3rd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals

University of Maryland –
Institute for Bioscience and Biotechnology Research (IBBR)
Rockville, Maryland, U.S.A.
September 27 - 28, 2022

This face-to-face meeting will focus on the recent expansion of scientific data and the current applications of next generation sequencing technologies for adventitious virus detection in biological products. This will include presentations on standardization and validation of the technical and bioinformatics steps involved in the NGS workflow and applications of different NGS strategies for characterization and safety evaluation of biologics, including human and animal vaccines, as well as gene therapy, and therapeutic products Current regulatory expectations will be discussed. The meeting will bring together representatives from industry, academia, contract research organizations, and international regulatory bodies for developing a scientific consensus regarding recommendations for using NGS for detection of adventitious viruses.

Scientific Committee

Name	Organization
Arifa S. KHAN	U.S. Food and Drug Administration, Center for Biologics Evaluation and Research FDA-CBER, U.S.A. Co-Chair, U.S.A.
Laurent MALLET	European Directorate for the Quality of Medicines & HealthCare (EDQM) – Co-Chair, France
Pieter NEELS	International Alliance for Biological Standardization (IABS) Chair, Human Vaccine Scientific Committee, Belgium
Johannes BLÜMEL	Paul-Ehrlich Institut (PEI), Germany
Jean-Pol CASSART	GlaxoSmithKline (GSK) Vaccines, Belgium
Miia JAKAVA-VILJANEN	Finnish Food Authority
Ivana KNEZEVIC	World Health Organization (WHO), Switzerland
Carine LOGVINOFF	Sanofi, France
Siemon NG	Notch Therapeutics, Canada
Michael WALL	Health Canada

AGENDA

Day 1 - Tuesday, September 27, 2022

8:00 am Registration & Welcome Coffee

8:30 am Welcome Remarks:
Rick HILL, IABS President
Pieter NEELS, Chair, IABS Human Vaccine Committee

8:45 am **Introduction** – Summary of Previous Meetings and Goals of the 3rd meeting
Arifa KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.
Laurent MALLET, European Directorate for the Quality of Medicines & HealthCare (EDQM), France

9:15 am **Keynote**
Latest Advances in Next Generation Sequencing and Their Impact on Biological Product Control
Domenico GENOVESE, Istituto Superiore di Sanità (ISS), Italy

Session 1 - Regulatory and Health Authority Perspectives on Using NGS for Adventitious Virus Testing

Chairpersons: **Robin LEVIS,** U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), USA
& Johannes BLÜMEL, Paul-Ehrlich-Institut, Germany

9:45 am FDA Perspectives and Ongoing Efforts on Next Generation Sequencing for Adventitious Virus Detection
Arifa KHAN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.

10:15 am EDQM Achievements and Perspectives on Next Generation Sequencing
Laurent MALLET & Gwenael CIREFICE, European Directorate for the Quality of Medicines & HealthCare (EDQM), France

10:45 am **Coffee break**

11:15 am Use of NGS in Adventitious Virus Screening of Biological Medicinal Products: Regulatory Requirements
Koen BRUSSELMANS, Sciensano, Belgium

11:45 am PMDA's Perspective and Discussion Points in Replacing Conventional Methods with NGS for Virus Testing in the Manufacturing Process of Pharmaceutical Products
Akira SAKURAI, Pharmaceuticals and Medical Devices Agency (PMDA), Japan

12:15 pm **Lunch**

1:15 pm High Throughput Sequencing for Detection of Adventitious Agents in Biological Products: WHO Approach
Ivana KNEZEVIC, World Health Organization (WHO), Switzerland *(virtual)*

1:45 pm **Panel Discussion**

2:30 pm **Break**

Session 2 - Reference Materials, NGS Qualification and Validation

Chairpersons: **Kathryn KING,** U.S. Food and Drug Administration, Center for Drugs Evaluation and Research (FDA-CDER)
& Jean-Pol CASSART, GlaxoSmithKline (GSK) Vaccines, Belgium

3:00 pm Updates on NGS Efforts in the Advanced Virus Detection Technologies Interest Group (AVDTIG)
Siemon NG, Notch Therapeutics, Canada

3:25 pm Reference Materials for Adventitious Virus Detection by NGS: Establishment of WHO International Reference Virus Reagents and Update on CBER's Reference Virus Database (RVDB)
Pei-Ju CHIN, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.

3:50 pm RNA Next-generation Sequencing Transcriptomics Analysis: A Validated Method to Assess Viral Safety of Cell Substrates
Pascale **BEURDELEY**, PathoQuest, France

4:15 pm **Break**

4:40 pm Towards the Use of NGS to Control Veterinary Master Seeds: A Challenging Method to Validate
Blandine De SAINT-VIS, Boehringer Ingelheim, France

5:05 pm Next Generation Sequencing Application for Adventitious Virus Testing: Limit of Detection Study in an Influenza Vaccine Background
Bradley HASSON, MilliporeSigma, U.S.A.

5:30 pm **Panel Discussion**

6:00 pm **Poster Presentation & Reception**

7:15 pm **End of Day 1**

Day 2 – Wednesday, September 28, 2022

8:00 am Registration & Welcome Coffee

Session 3 - NGS Applications for Adventitious Virus Testing

Chairpersons: **Robert CHARLEBOIS,** Sanofi, Canada
& Christophe LAMBERT, GlaxoSmithKline, Belgium

8:30 am Next Generation Sequencing Transcriptomics Analysis: An Alternative Method to Replace in Vivo Tests for Assessing the Viral Safety of Cells
Marc ELOIT – PathoQuest, France

8:55 am Developing Sample-tailored Procedures for In-Depth Analysis of Gene Therapy Products Using High-Throughput Sequencing
Katarina BAČNIK, National Institute of Biology, Slovenia

9:20 am Development of a Sample Preparation Pipeline Using Oxford Nanopore Technologies (ONT) Sequencing for the Rapid Detection of Adventitious Agents
Charles A. SWOFFORD, Massachusetts Institute of Technology, U.S.A.

9:45 am NGS Implementation Strategy for Adventitious Virus Detection in an Adenovirus Vaccine
Aurora SIGNORAZZI, Janssen Vaccines & Prevention B.V., Netherlands

10:10 am **Break**

10:35 am Next Generation Sequencing to Unravel a Positive Signal in QC Investigation: Pitfalls and Learnings
Noemie DENEYER, GSK Vaccines, Belgium

11:00 am Sensitivity of Latent Endogenous Virus Detection Using High-Throughput Genome Sequencing
Sandra FUENTES, U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.

11:25 am LABRADOR – a GMP Validated Workflow for Virus Detection
Izabela FABIANSKA, IDT Biologika GmbH, Germany *(virtual)*

11:50 am **Panel Discussion**

12:05 **Lunch**

Session 4 - Strategies for Optimization of NGS Virus Detection and Follow-up of NGS Signal

Chairpersons: **Alison ARMSTRONG,** MilliporeSigma, U.S.A.
& Siemon NG, Notch Therapeutics, Canada

1:05 pm Overview: Strategies to Optimize Next Generation Sequencing (NGS) Bioinformatics Pipelines for Virus Detection
Christophe LAMBERT, GSK Vaccines, Belgium

1:30 pm Overview: Deciding on Actionable Signals from an HTS-based Adventitious Virus Detection Assay
Robert CHARLEBOIS, Sanofi, Canada

1:55 pm NGS for Adventitious Agent Detection: Analysis Options and Consequences
Qiu RUAN, Genedata, Switzerland

2:20 Break

2:30 pm Reducing the Haystack to Find the Needle: Improved HTS Assay Sensitivity by Host Depletion in Biological Samples
Song SUN, Sanofi, Canada

2:55 pm Host RNA Depletion for Increased Sensitivity of Random Amplification for Next Generation Sequencing
David SUAREZ, Southeast Poultry Research Laboratory, Agricultural Research Service, USDA-ARS-SAA-SEPRL, U.S.A.

3:20 pm Panel discussion

3:35 pm **Break**

Session 5 – Expectations for NGS Implementation

Chairpersons: **Arifa KHAN,** U.S. Food and Drug Administration, Center for Biologics Evaluation and Research, (FDA-CBER), U.S.A.
& Laurent MALLET, European Directorate for the Quality of Medicines & HealthCare (EDQM)

3:55pm **Panel discussion**

Alison ARMSTRONG	MilliporeSigma, U.S.A.
Johannes BLÜMEL	Paul-Ehrlich Institut (PEI), Germany
Koen BRUSSELMANS	Sciensano, Belgium
Jean-Pol CASSART	GlaxoSmithKline, Belgium
Gwenael CIREFICE	European Directorate for the Quality of Medicines & HealthCare (EDQM)
Marc ELOIT	PathoQuest, France
Ivana KNEZEVIC	World Health Organization (WHO), Switzerland (*virtual*)
Robin LEVIS	U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.
Robert CHARLEBOIS	Sanofi, Canada
Siemon NG	Notch Therapeutics, Canada
Blandine de SAINT-VIS	Boehringer Ingelheim, France
Akira SAKURAI	Pharmaceuticals and Medical Devices Agency, Japan
Michael WALL	Health Canada (*virtual*)

5:25 pm **Summary & Conclusion**
Arifa **KHAN,** U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (FDA-CBER), U.S.A.
Laurent **MALLET,** European Directorate for the Quality of Medicines & HealthCare (EDQM**),** France

5:35 pm **Closing Remarks**
Pieter **NEELS**, International Alliance for Biological Standardization (IABS), Belgium

5:45 pm **End of meeting**

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Dr. Arifa Khan received her Ph.D. in Microbiology from the George Washington University in 1979 and joined NIAID/NIH, where she made primary contributions to murine retroviruses, SIV, and endogenous retroviruses. Dr. Khan joined the FDA as a tenured scientist in 1991 and is currently the Head of the Molecular Retrovirology Unit, Laboratory of Retroviruses in the Division of Viral Products, OVRR. She leads a research program addressing potential safety concerns related to adventitious and endogenous viruses in cell substrates and biological products. Her current efforts are focused on the development of viral reference materials for standardization of NGS platforms for adventitious virus detection in biologics. This includes detection of latent viruses using models of simian foamy retrovirus infection of human cell lines.

Dr. Khan is responsible for review of INDs and BLAs for a variety of candidate viral vaccines including influenza virus, HIV, Ebola virus, and SARS-CoV-2, and safety evaluation of novel cell substrates across different product categories. Dr. Khan is currently leading various FDA and external working groups related to NGS, adventitious viruses, and cell substrate safety. She is co-founder of the Advanced Virus Detection Technologies Interest Group and is the CBER Deputy Topic Lead for the ICH Q5A (R2).

Dr. Khan has over 100 peer-reviewed publications on her research works and on regulatory topics related to viral safety.

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Laurent MALLET, PhD

Head of Biological Standardisation,
OMCL network & HealthCare (DBO)
Department

Strasbourg, France
European Directorate for the Quality of
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Dr. Laurent MALLET obtained his Master Degree of Science in Biochemistry from Claude Bernard Lyon University in France. He completed his PhD work in Virology and Molecular Biology under the co-direction of Pr Michèle Aymard (National Reference Center for Enterovirus, Lyon, France) 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 has been the Global Head of Analytical Sciences within Sanofi Pasteur up to November 2019.

Since December 2019, he has joined the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, in Strasbourg, France. At EDQM, he is currently the Biological Standardisation, OMCL Network & HealthCare (DBO) Department Head.

He has been a member of several expert committees such as the EDQM Group 15 "Human vaccines and sera" at European Pharmacopoeia. In addition, he has been involved in several WHO working groups on vaccines including the WHO Study Group on Cell Substrates.

In his new role, he represents EDQM in the WHO Expert Committee on Biological Standardization (ECBS), in the NC3Rs working group to review animal testing requirements in WHO biologicals guidelines, in the COVAX Regulatory Advisory Group, in the IABS Board and in the ICH Q5 Expert Working Group.

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Dr. Domenico Genovese obtained a Master degree in Biological Science in 1982 and a Postgraduate Course in Microbiology from "La Sapienza" University in Rome. He started his career in the Italian National Institute of Health (ISS) in 1986 in the Virology Lab where he studied the molecular biology, the molecular epidemiology, and the effects of antiviral drugs on Picorna, HIV and hepatitis viruses. In 2009 he moved to the Pharmacology Department, in the "Global Health" units, where he studied therapy personalization in HCV and HBV patients and the human genome factors which could influence therapy response in viral hepatitis chronic patients. From 2017 he works in the National Centre for the Control and Evaluation of the Medicines (CNCF) of ISS, where he is involved in the analysis of viral safety of phase I clinical study and in EDQM and WHO collaborative studies in gene therapy; he is an EMA Assessor for Biological and Biotechnological products. His primary research interests are in the development of analytical procedure for the control of Biological and Biotechnological products.

Latest Advances in Next Generation Sequencing and Their Impact on Biological Product Control.

Dr. Domenico Genovese, Istituto Superiore di Sanità

Abstract

The market of biological products continues to be fast-growing due to the increase of authorised therapies and the emergency of new challenges for the prevention of old and new infectious diseases. Conversely, quality control does not develop with the same rate. The challenge is to develop quality control tools that meet the current level of production, reducing the cost, and accelerating the timelines of product release.

NGS could be utilised to verify the genome pattern of cell banks or for Adventitious Agents investigations. NGS scans millions of RNA/DNA molecules collecting data from all sequences present in a sample. Bioinformatics tools are then used to determine matches to known agents or genome, or to identify new, previously unknown pathogens. These approaches enable the testing of all biological media and other components, from the raw material to the batch release of marketed products, including investigation on contaminations.

Latest advances in DNA sequencing technologies, as long-read sequencing and nano-technology, result in sequence higher yield, faster turnaround time, and lower cost.

Progresses in NGS technology involve all the steps of the sequencing process: from sample preparation and extraction to library building, through target enrichment and specific extraction procedures as well as the use of longer nucleic acid fragment and specific barcoding.

These progresses have been followed by a rapid improvement in data analysis with the development of new software and innovative pipelines, combining together data from different techniques and platforms.

The improvement in the NGS technology, in the sequencing process, and in the data-analysis provide significant advantages in Biological Product Control.

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Gwenaël Ciréfice, PharmD

Scientific Programme Manager,
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Strasbourg, France

European Directorate for the Quality
of Medicines and HealthCare (EDQM)

Gwenaël Ciréfice holds a PharmD degree from the University of Strasbourg, France. He began his career in the vaccine industry in 2007 as a regulatory affairs manager before later joining the Quality of Medicines department of the European Medicines Agency in 2010, with responsibilities in the field of human vaccines and recombinant proteins. He joined the EDQM in 2014 as a Scientific Programme Manager in the European Pharmacopoeia Department, supporting the work of Expert Groups in the area of biologicals, including Group 15 (Vaccines and sera for human use), Group 6 (Biological substances), the Working Party on Host-cell protein assays, and more recently the Working Party on Bacterial endotoxin and pyrogen testing.

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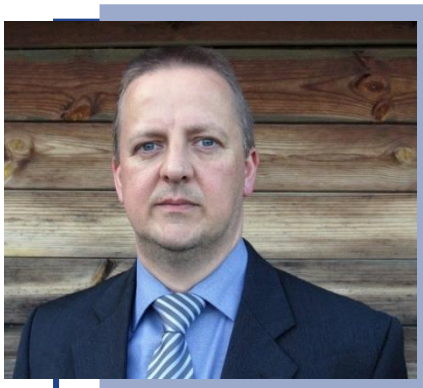

Koen Brusselmans

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Koen Brusselmans received a master degree in Bioengineering Sciences from the University of Leuven (Belgium) in 1996. Afterwards he started a PhD in medical sciences in the laboratory of Transgene Technology of Prof. Dr. Peter Carmeliet and Prof. Dr. Désiré Collen (University of Leuven), which focused on the role of hypoxia-inducible factors in the induction of angiogenesis during embryonic development and tumorigenesis.

After having obtained his PhD in 2001, he worked for 7 years as a post-doctoral fellow in the laboratory of Prof. Dr. Johan Swinnen and Prof. Dr. Guido Verhoeven (University of Leuven) on a research project studying the role of lipogenesis in cancer.

In 2008, he joined the group of 'Quality of vaccines and blood products' at Sciensano (the former Institute of Public Health, Brussels, Belgium), where he is currently working as senior quality assessor for biological medicines. He is involved in assessment of scientific advices and registration files of biological medicines (including vaccines, plasma-derived products and recombinant proteins), in collaboration with the Belgian Medicines Agency (FAMHP) and the European Medicines Agency (EMA). He also participates as expert in GMP inspections of manufacturers of biotech products and plasma-derived products.

Koen Brusselmans is Plasma Master File coordinator and alternate member for Belgium in the CHMP Biologics Working Party at the EMA.

Use of NGS in adventitious virus screening of biological medicinal products: regulatory requirements.

Koen Brusselmans (Sciensano, Belgium)

Background: According to current regulatory guidelines the virus screening of biological medicines and their starting materials requires a combination of both *in vitro* tests (in cell lines) and *in vivo* tests (in animals). As viral tropism of most cell lines and animals is limited, also additional testing for specific viruses (e.g. via PCR) is often needed. New analytical techniques such as next generation sequencing (NGS) are being used more frequently for the registration of biological medicines and the qualification of the materials used during production. Viral safety guidelines such as ICH Q5A(R1) and Ph.Eur.2.6.16 include the option to use NGS as a supplementary or alternative virus screening method. However, only limited information is currently available on validation requirements of NGS methods.

Relevant guidance: NGS is strongly recommended as a supplementary screening method for qualification of biological medicines and materials, as it combines high sensitivity with the ability to detect a broad range of viruses. If used as a supplementary test in addition to validated *in vitro* and *in vivo* virus tests, then there are currently no specific regulatory requirements for the use of NGS.

NGS may be used to replace the classic *in vivo* virus tests, which have limitations in terms of tropism, sensitivity, speed and animal welfare. In such case, adequate validation of the NGS method is essential, which should cover sample preparation, library preparation, sequencing and data analysis. Spiking experiments are expected using a broad and properly justified set of model viruses, as well as comparison with classic screening methods. However, in view of 3R principles no head-to-head comparison is needed for *in vivo* methods. If NGS is also used to replace the classic *in vitro* virus tests, then a more extensive validation may be required (larger set of model viruses in spiking experiments).

Complete validation of an NGS method does not necessarily need to be repeated for new medicines. If a proper and extensive validation has been performed on a particular product or matrix, then a product/matrix-specific validation for a new product could be limited to a smaller set of model viruses if appropriate justification is provided with regard to the material, matrix and manufacturing process.

In case virus screening by NGS yields positive results, then additional virus infectivity tests are required to confirm the presence of any infectious virus.

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Akira Sakurai is Senior Scientist for Biopharmaceutical Quality at the Pharmaceuticals and Medical Devices Agency (PMDA). He manages the evaluation direction of biopharmaceutical quality including gene therapy products and the "Cartagena Act" regulation. He received Ph.D. in 2004 from Hokkaido University an M.S. degree in 2001, and a Bachelor of Pharmacy in 1998 from Kyoto University. His specialty is Virology, and he worked as a scientist at the University of Wisconsin-Madison, National Research Institute of Police Science, and Tokyo Metropolitan Institute of Medical Science until he joined PMDA in 2014. He currently gets involved in the revision of ICH Q5A (Viral Safety Evaluation of Biotechnology Products) as deputy topic leader of MHLW/PMDA, Japan.

PMDA's Perspective and Discussion Points in Replacing Conventional Methods with NGS for Virus Testing in the Manufacturing Process of Pharmaceutical Products.

Akira SAKURAI, Pharmaceuticals and Medical Devices Agency (PMDA), Japan

NGS is the powerful tool to detect unknown adventitious viruses contaminated with culturing cells, has been expected to play an important role in the quality control tests at manufacturing process of pharmaceutical products, including regenerative medicines and advanced therapy medicinal products. NGS has comprehensiveness of viruses and the results could be gotten earlier than conventional virus detection tests with culturing cells. ICH Q5A guideline says that it is critical that detection of adventitious viruses at cell banks and unprocessed bulks of manufacturing process. The characters of NGS could fit the purpose of virus detection ICH Q5A guideline mentioned. In addition, regenerative medicines, particularly cell therapies, and medicines produced by continuous manufacturing sometimes cannot use conventional virus detection tests due to indicator cell culturing time for the tests. So, NGS may be an alternative method of virus detection in such situation.

Despite of these expectations, NGS has not been commonly accepted for virus detection tests at manufacturing processes. There are two reasons, one is that ICH Q5A guideline never mentions about utilization of PCR, including NGS, for virus detection. The other is that we don't have much knowledge about NGS for virus detection, and it is hard to decide the criteria or threshold. However, it is planned that ICHQ5A will be revised and mention about utilization of PCR for virus detection. So, we have to discuss how to accept the NGS for virus detection at manufacturing process of pharmaceutical products actually.

In Japan, Japanese regulatory authorities, MHLW and PMDA, have not officially provided any statement about NGS for virus detection yet. In this presentation, I'd like to show the points of discussion about NGS and current thinking of Japanese regulatory authorities.

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Ivana Knezevic, MD, PhD

Scientist, Technical Standards and Specifications Unit;

Group Lead, Norms and Standards for Biologicals; Health Product Policy and Standards Department (HPS);
Access to Medicines and Health Products Division (MHP);

World Health Organization (WHO), Switzerland

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 is a Scientist at the WHO Biological Standardization Programme, which she joined 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 also a 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 in the context of regulatory evaluation of biological products.

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 institutions that are playing a leading scientific and regulatory role in their countries (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.

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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 T cells 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.

Prior to joining Notch Therapeutics in 2022, Dr. Ng was the Head of the Molecular Biology Centre at Sanofi Pasteur Analytical Sciences. Dr. Ng completed his PhD at Simon Fraser University studying the Atlantic salmon genome. Subsequently, he worked on mouse genomics at The Jackson Laboratory. In 2008, Dr. Ng moved to Toronto to study the genomic landscape of stem cells at the Ontario Institute for Cancer Research and later joined Sanofi Pasteur in 2011 to support vaccine development before joining Notch Therapeutics.

Updates on NGS efforts in the Advanced Virus Detection Technologies Interest Group (AVDTIG)

Siemon Ng (on behalf of AVDTIG)

Background – Next generation sequencing (NGS) has the potential to detect and identify both known and unknown adventitious agents. The Advanced Virus Detection Technologies Interest Group (AVDTIG) was initiated by regulatory and industry scientists to address the knowledge and data gaps for using NGS for adventitious virus detection in biologics. AVDTIG was established with the support of the Parental Drug Association in 2012 and provides a forum for informal scientific discussions, knowledge-exchange and sharing of experiences on the use of NGS and other emerging methods for adventitious virus detection. Currently, AVDTIG comprises of over 200 scientific experts from industry, CROs, academia, and regulatory and government agencies.

Approaches – Five areas of focus were identified early on by members of AVDTIG, and subgroups A to E were formed to address each topic:

A. Evaluate different approaches for sample preparation and processing
B. Identify, characterize, and distribute virus reference standards
C. Develop a high-quality, complete viral database for confident identification of viruses
D. Advance the bioinformatics analysis for adventitious virus detection using NGS
E. Explore best practices for follow-up investigations for putative positive signals

In addition, five collaborative studies have been initiated to generate scientific data on experimental protocols, reference standards, and bioinformatics tools.

Results and Conclusions – The establishment of AVDTIG has advanced the adoption of NGS for adventitious virus detection. Five, well-characterized, viral stocks were accepted in 2020 as WHO Reference Reagents to support performance evaluation, standardization, and comparison to conventional virus detection assays. Two manuscripts were published in 2018, summarizing best practices for optimizing upstream sample processing and downstream bioinformatics analysis. In addition, a Reference Virus Database (RVDB) is now publicly available and continually updated to increase likelihood of novel virus detection, with reduced nonspecific cellular hits. Finally, the results from the first spiking study was published in 2018, while four other collaborative studies are underway to evaluate the sensitive in various background, long-read technologies and transcriptomics approaches for adventitious virus detection.

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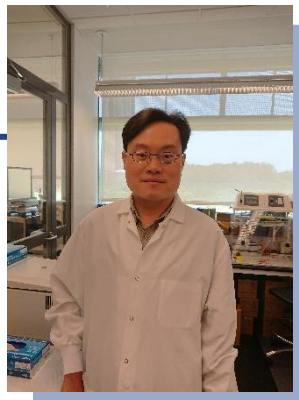

Pei-Ju Chin, M.S, Ph.D.

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U.S. Food and Drug Administration

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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 Interest Group (AVDTIG) Subgroup C working on database refinement, and is responsible for the update, maintenance, and refinement of reference virus database (RVDB), which is currently hosting 2,600 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.

Reference Materials for Adventitious Virus Detection by NGS: Establishment of WHO International Reference Virus Reagents and Update on CBER's Reference Virus Database (RVDB)

Pei-Ju Chin, Trent J. Bosma and Arifa S. Khan

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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, that could supplement or replace the currently recommended *in vivo* and *in vitro* assays. However, readily available reference materials to validate such NGS methods are lacking.

Reference materials are needed to evaluate the entire NGS workflow, including the upstream steps for sample preparation and extraction, cDNA synthesis, library preparation, sequencing and the downstream bioinformatics analysis pipeline. This talk will describe the characterization and studies of 5 currently available virus stocks that led to their approval by the Expert Committee on Biological Standardization as "WHO international reference reagents for adventitious virus detection in biological products by high-throughput sequencing". Additionally, an update on new virus stocks will be presented. Furthermore, the current status and ongoing work on CBER's non-redundant, reference viral database (RVDB; https://rvdb.dbi.udel.edu/) will be discussed. This was developed to include 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).

The reference viruses and the RVDB are available to facilitate NGS implementation for adventitious virus detection in biologics.

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Dr Pascale Beurdeley has 24 year of biotechnology industry with Extensive operational experience in GLP/GMP environments for Service Offerings and ISO:13485 for CE-IVD diagnostic assays/products developments. Prior to joining PathoQuest, Dr Beurdeley was the Executive Director R&D at Diaxonhit (formerly Exonhit Therapeutics) where she contributed to the development and the promotion of the company's proprietary platform for the analysis of gene expression and alternative splicing. Dr Beurdeley is graduated from the University Louis Pasteur Strasbourg-France and holds a Ph.D in Molecular and Cell Biology. She completed her post-doctoral fellow at Magainin Pharmaceuticals Inc-USA.

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She is graduated in Immunology (PhD in 1998) and spent several years in Immunology Research at Schering-Plough working on molecular biology to identify and characterize new genes.

In 2007, she joined Boehringer-Ingelheim in R&D veterinary vaccine department to bring her expertise with molecular biology techniques (PCR, sequencing, DNA chips) and bioinformatics. She led real-time PCR developments for all kind of microorganisms (numerous viruses, and bacteria) and all species to control vaccines and to perform clinical studies. In 2015, as Head of Analytical support, she was responsible of molecular biology methods development, seed controls and analytical support for new vaccines and Life Cycle products. As expert in molecular biology, she implemented NGS approaches for vaccine seeds controls. At present, in Global MSAT BI department, she is actively involved in several international projects to support the manufacture of vaccines.

Towards the use of NGS to control veterinary Master Seeds: a challenging method to validate

Presenter: Blandine de Saint-Vis, Senior Scientist, Boehringer Ingelheim Animal Health, Global MSAT Analytics, 813 cours du 3° millénaire, 69800 Saint- Priest FRANCE

Abstract:

The Next Generation Sequencing (NGS) is recognized as a promising tool for the detection of adventitious agents in viral seeds and cell banks during the production of human and veterinary vaccine. One of the challenges identified for using NGS was to assess the sensitivity and specificity of this method with model viruses spiked in different matrices.

To evaluate the performance of our method, we have conducted a spiking study using seven viruses at different concentrations in two master seed virus (MSV) matrices of veterinary vaccines. The viruses (3 DNA and 4 RNA viruses), representing the large diversity of veterinary viruses, were spiked simultaneously at decreasing concentrations in the two types of MSV, a baculovirus recombinant virus and an avian influenza virus, representing two modes of production.

Using the NCBI Viral Genome Database, 6/7 viruses were detected at the threshold of at least 10 CCID50/mL in both matrices (except 1 virus detected at 10 CCID50/mL in 1 matrix only). The Limit of detection (LOD) ranged from 0.1 to 10 CCID50/mL, depending on the virus and matrix. To complete the analysis of the set of raw data, and to compare different bioinformatics pipelines, the data were aligned against the c-RVDB database. The results demonstrated the good detection of the seven spiked viruses (7/7) in the two MSV matrices and strengthened the importance of the database for the analysis. Our results support NGS as a valuable and sensitive method for the detection of adventitious viruses in various complex virus seed matrices. The NGS method should be considered as a relevant tool for the prospective testing of biological products.

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Bradley Hasson is the Director of Lab Operations for Global Next Generation Sequencing Services at MilliporeSigma. Brad leads a team of Scientists, laboratory technicians and bioinformaticians that perform NGS-based biosafety testing services in support of product characterization, adventitious agent detection and product release worldwide. He has over 15 years of experience developing, implementing and commercializing molecular-based methods for the purposes of biosafety testing in a variety of biologically derived products.

Next Generation Sequencing Application for Adventitious Virus Testing: Limit of Detection Study in an Influenza Vaccine Background

Viral-based vaccines have become common place in the biotechnology and pharmaceutical industry. Adventitious virus testing for these products is an expectation of regulatory agencies for product release. Virus detection has historically been performed using in vivo and in vitro methodology, sometimes supplemented with targeted Molecular methods such as PCR. In vitro and in vivo methods are designed to identify potential virus contaminants by assessing viral replication in a cell-based system or animal model. The cell lines or animal models used in these studies must be susceptible to infection with the viral contaminant in order for it to be detected by the test system. These methods are designed to detect a wide variety of viral contaminants however it does not provide the identity of the adventitious agent.

Next Generation Sequencing (NGS) technology presents a unique approach to Adventitious Agent Detection in biologically-derived samples. Instead of identifying the presence or absence of a viral contaminant by its effect on a test system, NGS generates a profile of all sequences present within the test sample. The sequences are then bioinformatically assessed and qualified for the presence of potential viral contaminants using a suitable database of all known viral sequences. Using this method, the potential presence of a viral contaminant can be identified and characterized within the context of the test system.

Being that NGS is used as a broad virus screening sequencing technology, limits of detection are difficult to establish for each individual virus, therefore viruses from representative families are typically used in end-to-end spiking studies within the sample matrix in order to assess the limit of detection. AVDTIG has done a significant amount of work establishing a standard virus panel for the purposes of this testing over the past several years, most notably in CHO cells and Adenovirus 5 backgrounds. This presentation will describe the use of NGS technology for viral sequence detection in an Influenza vaccine sample consisting of allantoic fluid derived from avian eggs. The establishment of the limit of detection for viral contaminants in this specific matrix will be presented in support of the use of NGS technology as a suitable strategy for Adventitious Agent Detection.

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Marc Eloit is Professor of Virology at the Veterinary School of Maisons-Alfort and the head of the Pathogen Discovery laboratory at Institut Pasteur Paris, which is using Next Generation Sequencing as a routine method for discovering new viruses in human patients and animal reservoirs. He has been a member of the Virus Safety Committee, French Agency of Medicinal Products (1992-2012). He has served as a consultant for several companies in the field of virus safety of biologicals. He founded in 2010 Pathoquest, a spin-out of Institut Pasteur dedicated to the identification of pathogens using untargeted Next Generation Sequencing. He has been its CSO till the end of 2016 and acts currently as a scientific advisor for the company.

RNA Next-generation Sequencing transcriptomics analysis: an alternative method to replace *in vivo* tests for assessing the viral safety of cells.

Speaker: Marc ELOIT

In vivo viral safety testing in adult, suckling mice, and embryonated eggs, is a long and expensive procedure raising ethical concerns in the context of the 3-Rs principle on animal experimentation (Replacement, Reduction, and Refinement) and animal welfare. EP 2.6.16 *Tests for extraneous agents in viral vaccines for human use* requires justification of *in vivo* assays application in the testing program. In addition, EP 5.2.14 encourages the transition of *in vivo* to *in vitro* methods. In this context, next-generation sequencing (NGS) based testing can be an ethical alternative to replace such tests.

Pathoquest and Charles River decided to run a head-to-head comparison study focused on the analytical sensitivity and range of detection of the conventional *in vivo* testing and the transcriptomic NGS approach for the analysis of cell substrates. The aims were to support that RNA Next-generation Sequencing transcriptomics analysis is a suitable alternative method for adventitious virus detection.

Based on published data, we have defined a set of viruses for which the analytical sensitivity of *in vivo* assay was better than in the *in vitro* tests (Category A) or moderate and worse than the *in vitro* assay (Category B). We have included viruses that cannot be detected by in vivo tests (Category C). The results show that NGS is very sensitive and can detect few infected cells among hundred to millions of non-infected cells . The Limit of Detection was higher than that of *in vivo* assays for viruses of Category A, and was similar or much better for viruses of Category B. The transcriptomic assay detected viruses of category C not detected by *in vivo* tests.Therefore, NGS transcriptomic assays can replace *in vivo* tests for the analysis of cell substrates for viral contaminations

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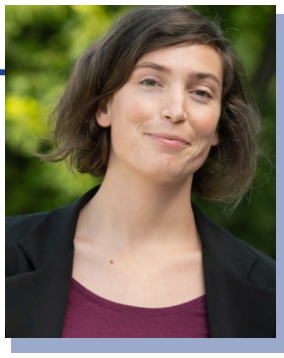

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Dr. Katarina Bačnik is a researcher at National institute of biology in Ljubljana and she is using high throughput sequencing for detection of viruses in complex samples from environment and industry. The work at our department is focused on innovative novel approaches of detection and characterization of viruses and joins experts in molecular methods, such as qPCR, ddPCR, high throuhput sequencing, and systems biology applied in various fields of life sciences.
We develop sample-tailored procedures starting from nucleic acids preparations, sequencing using different platforms (Oxford Nanopore, Illumina sequencing) and bioinformatics workflows for wide variety of samples originating from medical and biopharmaceutical projects, as well as from environmental studies.

Developing sample-tailored procedures for in depth analysis of gene therapy products using high-throughput sequencing

Katarina Bačnik, David Dobnik, Polona Kogovšek, Tjaša Jakomin, Maja Štalekar, Mojca Janc Tilen Kranjc, Maja Ravnikar, Denis Kutnjak

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Gene therapy-related research and development continues to grow at a fast rate, with a number of products advancing towards clinical development. In addition to precise quantification of purified recombinant adeno-associated viruses (AAV) using ddPCR and characterization of viral particles using TEM, we are applying shotgun high-throughput sequencing (HTS) for quality control in the development of AAV for gene therapy. HTS enables precise non-target identification of all nucleic acids in the samples, including non-product nucleic acids and characterization of residual DNA (plasmids, human genomic DNA, adventitious agents). The viral capsids of AAV have the potential to pack other nucleic acids besides the intended construct, therefore the generic nature of HTS-based analysis appropriately complements other methods for characterization of therapeutic viruses.

We developed sample-tailored HTS based procedure to investigate DNA impurities in the differently pre-treated samples, including DNase treatment and second strand synthesis, of purified AAV. The sequences which did not map to product, plasmids and host genome sequences were classified to characterize the metagenome, and sequences were further analyzed to search for possible chimeric reads. The metagenomics investigation of AAV samples is used to search for the presence of potential adventitious agents and expected background of nucleic acids originating from residuals of e.g., bacteria or expression vectors, which are probably introduced during the manufacturing process or sample processing. Furthermore, we focus on characterization of viral metagenome for discovery of possible adventitious viral sequences, which can be of potential concern in the development of AAV for gene therapy. Chimeric sequences that might be present as a result of recombination events within the same or between different plasmids used in the production of AAV are analyzed to search for most common chimeric reads and elucidate their identity. The complete procedure enables us to get better insights into purity of AAV-based gene therapy products.

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Charley Swofford works at the Center for Biomedical Innovation at MIT, where he is the Assistant Director for the Biomanufacturing@MIT-CBI program. In this role, he supports collaborative sponsored research projects, including investigating ONT sequencing for adventitious agent detection and developing a continuous manufacturing testbed for virus-like particle (VLP) vaccine production coupled with coupled with advanced process analytical tools. In 2014, he received his PhD in Chemical Engineering from the University of Massachusetts Amherst, where he focused on engineering microbes for targeted cancer drug delivery.

Development of a Sample Preparation Pipeline Using Oxford Nanopore Technologies (ONT) Sequencing for the Rapid Detection of Adventitious Agents

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Current adventitious agent testing methodologies are not adequate for the rapid detection of a broad spectrum of potential viral contaminants for the regular and rapid decision making required of continuous manufacturing. The *in vitro* virus (IVV) test is a cell-based assay that takes a minimum of 14 days to complete. PCR-based assays are significantly faster but can only detect a limited range of organisms and lack the broad-spectrum viral detection capabilities of the IVV test. Within the last six years, new third-generation sequencing technologies such as Oxford Nanopore Technologies (ONT) provide real-time, long-read sequencing of individual strands of DNA and RNA, allowing for broad-spectrum phylogenetic analysis of a sample with a relatively short time to detection compared to the IVV test. However, there is a lack of data demonstrating that the performance of sequencing methods is equivalent to a well-established and commonly accepted set of virus detection methods. Here, we develop and compare two viral concentration methods using either a microfluidic electrokinetic concentrator or commercially available Amicon® filters to detect minute virus of mice (MVM) in CHO cells using ONT sequencing. The optimized sample preparation methods can improve the detection limit of MVM over 100-fold compared to directly sequencing culture aliquots and has a level of detection on par with IVV tests. We also demonstrate that this sample preparation pipeline can be expanded beyond linear ss-DNA viruses such as MVM and used to detect the RNA-based feline lukemia virus (FeLV) and circular ss-DNA porcine circovirus (PCV).

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Dr Aurora Signorazzi obtained her MSc in Cellular and Molecular Biology from the University of Rome, Italy. For her master's internship, carried out at the Vaccinology lab of University Medical Center Groningen (the Netherlands) she investigated the effectiveness of physical inactivation techniques for whole inactivated influenza vaccines. After graduating she came back to Groningen to work on her PhD, which focused on developing *in vitro* assays for the potency assessment of the Tick-Borne Encephalitis vaccine. The project, part of the Vac2Vac consortium funded by the Innovative Medicine Initiative, sparked her interest in 3R principles. Since October 2020, she is working as viral safety scientist at Janssen Vaccine & Prevention, Leiden, where she focuses on the development and validation of innovative and animal-free viral safety tests.

ABSTRACT IABS 2022 (3rd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals)

Title: NGS implementation strategy for adventitious virus detection in an adenovirus vaccine

Authors: Aurora Signorazzi, Mark Kerstjens, Gilles Chenard, Femke Berkhoff

Affiliation: Janssen Vaccines & Prevention BV, Leiden (the Netherlands)

All Janssen vaccine products are currently tested, at various steps of the production process, for the presence of adventitious viruses through a combination of compendial and innovative assays. Due to the nature of the vaccine – based on a live adenovirus vector – the compendial assays require the use of animals or animal-derived products, such as adenovector-specific antiserum. Additionally, the compendial assays are the longest methods in Janssen vaccine safety portfolio. The use of an NGS-based method, highly sensitive and 3Rs compliant, is therefore highly advantageous for fast adventitious virus detection in adenovector vaccines.

The NGS implementation efforts at Janssen Vaccines & Prevention first centered on assessing the sensitivity of the method in our matrix. Using spikes representative of a broad range of viruses and various detection protocols, we identified suitable controls, optimized conditions and assessed level of detection. Next, viromic analysis was performed on the results obtained in the first experimental stage to screen for any adventitious viral sequences. Based on this data, we defined a workflow and set up an implementation plan for the NGS method to reduce the occurrence of false positive and to minimize business risks.

Our results illustrate the opportunities presented by an NGS-based adventitious virus detection method, as well as highlight the challenges associated with the method's application in live virus vaccines.

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Noémie Deneyer is a Senior Manager and Global Subject Matter Expert at GSK, with a focus on Next Generation Sequencing for Quality. In 2019, she started her journey at GSK where she was coordinating scientific projects aiming to deploy novel analytical methods for Quality Control testing. Since January 2021, she joined the Global Quality Control and Product Quality department where Noémie and her team work together to develop and implement Next Generation Sequencing in Quality Control to drive innovation and strengthen product safety. Noémie holds a PhD in Molecular and Cellular Biology from the UCLouvain, Belgium.

Abstract for the 3rd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals

Next Generation Sequencing to unravel a positive signal in QC investigation: pitfalls and learnings

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Invalid hemadsorption (HAD) test-results have been observed on a QC cell culture because of repeated and non-specific HAD of guinea pig blood cells in negative cell culture controls. An investigation was conducted to identify the root-cause of these positive HAD events. Several hypotheses were considered, including a cell culture contamination by an adventitious agent. In addition to classical testing to address mycoplasma, fungi or yeasts contamination, Next Generation Sequencing (NGS) was performed to screen for potential presence of extraneous nucleic acids from viruses and bacteria. Cell supernatants were collected after two, seven or fourteen days of cell culture and analyzed by NGS. The use of three timepoints enabled monitoring of the evolution of potential extraneous signals during the culture. No prominent bacterial or viral contamination was highlighted by the NGS analysis on cell supernatants, which therefore excluded the possibility that the root cause is an adventitious agent. This was confirmed by additional methods investigation. This investigation highlights that, although informative, NGS enables to address only specific questions that has been established from the start. NGS is a complementary tool that should not be seen as the only approach to apply in case of investigation. A comprehensive response plan based on robust observations is needed to quickly address the root-cause. Finally, this case-study highlights some pitfalls and difficulties of using NGS during an investigation, especially regarding sample management/preparation, selection of controls based on matrix, true hit definition or NGS approach to apply.

Topic area:

- Applications for virus testing in biologics
- NGS challenges and ongoing efforts

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Dr. Sandra Fuentes obtained her Ph.D. in Pathobiology from Pennsylvania State University in 2010. For her dissertation she investigated the functions of the respiratory syncytial virus (RSV) phosphoprotein and small hydrophobic protein. In 2016 she completed her postdoctoral fellowship at FDA where she contributed to the development of a luciferase reporter gene based microneutralization assay for RSV and investigated the antibody repertoires of RSV infected and vaccinated individuals to identify protective epitopes. She is currently a Microbiologist at the FDA where she contributes to the development of reference materials for the evaluation of HTS platforms aimed at the detection of adventitious viruses in vaccines.

Sensitivity of Latent Endogenous Virus Detection Using High-Throughput Genome Sequencing

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High-throughput sequencing has proven capabilities for detection of known and novel viruses in a variety of environmental, clinical, and research samples as well as some biological materials. Therefore, HTS is being considered by regulators and industry as an alternative method for the currently recommended tests for adventitious virus detection. This could accelerate development of vaccines and other biologics by reducing testing time and animal use. HTS has demonstrated detection of actively expressing known and novel adventitious viruses. We have evaluated HTS potential for detection of latent, endogenous viruses using a model of latent simian foamy retrovirus (SFV) infection in human A549 cells. To determine the limit of detection of the stably integrated provirus by HTS, uninfected A549 cells (10^6) were spiked with different levels of SFV-infected A549 cells (2 copies of SFV per cell). The reads obtained from whole genome sequencing of the spiked cell preparations were analyzed by direct mapping to the SFV genome (targeted analysis) and using blastn against the Reference Virus Database (RVDB; https://rvdb.dbi.udel.edu/). Both analyses identified SFV reads in the 10^3, 10^4 and 10^5 spiked samples. A single paired-end read in the 10^3 spike was identified through the targeted analysis and by blastn. The bioinformatics strategy that confirmed detection of the read as a true positive is presented here. Additionally, results comparing different Illumina platforms (MiSeq, Nextseq 550, and Novaseq 6000) for endogenous virus detection will be presented. These results demonstrate the capabilities of HTS for evaluation of latent viruses in cell-based biologics and for novel virus discovery of endogenous latent viruses.
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Dr. Izabela Fabianska joined the Analytical Development Department at IDT Biologika in 2020 to perform the computational analyses of sequencing data. Recently, she was involved in the development and validation of the analysis workflows for virus detection in biologics and for genomic identity testing.

Izabela completed studies in Biotechnology in 2013 at the University of Life Sciences in Poznan, Poland. Directly after, she joined the International Max Planck Research School (IMPRS) on Understanding Complex Plant Traits Using Computational and Evolutionary Approaches at Max Planck Institute for Plant Breeding Research in Cologne, Germany. In 2017, she received the PhD degree from the University of Cologne. During her PhD and short post-doc, she did the research in plant and soil microbiome including the plant molecular responses to environmental stresses, NGS sample preparation, development of NGS data analysis pipeline and multivariate data analysis.

LABRADOR – a GMP validated workflow for virus detection

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Background: High-throughput sequencing (HTS) is a perfect technology to determine whether the biological products, such as vaccines are free from the adventitious agents. HTS outcompetes the standard in vivo and in vitro assays or PCR methods in terms of time and the capacity to detect a broad spectrum of viruses. This makes it a promising and, probably, a leading method for virus detection in biologicals recommended by regulatory agencies in the nearest future. There is a need for standardized and reliable methods to manage HTS generated data for biologics, especially considering the complexity of bioinformatics. Therefore, we developed and validated LABRADOR software for adventitious virus detection with automated analysis of sequencing data and a program for viral hits evaluation by the research assistant.

Materials & Methods: LABRADOR consists of several third-party bioinformatic programs. The workflow was tested using published datasets.

Results: The core of LABRADOR software can be divided into reads filtering part and two workflows for virus detection: (i) direct reads classification based on the comparison of characteristic profiles between reads and sequences deposited in the database supported with alignment of to the best matching reference sequence and (ii) de novo assembly of contigs and their classification on nucleotide and amino acid levels. To meet the requirements published in guidelines for biologicals' safety, we additionally generated a custom nucleotide database with viral sequences.

Conclusions: Using LABRADOR we could reliably detect viruses in different samples such as in silico generated human microbiome or mixtures of model AVT viruses. We gained the valuable experience in GAMP5 software validation.

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Christophe Lambert is Associate Director in Data Science at GSK Vaccines in Belgium. He graduated B.Sc. and 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 leader of the AVDTIG Subgroup D exploring bioinformatics pipelines and reviewing best practices and perspectives about using HTS for virus detection.

Strategies to Optimize Next Generation Sequencing (NGS) Bioinformatics Pipelines for Virus Detection

Christophe Lambert, and AVDTIG

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Next Generation Sequencing (NGS) has demonstrated capabilities for broad virus detection based upon discovery of known and novel viruses in a variety of samples, including clinical, environmental, and biological. The Subgroup DE of the Advanced Virus Detection Technologies Interest Group (AVDTIG) has been exploring strategies for NGS bioinformatics pipelines optimization for virus detection in biologicals.

An important goal for NGS applications in biologicals is to establish appropriate parameters that can afford adequate sensitivity at an acceptable computational cost (computation time, computer memory, storage, expense or/and efficiency), and at critical steps in the bioinformatics pipeline. Those steps include initial data quality assessment, trimming/cleaning, and assembly to reduce data volume and increase likelihood of appropriate sequence identification.

The quality and reliability of the results depend on (1) the availability of a complete and curated viral database for obtaining accurate results; (2) appropriate selection of sequence alignment programs and their configuration to retain specificity for broad virus detection with reduced false-positive signals; (3) removal of host sequences without loss of endogenous viral sequences of interest; (4) follow-up up bioinformatic investigation of identified hits.

The objective of this presentation is to share collective considerations about factors related to detection sensitivity, data processing, database and reference genomes, de novo assembly and read mapping algorithms, and post-processing of unmapped reads.

Topic area:

- Bioinformatics strategies and pipelines

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Robert obtained his PhD in Biochemistry from Dalhousie University, where he studied the molecular evolution of Archaea. After a brief postdoc, he took a faculty position in Biology at the University of Ottawa, continuing his research in evolutionary molecular microbiology. At that time, he was also an Associate in the evolutionary biology programme of the Canadian Institute for Advanced Research. Robert joined Sanofi R&D in 2006, working on antigen selection and reverse vaccinology, then later on adventitious agent detection and genetic stability. He co-leads the bioinformatics and follow-up investigation strategies subgroup of the PDA Advanced Virus Detection Technologies Interest Group. He is currently the Head of the Molecular Biology Centre within Analytical Sciences at Sanof

Deciding on actionable signals from an HTS-based adventitious virus detection assay.

Robert L. Charlebois[1] and the AVDTIG[2]

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High-throughput sequencing (HTS) can be used for adventitious virus detection, showing competitive sensitivity relative to other approaches, and unsurpassed breadth of detection. It is a nucleic acid test, sampling from molecules present in the original test article, as well as those present in testing reagents or inadvertently introduced into the sampling and/or testing process. Even the nucleic acids present in the test article itself can include inert carryover molecules from raw materials.

Designing a decision tree that acts on contaminant signals but that also recognizes harmless background noise becomes a challenge when signals are at low levels. Concomitantly, weak signals are especially difficult to confirm in the laboratory as representing infectious particles. It may be even more difficult to identify their provenance in a root-cause analysis. Consequently, an HTS-based adventitious virus detection assay has a limit of actionability, that may be higher than the assay's limit of detection.

Patient safety is a paramount concern mitigated by adventitious virus detection, but release failures arising from false alarms may also compromise public health by frustrating the timely delivery of medicines. A risk-based approach to adventitious virus detection is needed, that leverages more information than just the list of viruses detected, by considering the context in which such signals were detected, and the accumulated experience outlining the background signal landscape expected from a given testing context. We will explore this landscape in this talk, and methods to accommodate any signals found with the intent of determining their risk, and hence, their actionability.

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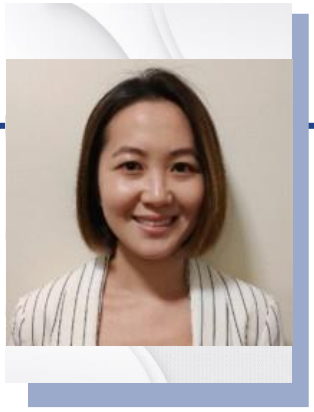

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Qiu Ruan obtained her Ph.D. degree in Pharmacology from Boston University School of Medicine in 2020. She comes from a background in molecular biology and neuroscience with a focus on unraveling and identifying genetic factors in psychostimulant addiction using mouse models. The scientific findings from her thesis project led to three first-author publications. During her Ph.D., she also received training in bioinformatics, particularly in the use of RNA-seq and CLIP-seq for studying RNA-binding proteins and RNA-protein interactions in the context of methamphetamine-induced addiction traits. Motivated to expand on her bioinformatics skills and contribute to drug discovery, Qiu joined Genedata in 2021 as a Scientific Account Manager. Since then, she has been engaged in the development and application of NGS- and omics-based approaches for advancing biopharmaceutical R&D processes, particularly in the areas of biosafety testing, cell line development, and cell & gene therapy characterization.

NGS for Adventitious Agent Detection: Analysis Options and Consequences

Co-authors: Qiu Ruan, Devon Ryan, Sebastien Ribrioux, and Thomas Hartsch

Background: Next-Generation Sequencing (NGS) is a transformative option for adventitious agent detection due to its speed, sensitivity, and unbiased nature.

Challenges: However, this technique generates a wealth of data leaving companies with an unclear path for how to accurately analyze and draw conclusions to guide future decisions. For example, to identify viral infection of the host genome, several possible viral databases could be used. It is therefore important to ask oneself "What are the benefits and drawbacks of each?". Another important strategy to consider is mapping the sequencing data. One could either directly map data to these viral databases or attempt to assemble the data beforehand. In this case, the impact of each approach on the specificity and sensitivity of the assay requires consideration. The added advantage of sequencing the production cell line for decreasing false positive rates should also be considered. For those setting up an analysis process for the first time, particularly those without a strong bioinformatics background, answering these questions can be extremely difficult.

Proposed approach: In the past few years, several studies have involved the introduction of viruses into production cell lines to mimic contamination. At Genedata Selector®, we use raw data from these studies to identify the effects of various analysis decisions, such as the choice of viral database to use or whether to use an assembly or direct mapping strategy. In this manner, we derive best practices. Examples of these include sequencing the host cell line for higher specificity, using assembly-based methods for higher specificity then direct-mapping methods without sacrificing sensitivity, and using the clustered RVDB for the best balance of sensitivity and specificity.

Conclusion: Adopting the proposed analysis practices should allow companies to quickly adopt NGS as a platform for adventitious agent detection, without the added uncertainty of how to analyze their results most accurately.

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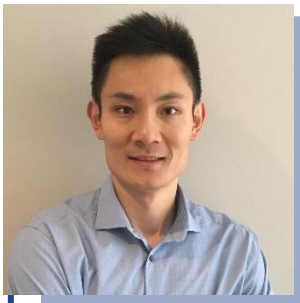

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Song Sun joined Sanofi Pasteur in 2019 as a Scientist at the Molecular Biology Centre, Analytical Sciences and has been the Head of High-throughput Sequencing (HTS) Unit since 2022. He received his bachelor's degree in Biochemistry from Shandong University in 2004, master's degree in Molecular Biotechnology and Bioinformatics from Uppsala University in 2007 and PhD in Medical Microbiology from Uppsala Univesity 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 epxerimental 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.

Reducing the haystack to find the needle: improved HTS assay sensitivity by host depletion in biological samples

Lauren Rodrigues[1], Jacek Remani[1], Artur Pedyczak[1], Robert Charlebois[1], Siemon Ng[1,2], Song Sun[1]

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High-throughput sequencing (HTS) is a promising new molecular technique with the potential to detect adventitious agents including viruses in biological samples with high sensitivity and large breadth of detection. However, the majority of nucleic acids extracted from biological samples are derived from host cells, which represents challenges for adventitious virus detection by HTS owing to the high proportion of host genome reads in relation to microbial reads (including potential adventitious viruses) and consequently can lead to a decreased sensitivity for adventitious virus detection.

We developed a sample-processing protocol to remove host nucleic acids and enrich sample matrices for viral DNA and RNA for metagenomic sequencing. A viral seed sample matrix, spiked with 17 model viruses, was processed using a nonionic detergent followed by treatment with DNase or benzonase prior to nucleic acid extraction. Both DNase and benzonase treatment significantly decreased reads mapped to the host genome and greatly improved the sensitivity of virus detection. The results showed that benzonase treatment outperformed DNase treatment in host depletion and could potentially increase the recovery of spike-in viruses by 15- to 126-fold.

We further applied benzonase pre-extraction treatment to other matrices. A cell bank and an unprocessed bulk, spiked with 17 model viruses, were processed with benzonase treatment. For the cell bank matrix, benzonase treatment increased the recovery of spike-in viruses by 55- to 290-fold. For the unprocessed bulk matrix, benzonase treatment increased the recovery of spike-in viruses by 12- to 224-fold.

Our results demonstrate a simple host depletion method in biological samples that improved HTS assay sensitivity for adventitious viruses.

This work also highlights that the effectiveness of sample treatment by benzonase is matrix-dependent and should be evaluated with respect to both the characteristics of the test item and the type of analysis in order to optimize adventitious virus detection.

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Dr. David Lee Suarez obtained a degree in Veterinary Medicine in 1988 from Auburn University. He obtained his Ph.D. degree from Iowa State University in Veterinary Microbiology in 1995. Dr. Suarez is board certified in the American College of Veterinary Microbiology in both Virology and Immunology. From 1988 to 1991, he worked as an Associate veterinarian at Quintard Veterinary Hospital in Anniston. He remains a licensed veterinarian in the state of Iowa. He joined the Southeast Poultry Research Laboratory, Agriculture Research Service, USDA in 1995 as a Veterinary Medical Officer in 1995. In 2005, he became Research Leader of the Exotic and Emerging Avian Viral Disease Research Unit with the same institution. He was Acting Laboratory Director from June 2010-October 2011. His primary research interests are avian influenza virus (AIV) and Newcastle disease virus (NDV). Since 1996, he has held the position of Adjunct Instructor in the Department of Infectious Diseases, University of Georgia. He is actively involved with numerous international collaborative projects.

Host RNA depletion for increased sensitivity of random amplification for next generation sequencing

David L. Suarez and Abhijeet Bakre
Southeast Poultry Research Laboratory, Agricultural Research Service, USDA-ARS-SAA-SEPRL

Next generation sequencing is becoming more frequently used as a diagnostic tool for veterinary samples, but sensitivity remains the biggest issue for practical use. Many approaches have been used to increase sensitivity, particularly in an effort to detect viral targets. A common approach is to use DNA probes targeted to host or bacterial rRNA and selectively degrade the RNA with RNase H. This approach is available commercially, but we have taken this strategy further by using custom probes targeted to both chicken and bacterial pathogens. This includes targeting both the 18s and 28s chicken rRNA, other chicken sequences found in high abundance, but also bacterial 16s and 23s rRNA to more effectively reduce non-target sequences. Using this custom approach has allowed more efficient removal of non-target sequences, but is cheaper than commercial applications. Ongoing analysis of sequence results to identify other host targets have shown mitochondrial and intergenic rRNA sequences can also be targeted. Using this approach we can increase 10 to 1000 fold the number of viral sequences identified from clinical samples, which allows for better coverage of important poultry pathogens including avian influenza, Newcastle disease virus and infectious bronchitis virus which allows pathotyping and lineage detection important in disease control. Additional methods to improve sensitivity are also being tested to continue to improve sensitivity of NGS.

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Dr. Alison Armstrong is Senior Director, Global Head of the Technical and Scientific Solutions team. Alison has a long history in academic research as a postdoctoral scientist and Research fellow and has been involved in examining the role of viruses in various pathogenic conditions. She has led a number of teams in different divisions within Merck; operational teams, validation and scientific development, and she is currently responsible for a scientific consultancy team with the remit to support clients in technical, scientific, and regulatory issues. During her career Alison has authored many different articles on trends in biosafety testing and is a member of regulatory taskforce groups related to microbiology and virology methods and alternate and rapid technologies. She is an invited speaker at international conferences. Dr. Armstrong holds a PhD in Molecular Virology from the University of Glasgow

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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 and recombinant DNA products such as monoclonal antibodies for clinical trials and marketing authorization. He participates as expert in EMA-Biologics Working Party (BWP) and EDQM TSE-certification procedure. Further, he is working in several research projects on virus inactivation and virus removal. 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.

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.

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Jean-Pol Cassart obtained his PhD in Molecular Genetics at the University of Namur (Belgium) in 1996. He worked on the transcriptional regulation in yeast in collaboration with the University of Uppsala where he conducted the final part of the lab work.

After his PhD, Jean-Pol was Professor Assistant in the University of Reims in France, working on the defense mechanism in plants.

Jean-Pol joined GSK Vaccines in 1998 where he was initially involved in cancer antigen discovery programs. In a second phase, Jean-Pol moved to the R&D Virology Department where he was responsible for the genetic characterization of viral vaccines and development of molecular clinical read-outs. In a third phase, he worked in the Quality Control Department of GSK Vaccines based in Wavre, Belgium where he was involved in the development and the implementation of novel analytical methods for QC testing. Currently, Jean-Pol is responsible for Viral Safety in the Global Quality Control Department of GSK Vaccines.

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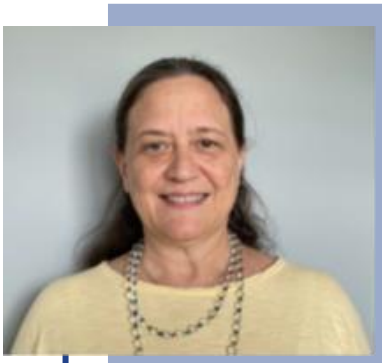

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Dr. Robin Levis has worked at the US Food and Drug Administration since 1995. She is currently the Deputy Director of the Division of Viral Products in the Office of Vaccines Research and Review at CBER/FDA; a position she has held since 2006. Prior to this position, she served as the Regulatory Coordinator for the Division of Viral Products (2002-2006) and served as a Senior Staff Fellow in the Laboratory of Vector Borne Viral Diseases (1995-2002). Her initial research work at the FDA related to flavivirus replication and the role of the NS1 protein. She then transitioned to be the lead CMC reviewer for licensed rabies virus vaccine products and rabies vaccine and related products under development. Her work with rabies virus vaccines was related to the development of an alternative, in vitro potency assay as an alternative to the currently licensed NIH potency test.

In addition to her work in the Office of Vaccines at CBER, she serves as the CBER representative to ICCVAM, as an observer to EDQM Group 15 for vaccines, and serves on several vaccine working groups for the Coalition for Pandemic Preparedness Innovations. Her role on these International working groups is to provide regulatory support to CMC development and product quality.

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Dr. Wall obtained his PhD in Biochemistry from the University of Ottawa, Canada in 2010 and was a post-doctoral fellow at the Atlantic Cancer Research Institute in Moncton, New Brunswick, Canada from 2011 - 2016. There he studied the role of ribonucleoproteins on cellular stress response and recovery.

Since 2016, Dr. Michael Wall has been a CMC Reviewer in the Biologics and Radiopharmaceutical Drugs Directorate at Health Canada.