USP Biologics Stakeholder Forum: Innovative Analytical Approaches to Cell and Gene Therapy – NGS and other methods, Rockville, MD

FDA Perspective on Using NGS/HTS for Adventitious Virus Testing

Arifa S. Khan, Ph.D.

Supervisory Microbiologist

Division of Viral Products

Office of Vaccines Research and Review

Center for Biologics Evaluation and Research

U.S. Food and Drug Administration

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Disclaimer

This presentation is an informal communication and represents my own best judgment.

The material in this presentation and my comments do not bind or obligate FDA or any other agency.

Viral Factors Influencing Safety and Quality of Biologics

EXTRANEOUS VIRUSES

- Manufacturing-related (all product types)
  - Adventitious viruses – *Raw materials (Cell substrate, cell culture reagents), personnel, equipment, facilities*
  - Endogenous retroviruses - *Cell substrate*
- Process-related (viral vectors and viral vector-based products)
  - Replication-competent viruses – *de novo* generation
  - Helper viruses – *used with some viral vectors*

VIRAL IMPURITIES

- Product-related
  - Viral nucleic acids from disrupted cells – free RNA or DNA; integrated DNA *(e.g., 293, HeLa)*
  - Encapsidated “irrelevant” sequences in viral vectors – viral or cellular (e.g., rAAV)
  - Plasmids used in transductions

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 Viral Adventitious Agents Tests for Product Safety

- **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 virus detection**
  - Extended and broader PCR-based assays
  - Expanded cell culture assays (*adding more target cell lines*)
  - Chemical induction assays: Latent viruses (endogenous and episomes)

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

Limitations of Currently Recommended Adventitious Virus Tests

- **Cell-culture assays**
  - Based upon susceptibility of target cells to virus infection
  - Assay read-out is a visible effect due to virus replication, such as cytopathic effect (CPE), hemadsorption, hemagglutination
  - Sample-related interference
  - 28-day observation period
- **Animal-based assays**
  - Unknown sensitivity for virus detection
  - Detection depends on susceptibility of animal species to virus infection
  - Based upon a measurable pathological effect due to a replicating virus
  - Sample-related interference
  - > 18 day-observation period depending upon the species
  - Use of animals globally discouraged (3 R's initiative!)
- **Molecular assays (PCR)**
  - Designed based upon available known virus sequences
  - Large number of assays needed for detection of different viruses
- **Additional assays: 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 for Broad Detection of Adventitious Viruses

- NGS was initially recognized as a powerful advanced technology for adventitious virus detection by **the identification of PCV1 in a licensed rotavirus vaccine** and the subsequent **discovery of a novel rhabdovirus in the insect Sf9 cell line** (used commonly for baculovirus-expressed vaccines and other biologics)

American Society for Microbiology | Journal of Virology®

VACCINES AND ANTIVIRAL AGENTS
June 15, 2010 Volume 84 Issue 12

Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus

Joseph G. Victoria[1,2], Chunlin Wang[3], Morris S. Jones[4], Crystal Jaing[5], Kevin McLoughlin[5], Shea Gardner[5], Eric L. Delwart[1,2,*]

JVI Journals.ASM.org

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Published ahead of print 26 March 2014

June 2014 Volume 88 Number 12

Identification of a Novel Rhabdovirus in *Spodoptera frugiperda* Cell Lines

Hailun Ma, Teresa A. Galvin, Dustin R. Glasner,* Syed Shaheduzzaman, Arifa S. Khan
Laboratory of Retroviruses, Division of Viral Products, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Maryland, USA

  - In both cases, routine testing had been done. Additional testing using degenerate PCR assays to detect various insect virus families was done for the Sf9 cells since it was a novel cell substrate.

- The testing gaps and complexity of the NGS technologies were recognized in CBER and by industry leading to their combined efforts for establishing the Advanced Virus Detection Technologies Interest Group in the the Paternal Drug Association (PDA)

General Challenges of NGS Applications for 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**

Advanced Virus Detection Technologies IG

(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** ***(initial focus: HTS)***

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

More than 216 participants from over >60 organizations:

- *Regulatory agencies*
- *Government agencies*
- *Industries*
- *Service providers*
- *Technology developers*
- *Academics*

Meeting/discussions by t-con once every 2 months

Co-chairs

Arifa S. Khan (FDA, U.S.A.; October, 2012)
Siemon Ng (Notch Therapeutics, Canada; June, 2022)
Ken Kono (National Institute of Health, Japan; October, 2023)
Noémie Deneyer (GSK, Belgium; November, 2023)

AVDTIG – Objectives

- Provide an **informal, scientific forum** for discussions, knowledge exchange, and scientific collaborations among scientists from different organizations
- Develop **consensus views**
- Promote and Conduct IG **collaborative studies**
- Publish **best practices, perspective and position papers** for considerations of NGS applications in biologics
- Interact with other consortiums to **enhance the IG goals and extend efforts.**

AVDTIG Subgroups

2012

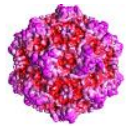
2018

Subgroup A

Sample selection/ preparation/processing

Subgroup B

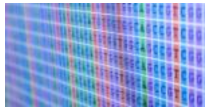
Virus standards and reference materials



Subgroup AB

Subgroup C

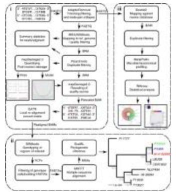
Complete and correctly annotated,
virus reference database



Subgroup C

Subgroup D

Bioinformatics pipelines analysis



Subgroup E

Follow-up strategies to confirm the identity of a “hit”

Subgroup DE

Achievements and Contributions of AVDTIG

Biologicals 67 (2020) 94–111

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

ELSEVIER

Report of the second international conference on next generation sequencing for adventitious virus detection in biologics for humans and animals☆

Arifa S. Khan[a,*], Johannes Blümel[b], Dieter Deforce[c], Marion F. Gruber[a], Carmen Jungbäck[d], Ivana Knezevic[e], Laurent Mallet[f,1], David Mackay[g], Jelle Matthijnssens[h], Maureen O'Leary[i,2], Sebastiaan Theuns[c], Joseph Victoria[j], Pieter Neels[d]

World Health Organization

WHO/BS/2020.2394
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 19 to 23 October 2020

Proposed 1st International Virus Reference Standards for Adventitious Virus Detection in Biological Products by Next-Generation Sequencing (NGS) Technologies (CBER-5)

Arifa S. Khan* and Study Group Participants

ELSEVIER

Biologicals

Volume 55, September 2018, Pages 1-16

Report of the international conference on next generation sequencing for adventitious virus detection in biologicals ☆

Arifa S. Khan, Luca Benetti, Johannes Blumel, Dieter Deforce, William M. Egan, Ivana Knezevic, Philip R. Krause, Laurent Mallet, Dietmar Mayer, Philip D. Minor, Pieter Neels, Guanhua Wang

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RESEARCH ARTICLE
Applied and Environmental Science

March/April 2018 Volume 3 Issue 2 e00069-18

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

Norman Goodacre,[a] Aisha Aljanahi,[a*] Subhiksha Nandakumar,[a] Mike Mikailov,[b] Arifa S. Khan[a]

F1000Research

F1000Research 2020, 8:530 Last updated: 31 MAR 2022

Check for updates

DATA NOTE

REVISED RVDB-prot, a reference viral protein database and its HMM profiles [version 2; peer review: 2 approved]

Thomas Bigot[1], Sarah Temmam[2], Philippe Pérot[2], Marc Eloit[2,3]

[1]Hub de Bioinformatique et Biostatistique – Département Biologie Computationnelle, Institut Pasteur, USR 3756 CNRS, Paris, France
[2]Pathogen Discovery Laboratory, Institut Pasteur, Paris, 75015, France
[3]École Nationale Vétérinaire d'Alfort, Maisons-Alfort, 94700, France

2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023

COMMENTARY Vol. 70, No. 6, November–December 2016

Advanced Virus Detection Technologies Interest Group (AVDTIG): Efforts on High Throughput Sequencing (HTS) for Virus Detection

ARIFA S. KHAN[1,*], DOMINICK A. VACANTE[2], JEAN-POL CASSART[3], SIEMON H. S. NG[4], CHRISTOPHE LAMBERT[5], ROBERT L. CHARLEBOIS[4], and KATHRYN E. KING[6]

[1]Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD; [2]Janssen Research & Development, LLC, Malvern, PA; [3]GlaxoSmithKline Vaccines, Wavre, Belgium; [4]Analytical Research and Development, Sanofi Pasteur. Toronto, ON, Canada; [5]GlaxoSmithKline Vaccines, Rixensart, Belgium; and [6]Office of Biotechnology Products, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD ©PDA, Inc. 2016

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

Arifa S. Khan,[a] Siemon H. S. Ng,[b] Olivier Vandeputte,[c] Aisha Aljanahi,[a*] Avisek Deyati,[d] Jean-Pol Cassart,[e] Robert L. Charlebois,[b] Lanyn P. Taliaferro[a*]

viruses MDPI

Perspective

Current Perspectives on High-Throughput Sequencing (HTS) for Adventitious Virus Detection: Upstream Sample Processing and Library Preparation

Siemon H. Ng[1,*], Cassandra Braxton[2], Marc Eloit[3,4], Szi Fei Feng[5], Romain Fragnoud[6], Laurent Mallet[7], Edward T. Mee[8], Sarmitha Sathiamoorthy[1,†], Olivier Vandeputte[9] and Arifa S. Khan[10]

viruses MDPI

Perspective

Considerations for Optimization of High-Throughput Sequencing Bioinformatics Pipelines for Virus Detection

Christophe Lambert[1,*], Cassandra Braxton[2], Robert L. Charlebois[3], Avisek Deyati[1], Paul Duncan[4], Fabio La Neve[5], Heather D. Malicki[6], Sebastien Ribrioux[7], Daniel K. Rozelle[8], Brandye Michaels[9], Wenping Sun[6], Zhihui Yang[10] and Arifa S. Khan[11]

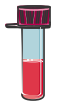
AVDTIG – Subgroup AB: Collaborative Spiking Studies

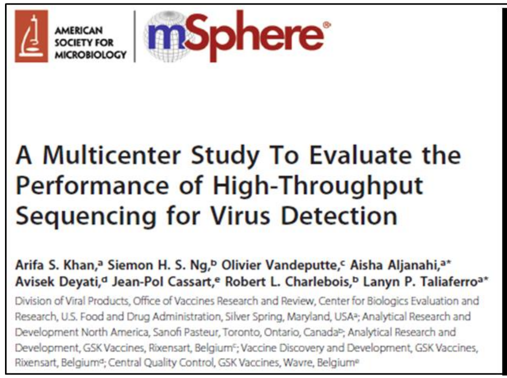
- **For performance evaluation and standardization of NGS**
  - Evaluate reference standards and bioinformatics tools
  - Compare and optimize experimental protocols

Spiking study 1
(2013-2016)

HeLa cell line

EBV *RSV* *FeLV* *Reo* *PCV1*





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

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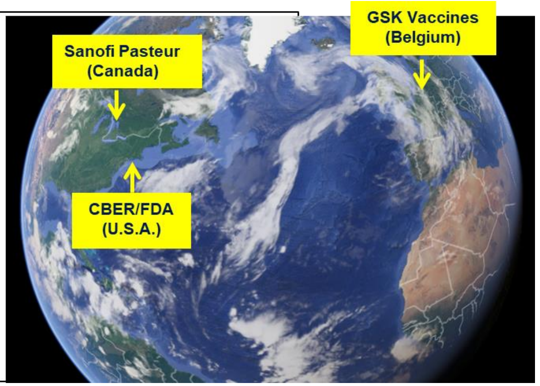
Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA[a]; Analytical Research and Development North America, Sanofi Pasteur, Toronto, Ontario, Canada[b]; Analytical Research and Development, GSK Vaccines, Rixensart, Belgium[c]; Vaccine Discovery and Development, GSK Vaccines, Rixensart, Belgium[d]; Central Quality Control, GSK Vaccines, Wavre, Belgium[e]



Spiking study 2A Started 2017	Virus in CHO background (short-read)
Spiking study 2B Started 2016	Viral seed/Viral vector background (short-read)
Spiking study 3 Started 2019	Transcriptomics (short-read)
Spiking study 4 Started 2020	Viral seed/Viral vector background: 2B (long-read)
Spiking study 5 Discussions- 2023	Cell substrate/high cellular background (long-read)

Development of Reference Viruses

❖ **Characterization**

- Infectious titer per mL (>10^6 $TCID_{50}$ per mL)
- Number of particles : TEM
- Genome copy number: ddPCR (>10^8 gc per mL)
- Adventitious virus analysis: Illumina HTS
- Host DNA copy number: ddPCR (*different species*)
- Reference virus genome sequence and variant analysis: HTS
- Stability studies: infectious titer; genome copy number

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

- CBER has supported storage and distribution at ATCC. Transferred to NIH/NIAID BEI Resources Repository from Sept 29, 2023 for long-term storage and distribution

WHO International Reference Reagents for Adventitious Virus Detection in Biological Products Using HTS *(Oct. 2020)*

- Developed by CBER based on the results of the first AVDTIG spiking study 1.
- Adopted as WHO reagents by ECBS based on results from the AVDITG Spiking Study 2B
- Characterization: Infectious titer, Particle count, Genome copy number and NGS analysis

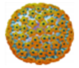
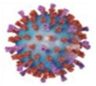
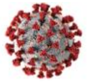
Virus	Genome type	Genome size	Particle size	Envelope	Chemical resistance
Reo (Lang)	RNA, double-strand; Linear (segmented)	23.6kb(1,196 –3,915nt	80nm	No	Medium-high
FeLV (KT)	RNA; single-strand; Linear (dimeric)	8.5kb	80-100nm	Yes	Low
RSV (A2)	RNA; single-strand; Linear	15kb	150-200nm	Yes	Low-medium
PCV-1*	DNA, single-strand; circular	1.8kb	16-18nm	No	High
EBV (B95-8)**	DNA, double-strand; Linear	172kb	122-180nm	Yes	Low-medium

* Also contains Porcine Endogenous Virus
** Also contains Squirrel Monkey Retrovirus

- Request for the 5 model viruses can be made via the NIAID BEI Repository catalogue number NR-59622
- Information regarding the virus stocks can be made by contacting CBER, Arifa.Khan@fda.hhs.gov

Proposed WHO Reference Virus Panel for NGS/HTS Adventitious Virus Detection

WHO Intl Ref Reagents *Currently Available*

		Particle size (nm)	Envelope	Genome topology	Genome size (bp/b)	Physical chemical resistance	
Epstein-Barr virus type 1		122-180	YES	ds-DNA li linear	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**
Human coronavirus HCoV-OC43		80-120	YES	ss-RNA linear	30,700	Low	**Coronavirus**
Minute Virus of Mice		26	NO	ss-DNA linear	5,100	High	**Parvovirus**

Proposed* 1st WHO Intl Ref Virus Panel – *Q1-2/2024

A Comprehensive Reference Virus Database (RVDB) for NGS Broad Virus Detection

- To address the deficiencies in the public databases, we developed a new reference virus database based upon semantic selection from GenBank and NCBI RefSeq + Neighbor Genomes *(Goodacre et al., mSphere, 2018)*
  - Contained all viral sequences regardless of size
  - Included endogenous viral and retroelements
  - Has a reduced cellular content
- The latest version RVDBv28.0 (*Nov 22, 2023*) 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/).

- *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!)*
- *Updated quarterly by the Khan Lab (Pei-Ju Chin)*
- *Ongoing work on annotation of sequences to remove misannotated sequences and enhance virus-specific detection (Pei-Ju Chin & Trent Bosma)*

RVDB Provides 4 Formats to Adapt Various Application Scenarios

- U-RVDB *fasta* file
  - Un-clustered, contain all viral sequences with redundancy
  - Higher computation-demanded. **Suitable for virus detection by *blastn/nhmmer***
- C-RVDB *fasta* file
  - Clustered, sequences share 98% similarity are collapse to one representative sequence for each clade
  - Lower computation-demanded. **Suitable for virus detection by *tblastx***
- SQLite DB Script
  - Create the entries (*fasta* header and the corresponding information) for advanced bioinformatic pipelines/workflows
- Proteic RVDB (provided by Institut Pasteur: https://rvdb-prot.pasteur.fr/)
  - **Hidden Markov Model (HMM)** profile of viral protein domains
  - Unknown viruses with remote homology by ***hmmsearch*** / ***hmmscan***

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

CBER/OVRR: Examples of Submissions Using NGS for Adventitious Virus Detection

Sample Type	NGS Application	Role of NGS	Outcome
2009- Novel CS- EOP cells and supernatant	To broaden detection of unexpected and unknown/novel viruses	Complementary – Additional; Full conventional testing done	2013-Results used for supporting submission
2011- Novel CS-WCB, EOPC	To broaden detection of unexpected and unknown/novel viruses	Complementary – Additional; Full conventional testing done	2015- Re-analysis of data with updated database
2019- MVS, Bulk Harvest	To potentially replace PCR assays	Supplementary – to fill gaps in vitro AV assays due to assay interference	Results as FYI.
2020- 2022 > 25; Cell Banks, MVS, WVS, Bulk Harvest	To replace *in vivo* or/and *in vitro* assays	Complementary, Supplementary, or Replacement due to assay interference	Method validation, Assay qualification/ validation
2023 – *Live, viral vaccine, License: MVS, Bulk DS	To replace *in vivo* adventitious virus assays	NGS for Replacement of the *in vivo* adv virus testing due to challenges of the in vivo assays	Performed acceptable assay qualification and validation

NGS Applications in the FDA

- NGS data is currently under review at **CBER**
    - Adventitious virus testing of Cell Banks, Virus Seeds, and Bulk Harvests
    - Genetic stability of vaccine virus
    - Cell substrate characterization
  - The CBER Advanced Technologies Team (CATT) coordinates scientific discussions on new technologies including NGS
  - Within CBER/OVRR we highly recommend that sponsors request a technical working group discussion related to the use of NGS for vaccine safety and characterization
    - Non-regulatory meeting to discuss "plans" for use of NGS
    - Reach consensus prior to initiating lengthy, expensive studies
  - NGS for AV testing is being applied in gene therapies (CBER/OTP)
- The Emerging Technologies Team in **CDER** is involved in discussions on NGS for adventitious virus detection in biotherapeutics
- Interest in considering HTS for AV detection has also been initiated for animal-based biologics (**CVM**)

ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (*Nov. 1, 2023*)

➢ The update reflects current scientific knowledge and biotechnology advances related to:

- **New classes of biotechnology products (*amenable to virus clearance*)** e.g., baculovirus-expressed VLPs and proteins; AAV vectors; helper-dependent [adenovirus, HSV] viral vector products (*ANNEX 6: Genetically-engineered viral vectors and viral vector-derived products*)
- Additional validation approaches for virus clearance e.g., modular validation
- **New virus assays and alternative analytical methods** e.g., PCR and NGS/HTS *(3.2 Recommended Virus Detection and Identification Assays 3.2.5 Molecular Methods)*
- Virus clearance validation and risk mitigation strategies for advanced manufacturing (e.g. continuous manufacturing)
- Aspects of virus clearance validation that have emerged or evolved

ICH Q5A(R2): New Nucleic Acid-Based Test Methods

- Guideline encourages use of new alternative tests (includes Next Generation Sequencing and PCR in discussion) -> **Aligns with the 3Rs initiative to reduce animals for testing**
- Specific opportunities to replace existing methods with targeted test (e.g. PCR or NGS) for replacing antibody production tests or non-targeted (agnostic/broad; such as NGS) for replacing *in vivo*/*in vitro*
  - Antibody Production Tests in Rodents (MAP, HAP, RAP)
  - *In Vivo* Assays
  - *In Vitro* Assay
  - Highlights that direct head-to-head comparison with existing methods is generally not expected (*in vivo and in vitro*)

It is recognized that these nucleic acid-based assays have limitations as they cannot distinguish between infectious and noninfectious particles and therefore detection of a signal may need a confirmatory test with an infectivity assay for risk-assessment.

General Acceptance of NGS for Adventitious Virus Detection in Biologics

- **Cell substrate characterization** – particularly for novel cell substrates or in case where there are concerns for occult and novel viruses
- **Supplementary to or replacement of *In vitro* AV assays** – particularly in case of assay interference due to lack of effective neutralization of vaccine virus
  - Potentially supplement *in vitro* AV assays as a read-out assay to broaden virus detection
- **Replacement assay (*in vivo* and PCR assays)**
  - *In vivo* AV assays – HTS can provide defined sensitivity and breadth of virus detection
  - Antibody Production Assays – MAP, RAP, HAP
    - *Reduce use of animals – meet the global objectives for 3Rs (reduction, refinement, replacement)*
  - PCR assays – HTS can have similar sensitivity to PCR assays
    - Single assay with broader virus detection

❖ As with any assay, implementation of NGS needs method qualification and validation

Current Thinking on Using NGS as an Alternative Adventitious Virus Detection Method

- **Replacement assay (*in vivo* assays; PCR assays)**
  - *In vivo* AV assays – NGS can provide defined sensitivity and breadth of virus detection
  - PCR assays – NGS can have similar sensitivity than PCR assays; broader virus detection; single assay
- **Supplementary or Replacement assay (*in vitro* assays)**
  - Cell substrate characterization – particularly in case where there are concerns for occult and novel viruses
  - *In vitro* AV assays – particularly in case of assay interference due to lack of effective neutralization of vaccine virus; as a read-out to reduce assay time

❖ ***Note***

- *Follow-up of a positive result is critical to determine biological significance of a signal for decision-making (as for any nucleic acid-based detection assay)*
- *NGS data may help design a "custom" assay to determine if signal due to infectious virus*

Follow-Up of NGS Signal

- Follow-up of a positive result is critical
  - Verification of results
    - *Can the results be confirmed by PCR or another assay?*
    - *Is a complete viral genome present?*
    - *Are particles present?*
    - *Are the particles infectious?*
    - *Is there a replication-competent virus?*
    - *Can the nucleic acid/particles be quantified?*
  - Determine biological relevance and significance (*as with any nucleic acid-based assay*)
  - NGS data can aid in design of a "custom" infectivity assay for risk management

Potential Applications of NGS for Safety and Characterization of Biologics

- **Testing to mitigate risk of adventitious virus introduction**
  - Raw materials for cell culture
  - Cell banks
  - Virus seeds
- **Monitoring absence of extraneous viruses during production**
  - Bulk harvest
  - Final product
- **Detection/Characterization of extraneous viral sequences in the final product**
  - Encapsidated extraneous sequences in viral vectors
  - Genome analysis
- **Characterization of viral vector sequences**
  - Identity testing
  - Persistence, Expression
  - Vector integration site analysis

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) (2023)*
  - *Ph. Eur. (2.6.41, public comments April 2023)*

Still More Work for Routine Implementation!

- Optimization of pre-treatment conditions to increase sensitivity of virus detection in complex matrices
- Development of SOPs and test datasets for establishing NGS by an early user of the technology
- Development of other types of standard materials (e.g., VLPs)

Collaborative Study- NIIMBL/GHF

- Study participants: FDA/CBER, GSK, Millipore Sigma (BioReliance and EMD Serono)
- A head-to-head study for evaluating NGS with *in vivo* and *in vitro* adventitious virus detection assays (*using same sample material*)
- Two model viruses from the WHO reference virus reagents were used to spike a complex matrix representing a bioreactor sample for protein production.
- Results are in preparation for publication
  - facilitate data-based, decision-making for NGS as an alternative method to replace *in vivo* assays and to replace or supplement *in vitro* assays

Ongoing Work on Reference Materials (Khan Lab)

- **Development of a viral reference reagent for cell-based biologics**
  - Human cell line with stably integrated retrovirus (cell clones with latent and active infection)
  - Well-characterized for viral genome copy number and integration site(s)
  - Can be used for spiking into an uninfected cell background to evaluate sensitivity of virus detection by NGS in cell substrates and cell-based products

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