PATH WEBINAR: Applying Next-Generation Sequencing for Vaccine Development and Testing.
September 17-18, 2025

Current Status of Using Next-Generation Sequencing for Adventitious Virus Testing and Regulatory Expectations

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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.

Outline

- Introduction of NGS for viral adventitious agent detection in biologics
- CBER's efforts towards NGS implementation
- NGS applications in OVRR's regulatory submissions
- Acceptance of NGS in global regulatory guidelines

Adventitious Agent: *Definition**

A microorganism that is unintended to be in the biological product

- Bacteria
- Fungi
- Mycoplasma / Spiroplasma
- Mycobacteria
- Rickettsia
- Protozoa
- Parasites
- TSE agent
- Viruses

**WHO Tech Rep Series No. 993, 2015, annex 2*

Routine Tests for Adventitious Agents

Non-Viral Agents

- Bacteria (aerobic and anaerobic) and Fungi
- Mycoplasma (cultivatable and non-cultivatable)/Spiroplasma
- Mycobacteria

Viral Agents

- **General 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)

US FDA: Guidance for Industry for Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases - 2010[5]

Additional Tests for *Viral* Adventitious Agents

- ***Case-by-case (e.g., novel cell substrates; unknown passage history)***
  - Extended PCR assays
  - Expanded cell culture assays (*adding more target cell lines*)
  - Chemical induction assays: Latent viruses (endogenous and episomes)

Conventional Virus Detection Assays - *Strengths*

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

- ***In vitro* cell culture assays** can be highly sensitive for general virus detection since large volumes are used
  - At least 3 cell types (*subculture at 14 d for total of 28 d test period*)

- ***In vivo* animal assays** may detect natural isolates
  - Embryonated chicken eggs, 2 routes, 18-day observation (blind passage)
  - Adult mice, 21-day observation
  - Suckling mice, 28-day observation (blind passage)

- **PCR assays** can be sensitive for specific virus detection
  - Viruses known to occur in host species of cell substrate origin (*e. g.* human)
  - Viruses known to occur in animal-derived reagents used (*e. g.* bovine serum; porcine trypsin)

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 based on virus replication, such as cytopathic effect (CPE), hemadsorption and 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
  - Detection is based on a measurable clinical 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

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 viral agents in the starting materials (cells, virus seeds, vector virus preparation)**
  - **Adventitious virus testing at different stages in manufacturing process and at steps with the greatest potential for contamination**
  - **Using various improved sensitive and broad virus detection assays**

Challenges for Testing Viral Adventitious Agents

- Large number of virus families
- High diversity in viral sequences (*especially RNA viruses*)
  - mutations, recombination
- Continuing increase of the virome due availability of next generation sequencing

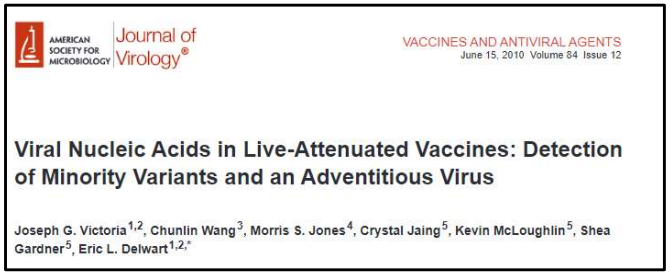
Need for Improved Analytical Methods for Broad Virus Detection

- **Known viruses**
- **Unknown, and unexpected viruses**
  - Tumor-inducing viruses (in case of tumor cell lines)
  - Latent viruses (infectious but silent and can become active)
    - DNA and RNA viruses
    - Endogenous retroviruses
  - Occult viruses (known, unexpected)
  - Novel viruses (unknown)
- **Reverse transcriptase (RT) activity**
  - Produced constitutively from avian and insect cells (endogenous retroviruses: retroviral particles/retrotransposons)

Industry and regulators recognized NGS as a powerful, new technology with capabilities to detect known and unknown viruses, without prior sequence knowledge.

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)



- 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.

Early Public Discussions on New Technologies for Adventitious Virus Detection in Biologics

- **2009:** PDA Cell Substrate Workshop. Discussed **broad virus detection technologies**. Conference Proceedings in PDA J Pharm Sci and Tech, 2010; PDA Technical Report on Emerging Methods for Virus Detection (71) 2015

- **2011:** IABS: Adventitious Agents, **New Technologies** and Risk Assessment. Conference Summary in Biologicals, 2011

- **Nov. 2011:** PDA/FDA Adventitious Agents and Novel Cell Substrates: Emerging Technologies and New challenges. Discussed applications and identified **knowledge and technical gaps** that needed to be address for further development and applications. Conference Proceedings in PDA Journal, 2012

- **Sept. 2012**: FDA VRBPAC discussions on use of human tumor cell lines and potential use of **advanced virus detection technologies** Transcript pdf available: www.fda.gov

- **May 2013:** WHO Cell Substrate Study Group (Beijing) **discussed DNA-based detection of** adventitious agents in cell substrates

- **Nov. 2013:** PDA/FDA Advanced Technologies for Virus Detection in the Evaluation of Biologicals: Applications and Challenges**. Identified challenges and priority areas** to be addressed for applications to biological products. Conference Proceedings in PDA Journal, 2014

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**

NGS Workflow

UPSTREAM PROCESSING

DOWNSTREAM PROCESSING

(PRE-TREATMENT)

- *Reduction of "free" nucleic acid using nuclease*
- *Enrichment of particles by WGA/filtration/size selection/ ultracentrifugation*

NUCLEIC ACID EXTRACTION

- Whole cells
- Cell lysate
- Supernatant (cell-free)

LIBRARY PREPARATION

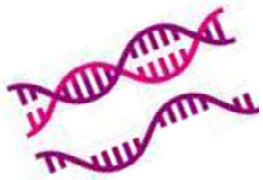
- rRNA depletion/ polyA+ selection
- cDNA synthesis
- Fragmentation

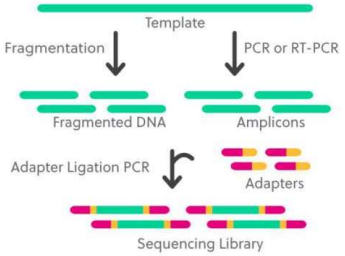
SEQUENCING

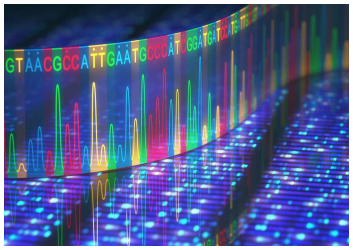
- Short-read
  - Illumina
- Long-read
  - PacBio
  - Nanopore

BIOINFORMATICS

- Assembly programs
- Analysis tools
- Databases







Advanced Virus Detection Technologies WG

(PDA "Users Group" in Oct. 2012; "Interest Group" in 2014; "Working Group since 2022)

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 270 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)
Simone Olgiati (Merck, Italy; August, 2025)

AVDTWG – 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.**

AVDTWG Subgroups

2012

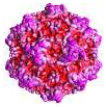
2018 ▬ · ▬ ▸

Subgroup A

Sample selection/ preparation/processing

Subgroup B

Virus standards and reference materials



Subgroup AB

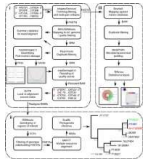
Subgroup C

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

AVDTIG – Subgroup AB: Collaborative Spiking Studies

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

Short-read NGS	
Spiking study 1 ***Published in 2017***	5 viruses in HeLa cell line background
Spiking study 2A *Started in 2017*	MVM in a CHO cell line background
Spiking study 2B *Published in 2025*	5 viruses (*CBER NGS Virus Reagents*) in high titer virus background
Spiking study 3 *Started in in 2019*	Transcriptomics: infected cells in uninfected cell background
Long-read NGS	
Spiking study 4 *Started in 2020*	5 viruses (*CBER NGS Virus Reagents*) in high titer virus background
Spiking study 5 *Started in 2024*	5 viruses (*CBER NGS Virus Reagents*) in high cellular background
Spiking study 6 *Started in 2024*	Transcriptomics: infected cellular transcriptome in uninfected cellular transcriptomic background

CBER/OVRR Efforts Towards NGS Implementation (Khan Lab)

- **AVDTWG collaborative studies**
  - Evaluate different NGS platforms to determine breadth and sensitivity of virus detection in different matrices

- **Development of virus reference stocks**
  - CBER NGS Virus Reagents to support NGS development (NIAID BEI cat no. NR-59622)
  - First WHO International Reference Panel for AV Detection in Biological Products (NIAID BEI cat. no. NR-59630) for NGS validation studies
  - Available for distribution to evaluate performance of NGS platforms

- **Generation of a comprehensive Reference Virus Database (RVDB)**
  - RVDBv30 is available at https://rvdb.dbi.udel.edu/ with link available for proteic RVDBs at Institut Pasteur (http://rvdb-prot.pasteur.fr/).

WHO International Reference Panel

- **Seven viruses established as "First World Health Organization International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing"**
  - Established based on a CBER collaborative study with participants from the AVDTWG
  - Characterization: Infectious titer, particle count, Genome copy number (ddPCR) and NGS.
  - Made specifically for qualification and validation studies (expects to be used as a panel of 7 viruses)

- **BEI Resource (Catalog #NR-59630)**
  - https://www.beiresources.org/Catalog/animalviruses/NR-59630.aspx
  - 1000 vials of the new virus stocks; general limit to 1 order per year.

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
Minute Virus of Mice (MVM)	DNA, single-strand; Linear	5.1 kb	26 nm	No	High
Human coronavirus (HCoV-OC43)	RNA; single-strand; Linear	30.7kb	80-120nm	Yes	Low

1st WHO International Reference Virus Panel for NGS AV Detection

		Particle size (nm)	Envelope	Genome topology	Genome size (bp/b)	Physical chemical resistance	
CBER Virus Reagents	Epstein-Barr virus type 1	122-180	YES	ds-DNA 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

1st WHO Intl Ref Virus Panel –*2024*

CBER Virus Reagents (previous WHO Intl Ref Reagents-2020): BEI cat. No. NR-59622 . *To support NGS development.*

Development of Reference Viruses*

❖**Characterization and Testing**

- Infectious titer per mL
- Number of particles
- Genome copy number: ddPCR
- Host DNA copy number: ddPCR (*different species*)
- NGS adventitious virus analysis
- Sterility and mycoplasma
- NGA reference virus genome sequence and variant analysis
- Stability studies: infectious titer; genome copy number

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

** Prepared by CBER at ATCC. Characterized at ATCC and Khan Lab*

Development of a New Reference Viral DataBase (RVDB)

- Work to create a new viral database for HTS data analysis was initiated in 2013

- Public databases had some limitations
  - NCBI Viral Genomes Resource (RefSeq and Neighbors) had under-representation of partial viral sequences and endogenous retroviruses
  - Nr/nt (non-redundant nucleotide collection) had viral diversity and included full and partial viral genomes but had abundant cellular and uncharacterized sequences and many were mis-annotated
  - NCBI protein database (non-redundant protein sequences) only contains complete protein sequence

- Limitations of the public databases were recognized by several including the Khan lab during discovery of the novel Sf9-rhabdovirus *(Ma et al., 2014*)

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

- 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 RVDBv30.0 (*June 9, 2024*) is available at https://rvdb.dbi.udel.edu/ with link for proteic RVDBs generated by Institut Pasteur (http://rvdb-prot.pasteur.fr/).

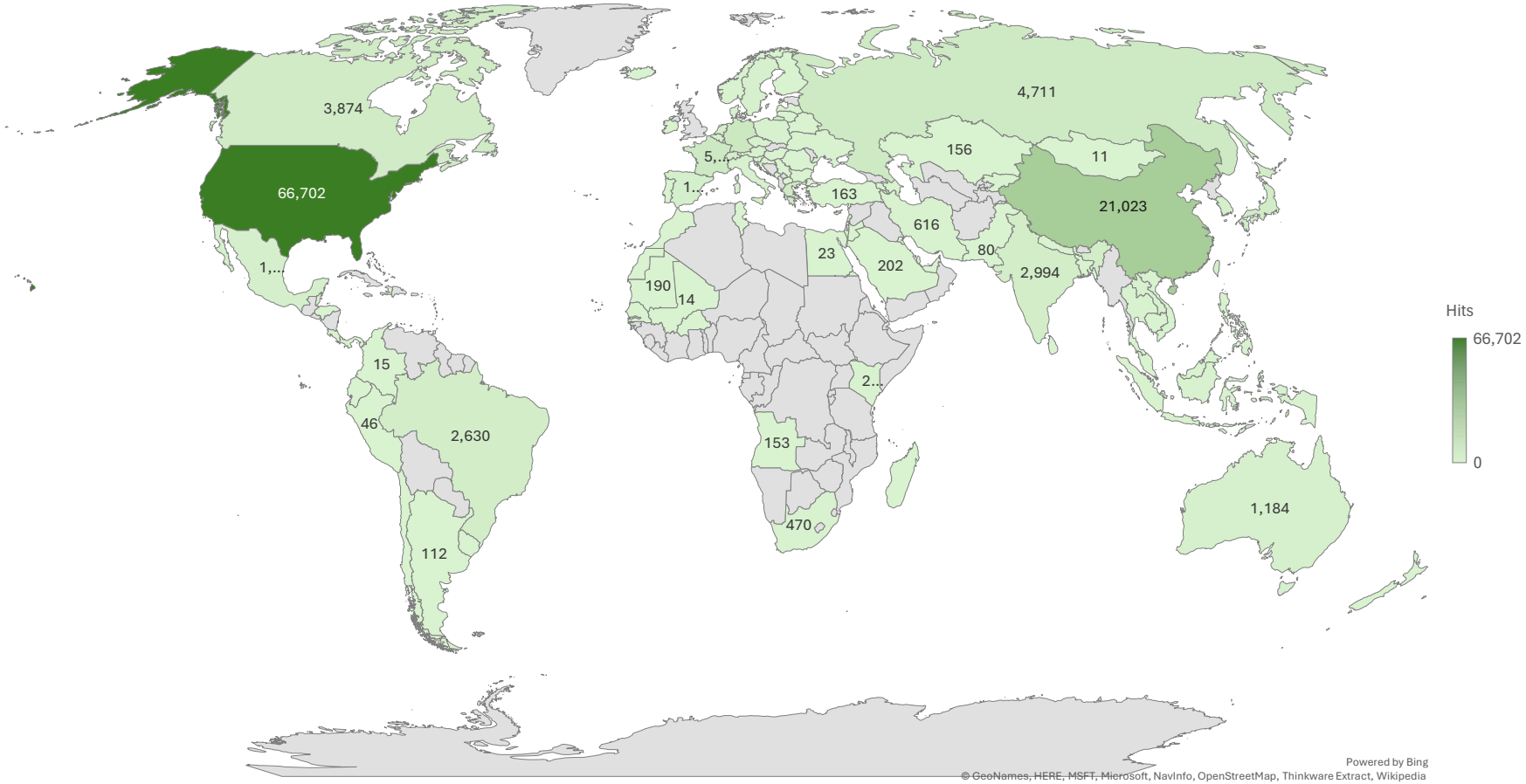
- *Provides high diversity of viral sequences to increase likelihood of novel virus detection, with reduced nonspecific cellular hits resulting in less data volume for bioinformatics analysis (and less computational time!)*
- *Updated twice a year by the Khan Lab (Pei-Ju Chin)*
- *Ongoing work on annotation of sequences to remove misannotated sequences and enhance virus-specific detection*

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***

Growing RVDB Community

RVDB User Demography by Hits (Jan – Sept 2025)



General Acceptance of NGS for Adventitious Virus Detection in OVRR

➢ **Replacement assay (*in vivo* assays; PCR assays)**

- *In vivo* Adventitious Virus detection assays and Antibody Product assay (MAP/HAP/RAP)– NGS can provide defined sensitivity and breadth of virus detection
  - *Reduce use of animals and meet the global objectives for 3Rs*
- PCR assays – NGS can have similar sensitivity than PCR assays; broader virus detection; single assay

➢ **Supplementary or Replacement assay (*in vitro cell culture* 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 confirm a true viral signal*

General NGS Follow Up Strategy

- Follow up of NGS signal to determine biological relevance and significance for decision-making
  - Calling criteria for including or excluding unexpected hit(s)
  - 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?*

General NGS Expectations in *OVRR* Submissions

- Use of references viruses: WHO Reference Virus Panel or equivalent
- Use of non-targeted NGS bioinformatics analysis using RVDB or equivalent for broad virus detection for replacing general virus detection assays (*in vivo* and *in vitro*)
- The method should be appropriately validated for its intended use. This includes the method **validation** and **matrix-specific qualification/verification** using suitable reference materials such using virus standards and a comprehensive viral database to demonstrate the limit and breadth of virus detection.
- Submit detailed reports and SOPs

❖A head-to-head comparison of NGS with existing methods is not required for *in vivo* assays.

➢ *Ph.Eur. General Chapter on HTS (expected pub. Oct. 2025):* on "High Throughput Sequencing for the detection of extraneous agents"

- Provide details on important considerations when implementing a NGS for adventitious virus detection assay
- A strategic frame-work for the NGS validation approach

Introducing HTS 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 guidelines 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): (Nov. 2023)*

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.

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 (verification, genetic stability of vaccine virus)
- **Characterization of viral vector sequences**
  - Identity testing
  - Persistence, Expression
  - Vector integration site analysis

Still More Work for Routine Implementation!

- Continued in-house efforts and AVDTWG efforts for evaluating performance of NGS in different matrices
- Optimization of pre-treatment conditions to increase sensitivity of virus detection in complex matrices (NIIMBL/BMG project)
- Development of SOPs and reference datasets for broader NGS implementation
- Development of other types of standard materials (e.g., VLPs, virus-infected cell lines, synthetic)
- Database enhancements for increasing accurate virus detection and reducing follow up of false positive signals

References

- **FDA Guidance for Industry:** Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications (February 2010)
  - "You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations"
- **WHO:** Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks, Annex 3, TRS No 978 (adopted 2010; revised May 2023)
  - Risk assessment strategy and new methodologies (*e.g.,* NGS)
- **Ph. Eur. Chapter 5.2.3** (Testing of vaccine cell substrates) **and Chapter 2.6.16 (**Tests for extraneous agent testing of viral vaccine seed lots/harvests (revised 2017)
  - **Test for specific viruses by NAT** (*e.g.,* PCR)
  - **Test for viruses using broad molecular methods** (*e.g.,* HTS) either as an alternative to in vivo tests and specific NAT, or as a supplement/alternative to in vitro culture tests based on the risk assessment.
- **Ph. Eur. Chapter 5.2.14**
  - Considerations for the **substitution to replace in vivo methods by broad molecular methods**
- **ICH Q5A(R2):** Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (original 1999; revised November 2023)
  - **Encourages use of new alternative tests (NGS/ HTS)** to replacing or supplementing the in vivo and in vitro adventitious virus detection assay, antibody production assays, and NATs (*e.g.,* PCR)

References (cont.)

- First WHO International Reference Panel for adventitious virus detection in biological products by high-throughput sequencing. In: **WHO Expert Committee on Biological Standardization**: seventy-ninth report. Geneva. World Health Organization; 2024: section 7.1.; pp. 29–31. WHO Technical Report Series, No. 1059
- **European Pharmacopoeia. (Draft) General Chapter 2.6.41**: High-throughput sequencing for the detection of viral extraneous agents. European Pharmacopoeia. 11th ed. Strasbourg: European Directorate for the Quality of Medicines & HealthCare, Council of Europe.
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). **ICH Q2(R2)** Guideline on validation of analytical procedures. 2023.

Thank You!