with conventional detection methods.

Biosketch

Laurent Mallet, PhD.

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Strasbourg, France European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe
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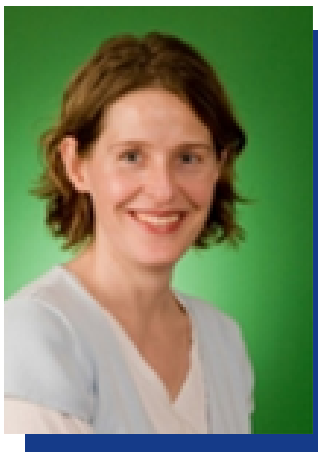
Laurent Mallet is the Head of the Biological Standardisation, OMCL Network and HealthCare Department at the European Directorate for the Quality of Medicines and HealthCare (EDQM) since 1 December 2019.
Laurent Mallet obtained his Master of Science in Biochemistry from Claude Bernard University, Lyon (France) and completed his PhD work in Virology and Molecular Biology under the co-direction of Professor Michèle Aymard (National Reference Center for Enterovirus, Lyon) and Dr François Pelloquin (Sanofi Pasteur, formerly Pasteur Mérieux-Connaught).

He obtained his PhD in 1996. After several positions within Sanofi Pasteur in France and in Canada, he was appointed Global Head of Analytical Sciences at Sanofi Pasteur, where he remained until November 2019. He joined the EDQM in December 2019. He was a member of several expert committees, including EDQM/European Pharmacopoeia Group of Experts 15 (Human vaccines and sera), EDQM/European Pharmacopoeia Mycoplasma Working Party, and a stakeholder in the French Pharmacopoeia Committee for "Biological Products and Innovative Therapies". He was also involved in several WHO working groups on vaccines, including the WHO Study Group on Cell Substrates.

He currently represents the EDQM on the WHO Expert Committee on Biological Standardisation (ECBS), and in the NC3Rs and HSI Steering Committees, the Regulatory Advisory Group (formerly called COVAX RAG) and the implementation working group for the ICH Q5A(R2) revision. He is also a member of the IABS Board.

Biosketch



Marie Murphy, Ph.D.

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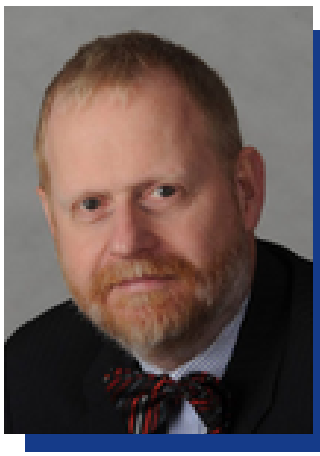
Marie works for Eli Lilly & Co., providing technical support for the microbiological control and viral safety aspects of the biotechnology drug substance manufacture platform.
Marie is representing the European Federation of Pharmaceutical Industries and Associations (EFPIA), supporting the recent ICH Q5A(R2) viral safety guideline revision. The EFPIA team are an industry stakeholder within the ICH implementation working group, tasked with development of training materials to support the implementation of the guideline. Marie has a B.Sc. in Microbiology from University College Cork and a Ph.D. in Virology from Trinity College, Dublin, and is currently based in Ireland.

Biosketch



Pieter Neels, MD

Ex-CHMP member
Ex-EMA Vaccine Working Party Vice-chair
Vaccine-Advice BVBA Founder
Ex-Associate Professor University of Namur
Chair – Human Vaccine Committee of IABS
Ex officio member – Executive Committee
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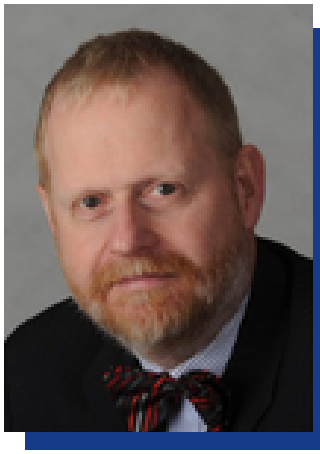
Dr Pieter Neels is a native of Belgium where he trained as an MD (University of Antwerp, 1985) and was boarded as a general practitioner. In 1994, his interest for medical research led him to work for a pharmaceutical company. In 1997, he joined the Belgian Ministry of Public Health as a senior evaluator of the clinical part of registration files in the field of cardiology, nephrology, endocrinology (diabetes), ...

In 2001 he was appointed CPMP member. In 2002 he was asked to take over all Belgian central vaccine rapporteurships. During this year he became infected by the world of vaccines and until June 2013 he was the rapporteur of more than 15 vaccines.

After being an observer for more than 5 years at the Vaccine Working Party, he was elected vice-chair of this CHMP Working Party for discussion on development and evaluation of registration files for vaccines until June 2013.

The Belgian agency started a spearhead policy in 2007 and Dr Neels was appointed co-ordinator for the spearhead domain vaccines.

Biosketch



Pieter Neels, MD

Ex-CHMP member
Ex-EMA Vaccine Working Party Vice-chair
Vaccine-Advice BVBA Founder
Ex-Associate Professor University of Namur
Chair – Human Vaccine Committee of IABS
Ex officio member – Executive Committee
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EMA/CHMP has asked Dr Neels has be an observer at the SAGE/WHO meetings and to attend several scientific meetings on vaccines until June 2013. WHO has asked Dr Neels to attend many meetings on vaccine development all over the world in order to share the EU regulatory requirements/competence in vaccinology.

Dr. Neels is also a member of the worldwide network on vaccine promotion as he is asked to attend the ADVAC course (Foundation Mérieux) and the IABS conferences.

Biosketch

Siemon Ng, PhD

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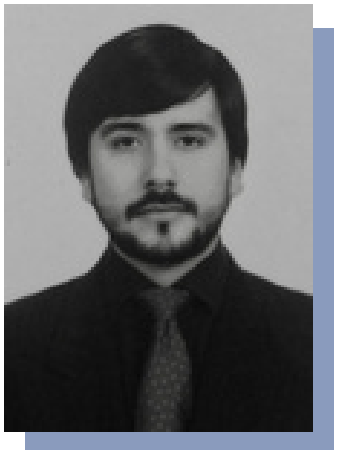
Dr. Siemon Ng is the Senior Director, Analytical Development and Quality at Notch Therapeutics and is responsible for developing and implementing the analytical strategy for Notch's iPSC-derived immunotherapies. Dr. Ng has extensive experience with analytical development including next generation sequencing and NGS data analysis, digital PCR, qPCR, and other molecular and immunological techniques. Dr. Ng is an active member of the PDA Advanced Virus Detection Technologies Interest Group and has participated in multiple collaborative studies involving the use of NGS.

Biosketch



Gibran Horemheb Rubio Quintanares

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I´m an MD-PhD specialized in virology with experience in NGS workflow establishment for sequencing virus and transcriptomics. I worked as postdoc in the Virology Institute from the University Clinics of Cologne, establishment a COVID diagnostic method for schools. Since 2022 I work in the viral safety department of the Paul Ehrlich Institute for the establishment of a system for adventitious and vertebrate pathogenic virus detection. In collaboration with Dr. Ian Lipkin group (Columbia University, NY) we adopted his capture system technique and modified for our proposes, increasing sensitivity and reducing cost for adventitious virus searching in blood products. I´m an voluntary external advisor for the establishment of a translational medicine program in the Mexican Institute of Social Security, I´m a certified professional in translational medicine, member of the National Research System of Mexico (level 1), member of the Consortium of Mexican Researchers Against the COVID-19, co-director of SPARK Mexico, a translational medicine program, member of the HTS (high throughput sequence) group of experts for the EDQM, and member of the Global Alliance for Pandemic Prevention (GAPP).

Abstract

Gibran Horemheb Rubio Quintanares

Comparison of Non-Targeted and Broad-Spectrum Targeted NGS for Adventitious Virus Detection

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Introduction

Next generation sequencing (NGS) is a promising method to detect contamination by known and novel viruses.. Each step of the workflow can introduce a bias on the breadth of detection and impact the Limit of Detection (LoD). We present a comparison of the LoD and breadth of detection using a non-targeted and targeted (VirCapSeq-VERT) approach. The VirCapSeq-VERT system is based on a comprehensive library of oligonucleotide probes that have been designed to bind and capture sequences to all known and novel vertebrate viruses.

Objectives

To compare the LoD and breadth of detection between the non-targeted mNGS approach and the VirCapSeq-VERT system.

Abstract

Gibran Horemheb Rubio Quintanares

Comparison of Non-Targeted and Broad-Spectrum Targeted NGS for Adventitious Virus Detection

Material and Methods

To determine the LoD and breadth of detection, we tested serial dilutions (10^6 to 10^1 GE/ml) of the WHO international Reference Panel in a background of Ad5 DNA (10^9 GE/ml) and pooled human plasma matrix.. For the targeted approach, viral nucleic acids were captured by hybridization after library preparation and re-amplified before sequencing.
To verify the breadth of detection we spiked 21 virus species at different TCID50 in pooled human plasma.
Sequencing was performed using Illumina, with an average of 100, and 17 million reads per sample for non-targeted and targeted respectively. Bioinformatics were performed using PEI pipeline, and targeted analysis.

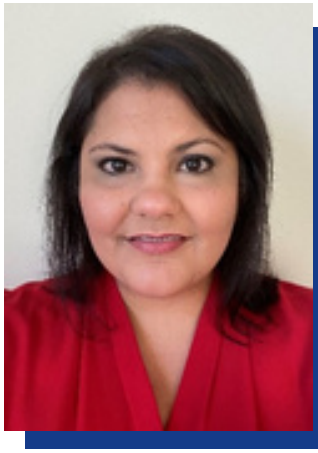
Results

Both systems were able to detect all the viruses of the WHO panel. Targeted approach exhibited lower LoD and longer coverage. Both approaches detected the 21 viruses from the PEI collection.

Conclusion

The use of a broad-spectrum targeted approach like the hybridization/capture VirCapSeq-VERT allows an increase of LoD between 100-1000 folds without affecting the breadth of detection. The use of these approaches can reduce the cost and time of sequencing. These advantages will allow a wider use of this technology in the interest of adventitious virus detection and public health.

Biosketch



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Vanessa Sarathy is a Principal Scientist in MSD in Analytical R&D. Vanessa earned her Ph.D. in Microbiology and Biochemistry. During her Postdoctoral and Assistant Professor research appointments she studied viral pathogenesis including mouse models for evaluation of vaccines and therapies and next-generation sequencing to tackle challenges in the characterization of wild-type and live-attenuated viruses. Since coming to MSD in 2020, Vanessa has held appointments in vaccine potency and molecular virus safety. As lead for high-throughput sequencing characterization of vaccines, she has performed studies to support regulatory filings and contributed to the implementation of a virus sequence analysis software application. Vanessa is active in the Advanced Virus Detection Technologies Working Group as part of her commitment to the development of safe and efficacious vaccines.

Abstract

Vanessa V. Sarathy

Viral metagenomic analysis to complement the viral risk assessment and adventitious agent testing of live virus vaccines

Authors:
- Vanessa V. Sarathy
- Jack Baker
- Julia Maritz
- John Connor

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NGS-based virus metagenomic testing of our live virus vaccines has been performed at the characterization level for regulatory filings.These studies furtherinform the viral risk assessment for rapid development of accelerated programs, complement traditional in vivo and in vitro testing for contaminating viruses, and supplement late-stage adventitious virus agent testing.

Bioinformatic analysisfor metagenomic studieswas executed using the High-performance integrated virtual environment (HIVE). Recent efforts to automate analysis applicable to GMP studies led to the development of an internal application, ViruScreen, with customized workflows for shortnucleic acid sequencing reads. ViruScreen provides user-friendly solutions for complex computations. Analysts perform basic curation of the referenceviral database priorto execution of read alignments. Further, ViruScreen has built-incapability for de novo assembly to align longer contigs to the viral database. In addition to a graphic user interface, a downloadable report is generated.

We used in silico datasets and nonGMP Illuminasequencing data of the dengue virus vaccineto compare the HIVE and ViruScreen pipelines. Datasets were used to assess detection of the WHO viral reference panel available for adventitious virus detection studies. Results of short read alignments demonstrated similar specificity and sensitivity between HIVE and ViruScreen indicating that our internal application is suitable to perform bioinformatic analysis to identify potential contaminants.

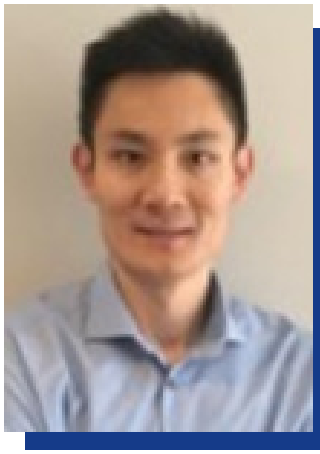
Abstract

Vanessa V. Sarathy

Viral metagenomic analysis to complement the viral risk assessment and adventitious agent testing of live virus vaccines

Because bioinformatic analysis varies depending on the specific study needs, ViruScreen includes an additional option of de novo assembly on the sequence reads. ViruScreen data indicate that using both short read and contig alignments provides comprehensive analysis for detection of potential low-level adventitious viruses and increases the specificity of detection. Taken together, the present study charts progress to implement a robustvirus detection analysisfor products such as live virus vaccinesas health authorities and new guidelines support the use of molecular technologies for regulatory filings.

Biosketch



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Song Sun joined Sanofi Pasteur in 2019 as a Scientist at the Molecular Biology Centre, Analytical Sciences and has been the Head of High-throughput Sequencing (HTS) Unit since 2022. He received his bachelor's degree in Biochemistry from Shandong University in 2004, master's degree in Molecular Biotechnology and Bioinformatics from Uppsala University in 2007 and PhD in Medical Microbiology from Uppsala University in 2012. As a PhD student with Dr. Dan Andersson, his research focused on dynamics and mechanisms of adaptive evolution in bacteria, and during his late graduate career he developed a HTS-based method to identify spontaneous genomic rearrangements in bacterial populations. In 2013, he received a prestigious research grant from Swedish Research Council for International Postdoc. To continue pursuing his research interest in experimental and computational genomics, he joined Dr. Frederick Roth's research group at University of Toronto as a postdoctoral fellow. His postdoctoral research contributions focused on functional analysis of disease-associated gene variants using HTS, and whole genome sequencing, amplicon-sequencing and barcode-sequencing applications by HTS.

Abstract

Carine Logvinoff & Song Sun

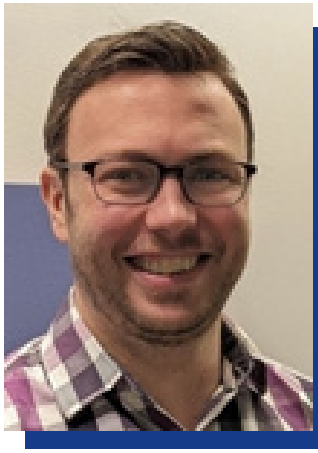
Selecting and developing approaches for adventitious virus detection by HTS, contrasting release testing with investigational follow-up

Multiple approaches for adventitious virus detection by HTS (e.g., viromics, genomics, transcriptomics) are described in Ph.Eur 2.6.41. Within this presentation, we will discuss Sanofi Vaccines' rationale for approach selection based on sample type, considering both validated release testing as well as investigational testing. We will also discuss how we designed and configured our HTS pipeline for testing under both scenarios.

This work was funded by Sanofi.

Carine Logvinoff and Song Sun are Sanofi employees and may hold shares and/or stock options in the company.

Biosketch



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Charley Swofford has been at the MIT Center for Biomedical Innovation since 2022, where he is the Assistant Director for Biomanufacturing Initiatives. In this role, he manages collaborative sponsored research projects, including investigating Oxford Nanopore sequencing for adventitious agent detection. He also supports joint research projects among members of the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB), a pre-competitive biopharmaceutical industry consortium focused on identifying and sharing best practices to mitigate the risk of adventitious agent contamination in biopharmaceutical manufacturing.

Abstract

Charles Swofford

Perspectives from the CAACB on the Current Benefits and Challenges of NGS Adoption for Viral Safety Testing

Authors:
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The recent ICH Q5A(R2) guidelines and upcoming Eur. Ph. 2.6.41 chapter both support the use of next-generation sequencing (NGS) as a replacement or orthogonal assay for viral safety testing, yet implementation and validation of NGS has been slow at best. To better understand the benefits and challenges of NGS adoption, this presentation will focus on lessons learned from the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB). Since 2010, 75% of CAACB member companies that experienced a contamination report NGS as the most useful tool for viral identification. When it comes to viral detection, however, CAACB members report continued use of the in vivo virus test, with 94% of members using it for cell line characterization and 50% of members using it for virus seed testing and/or lot release testing, despite the fact that the in vivo virus test has not been reported to detect a virus contamination that was not also detected in another concurrent assay. For CHO-based products specifically, only two CAACB members have transitioned to using NGS for cell bank testing and no members have reported its use for lot release. CAACB members, however, demonstrate a clear interest in NGS as an alternative method, with 69% of member companies actively evaluating replacement of in vivo assays and 63% evaluating supplementing in vitro assays with NGS. Some members are also including NGS in their IND filings. This is mainly motivated by reducing animal use, but also due to NGS having a greater breadth of detection and the expectation that NGS will have greater sensitivity than the in vivo virus test, ensuring a safer product. Companies still face significant challenges to adoption, including concerns of high false positive rates, buy-in from upper management, and lack of harmonization across regulatory authorities.

Biosketch

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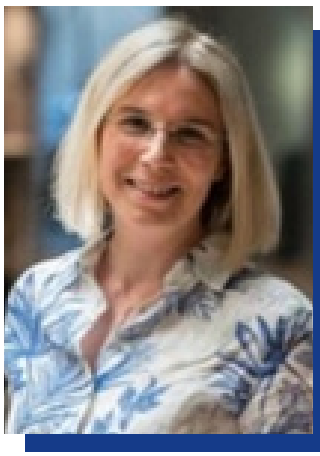
Dr. Michael Wall is a Senior CMC Reviewer within the Vaccines Division of the Biologics and Radiopharmaceutical Drugs Directorate (BRDD) at Health Canada, and supervisor of the Pneumococcal and mRNA Vaccines lot release laboratory.

Biosketch



Valeria Maria Zanda

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Valeria Maria Zanda, PhD, is the head of Innovative Sequencing & Data Science Lab within the Healthcare business of Merck. In her role, she is involved in the development and validation of innovative molecular methods based on sequencing technologies.

She holds a Master Degree in Biotechnology from the University of Milano-Bicocca and a PhD in Industrial Biotechnologies. She has been part of Merck since 2008, first within the R&D organization, then moved to Quality Control, where she has supported the implementation of molecular technologies for several applications. In particular, since 2011 she has been a subject matter expert working on the implementation of NGS technologies, leading or contributing to several efforts in method development in both viral safety and genetic characterization.

Abstract

Valeria Maria Zanda

AVDTIG Spiking Study 4 - Evaluation of Long-Read Sequencing for Adventitious Virus Detection in a Low Complexity Viral Background

Authors:
Valeria Maria Zanda1, Simone Olgiati1, Arifa Khan2, and Spiking Study 4 participants

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*Study co-leaders

The ICH Q5A(R2) revision recognizes High-Throughput Sequencing (HTS, also known as Next Generation Sequencing) as an alternative method to some of the classical safety assays for adventitious virus detection in biologics. Most HTS applications for viral safety have focused on using short-read sequencing technologies, while there is limited experience of the performance of long-read sequencing. To address this gap, in 2020, the Advanced Virus Detection Technologies Interest Group (AVDTIG), sponsored by the Parenteral Drug Association (PDA), launched an international collaborative study referred to as "AVDTIG Spiking Study 4". This study involved twelve laboratories from regulatory agencies and national institutes, biologics manufacturers, academia, and contract research organizations.

The main aim of the study was to evaluate the application of long-read sequencing to detect adventitious viruses in a high viral titer background. The experimental design mimicked a virus seed or a viral vector, and was similar to a previous short-read sequencing study ("AVDTIG Spiking Study 2B"). Samples were spiked with 5 viruses developed by CBER and adopted by the WHO as International Reference Reagents for adventitious virus detection by HTS.

Abstract

Valeria Maria Zanda

AVDTIG Spiking Study 4 - Evaluation of Long-Read Sequencing for Adventitious Virus Detection in a Low Complexity Viral Background

Each participant tested at least 2 spike levels of the 5 model viruses in a fixed amount of Human adenovirus 5 (109 GC in 200µL) and the non-spiked adenovirus background, using a common protocol for virus dilutions and spiking levels. To process and sequence samples, each participating lab applied either its individual, or a common protocol shared within the team. Data analysis was performed by individual bioinformatic workflows using both targeted and non-targeted approaches.

The study will present preliminary results, share overall findings from the targeted bioinformatic analyses, and provide an initial assessment of the long read sequencing performances. The findings will offer insight into the ability of long-read HTS to detect viral contamination across different viral species.

POSTERS

Poster

David Yarmosh

A Transcriptomic Workflow for Adventitious Agent Detection

Authors: David Yarmosh; Kumari Karna; Laura Le; Sophia Huff; Joesph Petrone; Jeffrey Tokman; Anjan Panthee; Stephen King; Ana Fernandes; John Bagnoli; Heather Couch
Affiliation: American Type Culture Collection (ATCC), Manassas, Virginia, USA
Topic: "Optimization of NGS sample preparation and processing for virus detection" or "Bioinformatics strategies and pipelines."

Adventitious agents represent one of several key challenges facing the manufacture of any biological product. Current best practices in the detection of adventitious agents such as qPCR techniques or application of live-animal models are becoming recognized as inadequate. PCR and comparable techniques are highly accurate but target fixed sets of organisms; they cannot reliably cover the scope of all agents. In vivo analyses are expensive, time consuming, and conflicts with the three Rs (replacement, reduction, and refinement) principles. Recently, Next Generation Sequencing (NGS) has been proposed as an alternative to both methods for its broad-spectrum analytical capacity in response to PCR methods, its speed, and affordable costs. Determining a true detection is an ongoing conflict as classification programs routinely report so-called "background noise," comprising nonspecific read assignments due to homology, incomplete databases, algorithmic biases, and unoptimized parameterization. ATCC's Sequencing and Bioinformatics Center developed an NGS approach for arbitrary adventitious agent detection; taking lessons from the ongoing genomic and transcriptomic characterization efforts of its diverse set of organisms and sample types. ATCCs method is multi-tiered to account for sequencing and analysis biases, including metagenomic classification with a minimally redundant database, multi-organism assembly, annotation, and quantification. Each component is considered as a separate factor in determining the presence of an adventitious agent.

Poster

David Yarmosh

A Transcriptomic Workflow for Adventitious Agent Detection

This approach begins with RNA-Seq to reduce the sequencing of material that is not actively transcribing, thereby increasing the signal available of both the host organism and any adventitious agent. Post-sequencing metagenomic classification is performed, narrowing the list of potential adventitious agents for differential expression analysis relative to a control sample, amplifying adventitious agent signal and diminishing erroneous assignments. Finally, results from each stage are collated into a single report for review. ATCC presents our method for NGS triage of biological products for adventitious agent detection.

Poster

Alexander Van Uffelen

In silico strategy to evaluate the feasibility of nanopore metagenomic sequencing for viral adventitious agents detection in vaccines

Authors: A. Van Uffelen*,1,2, A. Posadas*,1,2, L. Stradiot3, N. Desmet3, N. H. C. Roosens1, K. Marchal2, G. Waeterloos3, S. C. J. De Keersmaecker†,1, K. Vanneste†,1

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4. *,†These authors contributed equally

Vaccines carry the risk of containing viral adventitious agents among others due to the use of cell cultures or animal-based reagents in manufacturing. Traditionally, testing of these agents has relied on cell cultures or in vivo tests. However, through technological advancements of Next-Generation Sequencing (NGS), metagenomic sequencing has emerged as a promising non-targeted technique capable of detecting both known and unknown adventitious agents. Shotgun metagenomics involves sequencing all nucleic acids extracted from a sample, enabling unbiased microbial detection without prior assumptions. Especially the use of 3rd-generation sequencing technologies, through the production of long reads, can be advantageous for characterizing vaccine matrices. However, potential contaminations are likely present at (extremely) low levels, complicating reliable detection. In a first step to evaluate the feasibility of long read metagenomics for detecting adventitious agents, we propose an in silico strategy based on creating multiple in silico mixes with different concentrations of possible viral adventitious agents within a matrix background. Therefore, several pure viruses and the matrix background (pre-treated in different ways) should undergo nanopore sequencing separately to exhaustively characterize their genetic material. Subsequently, the reads from these viruses and matrices can be mixed in silico at different concentrations, correcting for both the yield and read length, to ascertain the limit of detection for the adventitious agents.

Poster

Alexander Van Uffelen

In silico strategy to evaluate the feasibility of nanopore metagenomic sequencing for viral adventitious agents detection in vaccines

To this end, a bioinformatic pipeline will be developed to taxonomically classify the reads with state-of-the-art classifiers and the Reference Viral DataBase. A subset of in silico mixes should next be created in vitro to validate our in silico approach. We will present our comprehensive strategy for defining the limit of detection of adventitious viral agents in vaccines using shotgun metagenomics, which will moreover has the potential to yield valuable insights into the viability and efficacy of long read metagenomics for detecting unforeseen contaminants in a vaccine matrix.

Poster

Sandra Fuentes

Cell Standards for evaluating sensitivity of HTS viral transcriptomics

Authors: Sandra M. Fuentes, Pei-Ju Chin and Arifa S. Khan

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Clones of A549 cells stably infected with simian foamy retrovirus (SFV) were characterized for viral RNA expression. Quantitative RT-ddPCR was used to identify three clones with differing levels of RNA expression ranging from low to medium-high. To determine the suitability of the cell as standards for HTS transcriptomics, the cells were spiked at 105, 104, 103 and 102 infected cells in the background of 106 uninfected A549 cells to determine the limit of virus RNA detection by HTS for each of the cell clones. The LOD of virus detection using targeted bioinformatics analysis will be presented.

Poster

Sofus Haljaer Wiisbye

High throughput sequencing, a rapid method for virus safety testing in cell therapy products

We have developed an automated high throughput sequencing method to GMP release test for adventitious virus in cell based advanced therapeutic medicinal products. This method detects the entire WHO5 virus panel at a spike-in ratio of 104 viral particles in a total of 106 human stem cells. We are validating the method in a GMP setting.

The method is fully automated with the following key steps; host cell lysis and virus-like control spike-in, host DNA depletion, total nucleic acid extraction, cDNA synthesis, fragmentation, library preparation, library quality control, Illumina sequencing and a GMP-adhering bioinformatic analysis. Key steps in the bioinformatic pipeline are adapter and quality trimming, host removal, spike-in control detection, reference funneling, and mapping to the RefSeq viral database to detect contaminants. All steps are optimized to decrease host nucleic acids and maximize conversion of all viral genome types to sequencing libraries. Using our defined bioinformatic pipeline, the method condenses the results into a qualitative measure; contaminants detected/not detected. This strategy leaves follow-up investigations only to cases where contaminants are detected. The full method, from sample receival to result, is performed in 3-4 calendar days.
While this specific method is developed to detect virus in a human cell matrix, it has provided important groundwork for expanding the GMP use of high throughput sequencing methods to other biological contaminants and matrixes.

Poster

Sakthi Balaji

To ensure the safety of patients receiving biological medicines manufactured from mammalian systems, the risk of potential adventitious viral contamination is highly controlled according to the ICHQ5a guidelines. Traditionally, the cell banks used for GMP manufacturing must be qualified for their use in production by performing appropriate in vivo and in vitro testings for the presence of adventitious viruses. To meet the global 3Rs (reduce, replace, refine in animal research) requirement, as well as leveraging novel advanced molecular technologies (such as PCR and NGS), the ICHQ5a guidelines have been revised recently to include non-targeted Next Generation Sequencing (NGS) as a replacement for traditional in vitro and in vivo testing methods as well as PCR based methods. Here, we will present AbbVie's efforts on the development of NGS viral safety testing by genomics approach using reference viruses and Genedata's Selector for bioinformatic analysis.

Viral genomes are diverse, and they can be single or double stranded RNA or DNA with different sizes and can be enveloped or non-enveloped. To maximize the likelihood of detecting potential contaminants, an unbiased and efficient sample preparation method was developed, designed to cater to all diverse viral types. Method standardization and method validation was performed using World Health Organization (WHO) virus reference panel containing different model viruses, as well as using Genedata's Selector pipeline to process the raw data for virus detection. Negative control was used for determining the specificity of the virus detection and sensitivity was determined by spiking in model viruses at different concentrations into the cell substrate matrix to assess the limit of detection as well as the robustness of the Selector pipeline.