Reference Materials for Next Generation Sequencing for Adventitious Virus Detection

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Outline of the Talk

- Currently recommended assays for adventitious virus detection and need for advanced technologies
- Considerations for using NGS/HTS (high-throughput sequencing) for supplementing or replacing the conventional assays for virus detection
- Challenges and efforts on using NGS for adventitious virus detection in biologics
- Considerations for regulatory applications for NGS

An Integrated Strategy for Adventitious Virus Risk Mitigation

- **PREVENTION**
  - **Risk assessment-** Identify potential sources of virus introduction to develop a comprehensive risk mitigation strategy and testing plan
    - Know the spectrum of infectious viruses that could potentially be in the host species of source materials (naturally-occurring, animal vaccines)
    - Gain cell culture passage history and characterization
    - Examine potential for virus exposure in the supplier's facilities (*including chemically-derived materials*)
  - **Use qualified materials**
    - Well-characterized cell banks
    - Certified/tested animal-derived biological materials (e.g. serum, trypsin, antibodies)
- **CLEARANCE *(not applicable for all products!)***
  - **Incorporate robust viral clearance steps** during manufacturing to validate the process
    - Viral inactivation and removal
    - Product purity: reduction of residual cellular materials (DNA, RNA, proteins)
- **TESTING**
  - **Extensive testing for known and unknown agents in the starting materials (cell substrate, virus seeds, vector virus preparation)**
  - **Adventitious agent testing at different stages in manufacturing process and at steps with the greatest potential for contamination**
  - **Using various improved sensitive and broad detection assays**

Routine Tests for Non-Viral Adventitious Agents

- **Bacteria (aerobic and anaerobic) and Fungi**
  - 21CFR 610.12
- **Mycoplasma (cultivatable and non-cultivatable) / Spiroplasma**
  - agar and broth media culture method (21CFR 610.30)
  - indicator cell culture procedure
  - PCR
- **Mycobacteria**
  - culture method
  - guinea pig test
  - PCR

Routine Tests for Viral Adventitious Agents

- **General virus detection assays**
  - *In vivo* assays (adult mice, suckling mice, embryonated hens' eggs, guinea pigs)
  - *In vitro* cell culture tests in cell lines of 3 species (same as cell substrate, monkey, human)
  - Transmission electron microscopy (TEM)
  - Reverse transcriptase assay for retroviruses (PERT)
- **Species-specific assays**
  - *In vitro* tests for animal viruses e.g. bovine, porcine (9CFR 113.47 and 113.53)
  - *In vivo* antibody-production assays for rodent viruses (MAP, including LCMV challenge; HAP; RAP)
  - Assays for known viruses (PCR, Infectivity)
- **Additional assays for novel cell substrates** *(OVRR/CBER: recommended case-by-case)*
  - Extended PCR assays
  - Oncogenicity assays: Tumor-inducing viruses
  - Chemical induction assays: Endogenous retroviruses, latent DNA viruses

The currently recommended assays have been generally effective in demonstrating the absence of adventitious viruses for product safety

Currently recommended adventitious virus tests have some limitations

❑ Conventional assays for routine testing

- **Cell-culture assays** are based upon detection of a visible effect due to virus replication, such as cytopathic effect (CPE) or hemadsorption / hemagglutination; > 28 days
- **Animal-based assays** are based upon a measurable pathological effect due to a replicating virus; > 18 days
- **Molecular assays** are designed based upon available known virus sequences

❑ Additional Testing for novel cell substrate

- **Chemical induction** can activate latent viruses, but detection of induced, unknown viruses would be missed due to using the conventional methods for virus detection

NGS Adventitious Virus Detection in Biologics

- **NGS is an advanced nucleic acid-based technology that can broadly detect known and unknown viruses, without prior sequence knowledge.**

- **Potential applications of NGS in biologics was demonstrated by:**
  - **Finding of porcine circovirus type 1 (PCV1) in a licensed rotavirus vaccine *(Victoria et al., 2010)***
  - **Discovery of a novel rhabdovirus in the Sf9 insect cell line used for baculovirus-expressed products *(Ma et al., 2014).***

- ***In both cases, extensive testing was done using currently recommended assays to demonstrate absence of adventitious viruses.***

General Challenges of NGS Applications for Adventitious Virus Detection

❑ Standardization and validation

- **Appropriate reference viruses and other standards (*for spiking studies*)**
  - Efficiency of the different steps involved in the methodology
  - Sensitivity and specificity

❑ Bioinformatics

- **Data analysis**
  - Pipeline optimization
    - Reference datasets
    - Criteria for acceptable quality of reads
    - Parameters for short read assembly; hybrid assembly to correct high error-rate currently seen in long-read sequencing
  - **Development of a complete and correctly annotated, publicly available, Reference Virus Database**
  - Develop strategies for novel virus detection
- Data submission, storage, and transfer
  - Format
  - Security

❑ Follow-up strategy

- Confirmation of a “true” hit
- Determination of biological relevance and significance of a positive signal

FDA/Industry Efforts on Advanced Virus Detection Technologies

Advanced Virus Detection Technologies Interest Group (AVDTIG)

(PDA sponsored "Users Group" in Oct. 2012; "Interest Group" since 2014)

"Mission" – To advance the tools for the next generation of viral risk evaluation by providing an informal, scientific forum for discussions and scientific collaborations

-> *Through scientific discussions, knowledge exchange, and collaborative studies.*

Co-chairs

- Arifa Khan: FDA, U.S.
- Jean-Pol Cassart: GSK, Belgium
- Keisuke Yusa: National Institute of Health Science, Japan
- Siemon Ng: Notch Therapeutics, Canada
- *(Dominick Vacante: Janssen R & D, U.S.: 2012-2022)*

➢ **More than 180 participants (mostly in U.S. and Europe) Includes: industry (vaccine and therapeutics), regulatory and other government agencies and national authorities, academia, CROs, and others**

- Meetings/discussions by t-con every other month
- Five focus subgroups on identified priority areas, with additional meetings

PDA J Pharm Sci and Tech 2016, 70 591-595

Current AVDTIG Subgroups – *since 2018*

❖**Subgroup AB:** **Sample selection/preparation/processing and Reference Virus Reagents**
Sng@notchtx.com and Megan.Cleveland@nist.gov

Collaborative Spiking Study Groups

- 2a: Cassandra.braxton@biogen.com
- 2b: Shanaz.Gilchrist@sanofipasteur.com and Arifa.Khan@fda.hhs.gov
- 3: Noemie.x.deneyer@gsk.com
- 4: Alessia.Bachmann@merckgroup.com and Simone Olgiati@merckgroup.com

❖**Subgroup C:** **Databases: Development of a complete and correctly annotated, public Reference Virus Database.** Pei-Ju.Chin@fda.hhs.gov and Vanessa.Sarathy@merck.com

❖**Subgroup DE:** **Bioinformatics pipelines analysis; Follow-up strategies to confirm a hit.**
Christophe.G.Lambert@gsk.com and Robert.Charlebois@sanofipasteur.com

KHAN Lab Efforts on NGS Standardization and Reference Materials

- **First Collaborative study to evaluate virus detection by different NGS platforms** *(FDA, GSK, Sanofi)*
  - Model viruses for NGS studies were identified based on subgroup discussions in AVDTIG
  - Performed by spiking 4-5 model viruses in different matrices relevant to biological materials datasets available in NCBI

  mSphere **September/October 2017**

  A Multicenter Study To Evaluate the Performance of High-Throughput Sequencing for Virus Detection

  Arifa S. Khan, Siemon H. S. Ng, Olivier Vandeputte, Aisha Aljanahi, Avisek Deyati, Jean-Pol Cassart, Robert L. Charlebois, Lanyn P. Taliaferro

- **Development of virus reference stocks**
  - 5 large scale, well-characterized virus stocks were prepared at ATCC

- **Generation of a complete Reference Virus Database (RVDB)**
  - In-house lab efforts in consultation with AVDTIG scientists have resulted in development of a word-based viral database that includes all viral, viral-related, and viral-like sequences

Development of Well-Characterized Reference Virus Stocks for NGS

- 5 viruses were selected based on distinct physical, chemical, and genome properties
- Representing virus families of potential safety concern in biologics
- Large scale preparation
  - Infectious titer
  - Genome copy number
  - Number of particles
  - Virus genome analysis
  - Residual host nucleic acids
- Stability studies

		Particle size (nm)	Envelope	Genome topology	Genome size (bp/b)	Physical chemical resistance	
Epstein-Barr virus type 1		122-180	YES	ds-DNA circular	172,281	Low to Medium	**Herpesvirus**
Feline leukemia virus		80-100	YES	ss-RNA dimeric	8,448	Low	**Retrovirus**
Human respiratory syncytial virus type A		150-300	YES	ss-RNA linear	15,158	Low to Medium	**Paramyxovirus**
Human reovirus type 1		60-80	NO	ds-RNA segmented	1,196 3,915	Medium to High	**Reovirus**
Porcine circovirus type 1		16-18	NO	ss-DNA Circular	1,758	High	**Circovirus**

❖**Vialed individually** to allow freedom for custom-mixing, as needed by user

Virus Stocks for NGS Studies

Virus Name	Total vials prepared*
Porcine circovirus type 1	392
Human orthoreovirus type1	403
Feline leukemia virus	503
Human respiratory syncytial virus	388
Epstein-Barr virus	490

- Virus Stocks are currently being used in additional Virus Spiking Studies for NGS standardization (*including Khan Lab*)
- Available externally for NGS establishment/standardization/validation studies : *arifa.khan@fda.hhs.gov*
- **Adopted by WHO as International Virus Reference Reagents for Adventitious Virus Detection by NGS (Oct. 2020)**

Generation of a complete Reference Virus Database (RVDB)

- **Need for a "complete" database was realized during our discovery of a novel rhabdovirus in Sf9 cells (*Ma et al., 2014*)**
- **To address the deficiencies in the public databases, we developed a new reference virus database**
  - **Based upon semantic selection from GenBank and NCBI RefSeq + Vrial Genomes**
  - **Contained all viral sequences regardless of size**
  - **Included endogenous viral and retroelements**
  - **Has a reduced cellular content**
- ***Provides high diversity of viral sequences to increase likelihood of novel virus detection, with reduced nonspecific cellular hits resulting in less data volume for bioinformatics analysis (and less computational time!)***

March/April 2018 Volume 3 Issue 2 e00069-18 mSphere

A Reference Viral Database (RVDB) To Enhance Bioinformatics Analysis of High-Throughput Sequencing for Novel Virus Detection: RVDB 10.2

Norman Goodacre, Aisha Aljanahi, Subhiksha Nandakumar, Mike Mikailov, Arifa S. Khan*

- RVDB was found to be more sensitive and specific for virus detection and is expected to enhance HTS investigations for product safety by increasing efficiency of virus detection, particularly for novel viruses

- Unclustered (U-RVDB) and Clustered (C-RVDB) versions are available

- RVDB is regularly updated by CBER/Khan Lab with new entries deposited in Genbank to aid in detection of emerging viruses

- The latest version RVDBv24.1 is available at https://rvdb.dbi.udel.edu/ with link for proteic RVDBs generated by Marc Eloit and Thomas Bigot (http://rvdb-prot.pasteur.fr/).

Introducing NGS for Improving Viral Safety Testing

- Increased efficiency (time)
- Ethical (reduce animal use)
- Superiority (LOD, specificity, repeatability, accuracy)

❖ Current cell substrate and viral safety guidances and regulatory documents provide flexibility for using alternative approaches with broad virus detection capabilities and “fit-for-purpose”

- *US FDA (2010)*
- *WHO (2010, pub. 2013)*
- *Ph. Eur. (2017)*
- *ICH Q5A(R2): (in progress)*

Potential Role NGS for Adventitious Virus Detection in Biologics

- **Strategy to mitigate risk of AV introduction**
  - Raw materials for cell culture
  - Cell banks
  - Virus seeds
- **Monitor absence of AV during production**
  - Bulk harvest
  - Final product

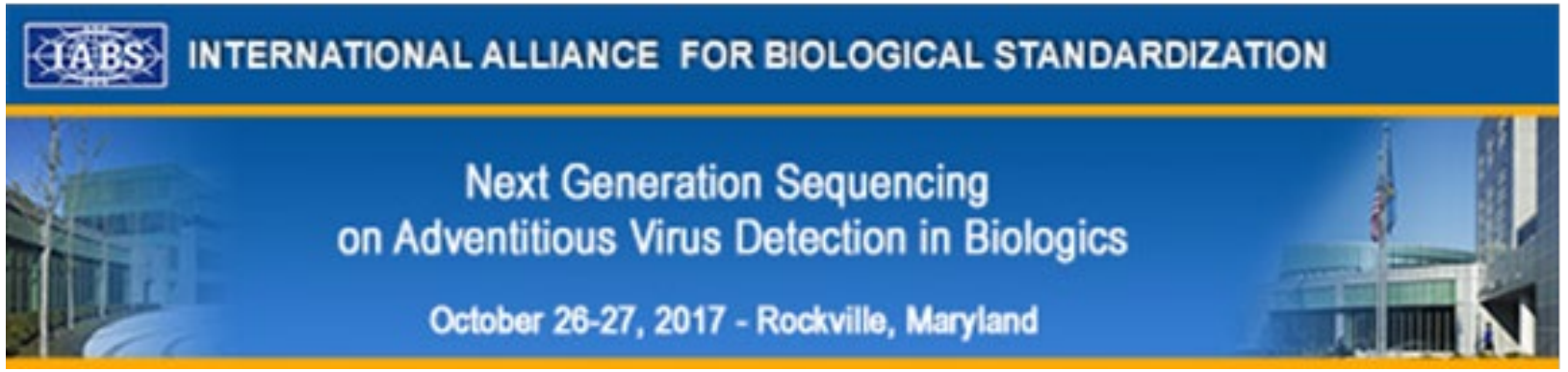
Assay Validation

- **You should demonstrate the reliability of assays or tests used to evaluate your cell substrate in the context of intended use.**
  - Assays related to assurance of safety should be scientifically valid (for example, by formal validation and/or inclusion of appropriate controls or standards) prior to initiation of clinical trials.

- This may include assessment of assay accuracy, precision, limits of detection, limits of quantification, specificity, linearity and range, ruggedness and robustness, and system suitability.

The Changing Landscape of NGS Applications in OVRR: *COVID-19 Era*

- **OVRR has been receiving submissions requesting use of NGS as a broad adventitious virus test (*pre-COVID*).**
- **The number of requests have increased in 2020 for using NGS an alternative adventitious virus detection assay to accelerate SARS-CoV-2 vaccine development.**
  - **Increase in the number of sponsors using NGS**
    - **Increased in-house capabilities and commercial availability**
  - **Expanded use of NGS for product characterization and testing**
    - **Cell substrate characterization**
    - **Testing of Master and Working Virus Seeds and DS Harvest**
    - **Genetic stability of vaccine virus**
  - **Extended use of NGS for a virus detection**
    - **Complementary or supplementary assay -> Replacement of one or more conventional virus detection assays**



Scientific Committee

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Philip Minor National Institute for Biological Standards and Control, United Kingdom
Gayle Pulle Health Canada

- **NGS for adventitious virus detection in biologics with focus on applications for human vaccines and lessons learnt from veterinary vaccines.**
- **The meeting included data presentations and discussions for developing a scientific consensus for using NGS for virus detection in selected applications of biologics.**

FDA U.S. FOOD & DRUG ADMINISTRATION

Meeting report is available in Biologicals, Sept. 2018

- **Bring together industry, academia, technology providers, and international regulatory bodies to discuss current status of NGS for adventitious virus detection in biologics**

- **Present ongoing efforts on standardization and validation of the technical and bioinformatics steps in NGS for its applications in characterization and safety evaluation of biologics, including human and animal vaccines.**

- **Develop a scientific consensus regarding readiness of NGS for detection of adventitious viruses in biologics.**

** Full meeting report is available online since July 11, 2020 in Biologicals*

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

Sept 27 - 28, 2022

IBBR / University of Maryland, Rockville, U.S.A.

- **Face-to-face meeting**
- **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**
- **Focus on developing a scientific consensus regarding recommendations for using NGS for detection of adventitious viruses.**

Current Considerations for Using NGS

- **NGS may be considered for supplementing or replacing the conventional assays for adventitious virus detection based upon justification for suitability and fit for purpose (*currently, on a case-by-case basis*).**
- **Use of NGS reference materials can provide confidence in NGS results and facilitate method implementation**
- **Early discussions between industry and regulatory authorities is encouraged to since the technology is still evolving**

Thank You!