

Impact of COVID-19 on Next Generation Sequencing Applications for Adventitious Virus Detection in Vaccine Manufacturing

Arifa S. Khan, Ph.D.

Division of Viral Products

Office of Vaccines Research and Review

Center for Biologics Evaluation and Research

U.S. Food and Drug Administration

January 28, 2021

Overview

- Sources of viral contamination and viral risk mitigation
- Current recommendations for adventitious virus detection and limitations
- Need for new technologies
- Considerations for NGS applications
- FDA and other efforts for NGS implementation
- Acceleration of NGS applications for adventitious virus testing
 - *COVID-19 Era*

Origins of Adventitious Virus Contamination in Biologics

- ❑ **Viruses in donor species of source materials** (*virus seed, cell substrate, or animal-derived cell culture reagents*)
 - Naturally-occurring exogenous and endogenous viruses
 - Acquired by specific-exposure to infectious agents (e.g., animal vaccines, environmental)

- ❑ **Cell substrate passage history**
 - Cross-contamination in previous facilities (*if applicable*)
 - Propagation in different labs
 - Other viruses or cell lines used
 - Raw materials (serum, trypsin, etc.)
 - *De novo* generation of novel recombinant viruses

- ❑ **During product manufacturing**
 - Potential introduction due to: Handling; Equipment; Raw materials
 - Potential activation of endogenous retroviruses or reactivation of latent DNA viruses due to production conditions

Integrated Strategy for Adventitious Virus Risk Mitigation

☐ PREVENTION

- **Risk assessment-** Identify potential sources of virus introduction
 - 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
 - Viral inactivation and removal
 - Product purity: reduction of residual cellular materials (DNA, RNA, proteins)

☐ TESTING

- **Known and unknown agents** in the starting materials (cell substrate, virus seeds, vector virus)
- **Different stages** in the manufacturing process and at steps with the greatest potential for contamination
- **Sensitive and broad detection assays**

Viral Vaccines and Adventitious Virus Testing

Comprehensive adventitious virus testing is recommended for all viral vaccines grown in a cell substrate

- Live-attenuated virus
- Recombinant-vector virus
- Virus-like particle
- Protein subunit

Exception

- *Whole-inactivated virus*

Conventional Adventitious Virus Tests

❖ *In vitro* assays

- *In vitro* AV assays in cell lines of 3 species (same as cell substrate, monkey, and human)
- Transmission electron microscopy (TEM)
- Reverse transcriptase assay for retroviruses (PERT)
- Tests for animal viruses e.g. bovine, porcine (9CFR 113.47 and 113.53) (*if applicable*)

❖ *In vivo* assays

- *In vivo* AV assays (adult mice, suckling mice, embryonated hens' eggs)
- Antibody-production assays for rodent viruses (MAP including LCMV challenge, HAP, RAP) (*if applicable*)
- Assays for known viruses (PCR, DNA hybridization, Infectivity, antibody detection)

❖ Additional assays (CBER/OVRR: recommended case-by-case, *if applicable, e.g. novel cell substrate*)

- Extended PCR assays
- Oncogenicity assays: Tumor-inducing viruses
- Chemical induction assays: Endogenous retroviruses, latent DNA viruses

Currently recommended adventitious virus tests have some limitations

❑ Conventional assays for routine testing

- **Cell-culture assays** are based upon on virus replication resulting in a visible effect, such as cytopathic effect (CPE) or hemadsorption / hemagglutination
- **Animal-based assays** are based on virus infection producing a measurable pathological effect due to a replicating virus
- **Molecular assays** are designed based upon available known virus sequences

❑ Additional Testing for novel cell substrate

- **Oncogenicity assays** does not have positive controls
- **Chemical induction** can activate latent viruses, but detection of induced, unknown viruses would be missed due to using the conventional methods for virus detection

Next Generation Sequencing has Capabilities for Detection of Known and Novel Viruses

NGS (or high-throughput sequencing) is an advanced nucleic acid-based technology for broad virus detection, without prior sequence knowledge

- 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)

NGS has Demonstrated Potential for Adventitious Virus Detection in Biologics

- ❑ Potential applications of NGS in biologics for detection of known and novel viruses 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.*

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

FDA Efforts on NGS for Applications in Biologics

- Establishment of FDA and CBER Genomic WGs to support a research and regulatory infrastructure to support policy development and decision-making related to applications of NGS
- Strengthen in-house FDA laboratory and bioinformatics expertise for NGS analysis
- Establishment of the FDA/Industry led PDA-Advanced Virus Detection Technologies Interest Group (AVDTIG) with focused efforts on NGS standardization and implementation for adventitious virus detection in biologics (*PDA J Pharm Sci and Tech 2016, 70 591-595*)

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 next generation sequencing for viral risk evaluation by providing an informal, scientific forum for discussions and scientific collaborations

Co-chairs

- Arifa Khan: FDA, U.S.A.
 - Dominick Vacante: Janssen R & D, U.S.A.
 - Jean-Pol Cassart: GSK, Belgium
 - Keisuke Yusa: National Institute of Health Science, Japan
- **More than 180 participants : industry (*vaccines, cell and gene therapies, 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
 - Sample preparation and processing
 - Standards

AVDTIG - Subgroups

- **Subgroup A**
Sample selection/ preparation/processing Siemon.Ng@sanofipasteur.com
- **Subgroup B**
Virus standards and reference materials Jean-Pol.Cassart@gsk.com
- **Subgroup C**
Complete and correctly annotated, virus reference database Arifa.Khan@fda.hhs.gov
- **Subgroup D**
Bioinformatics pipelines analysis Christophe.G.Lambert@gsk.com
- **Subgroup E**
Follow-up strategies to confirm the identity of a “hit” Robert.Charlebois@sanofipasteur.com

Study leaders

Collaborative studies

- Evaluation of different NGS platforms
- Determination of LOD for different virus types in matrices mimicking virus seed/vector virus preparation, cell substrate, bulk harvest

Development of NGS Reference Virus Stocks for NGS evaluation and standardization

➤ Based on previous data (*Khan et al., 2017, mSphere*) and discussions in AVDTIG, efforts were initiated by the Khan Lab for large-scale preparation of five Reference Virus Stocks at ATCC

❖ Well-Characterized






- Infectious titer
- Viral genome copy number
- Reference virus genomes
- Residual host cell nucleic acids

❖ Stability studies

- 24-month

❖ Vialled individually

- Freedom for custom-mixing by user

		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

Availability of NGS Reference Virus Stocks

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 for virus spiking studies in the AVDIG regarding NGS standardization (*including Khan Lab*)
- Provided upon request for NGS for NGS qualification and validation studies for adventitious virus detection
- Established as **WHO international virus reference standards** for adventitious virus detection by NGS (*ECBS, Oct. 2020*)
- Contact: arifa.khan@fda.hhs.gov

Development of a Reference Viral Database (RVDB) to enhance NGS bioinformatics analysis for novel virus detection

- Need for a comprehensive viral database was recognized and efforts were initiated in the Khan lab to create RVDB to include sequences of all virus species from eukaryotes (based on cell substrates used in biologics), regardless of size (*RVDB v10.2: Goodacre et al., mSphere, 2018*)
- RVDB was found to be more sensitive and specific for virus detection and is expected to enhance NGS investigations for product safety by increasing efficiency of virus detection, particularly for novel viruses
- The reduced nonspecific cellular hits resulting in less data volume for bioinformatics analysis and less computational time!

RVDB Current Status

- Available publicly at the University of Delaware
 - <https://rvdb.dbi.udel.edu/>
 - Updated quarterly and coincident with a GenBank update along with an annotation file to aid in detection of emerging viruses (*Pei-Ju Chin, Trent Bosma – Khan Lab*)
 - Efforts to refine RVDB were initiated with v19.0
- **RVDBv20.0 and Annotation File**
 - Available since Sept 3, 2020
 - Based on GenBank Aug. 2020 release version 239 and RefSeq Aug. 2020 release version 201
 - Number of sequences
 - Unclustered RVDB= 3,180,577 (with 16,236 sequences of SARS-CoV-2, compared with 1,013 in v19.0)
 - Clustered RVDB= 781,728
 - Link to Proteic RVDB is available: <https://rvdb-prot.pasteur.fr/> (*Marc Eloit and Thomas Bigot; Bigot et., F1000Research, 8:530, 2019*)
- **RVDBv21.0 and updated Annotation File is expected to be available in early Feb. 2021**

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

- **Oct. 2017: IABS/FDA Conference** on NGS for Adventitious Virus Detection in Biologics. Applications for human vaccines and lessons learnt from veterinary vaccines. (*Meeting Report: Khan et al., Biologicals, 2018*)
- **Sept. 2019: NIST/FDA Workshop** on Standards for NGS Detection of Viral Adventitious Agents in Biologics and Biomanufacturing. (*Meeting Report: Cleveland et al., Biologicals, 2020*)
- **Nov. 2019: IABS 2nd Conference** on NGS for Adventitious Virus Detection in Human and Veterinary Biologics. Standardization, validation and readiness of NGS. (*Meeting Report: Khan et al., Biologicals, 2020*)

Potential Applications of NGS for Adventitious Virus Detection in Biologics

- Strategy to mitigate risk of AV introduction
 - Raw materials used for cell culture
 - Cell banks
 - Virus seeds

- Monitor/test absence of AV during production
 - Bulk harvest
 - Final product

NGS for Virus Detection in Biologics

➤ Substitution/Replacement assay

- ***In vivo AV assays*** – NGS can provide defined sensitivity and breadth of virus detection
 - Reduce use of animals – meet 3R's objectives
 - Minimum numbers for 1 Test Article = 30 eggs; 20 adult mice; 40 suckling mice
 - Reduce testing time - months to days
- **PCR assays** – NGS can have similar or greater sensitivity than PCR assays
 - Single assay with broader virus detection

➤ Supplementary assay

- **Cell substrate characterization** – particularly in case where there are concerns for occult and novel viruses
- ***In vitro AV assays*** – can shorten testing time from weeks to days
 - In case of assay interference due to lack of effective neutralization of vaccine virus
 - As a read-out assay for early and broad virus detection

➤ Follow-up of a positive result is critical

- Determine biological relevance and significance (as with any nucleic acid-based assay)
- NGS data can aid in design of a “custom” infectivity assay to determine if signal due to infectious virus

Introducing NGS for Improving Viral Safety Testing

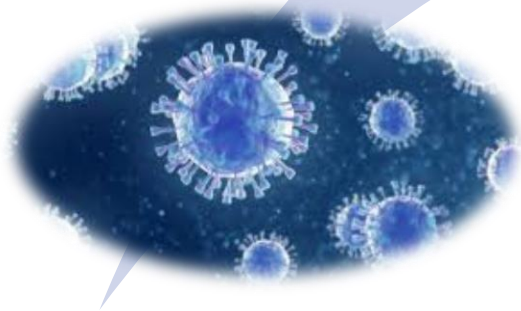
- Increased efficiency (reduce number of tests, effort, time)
- Ethical (reduce animal use)
- Superiority (LOD, specificity, repeatability, accuracy)

- ❖ 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 revision)

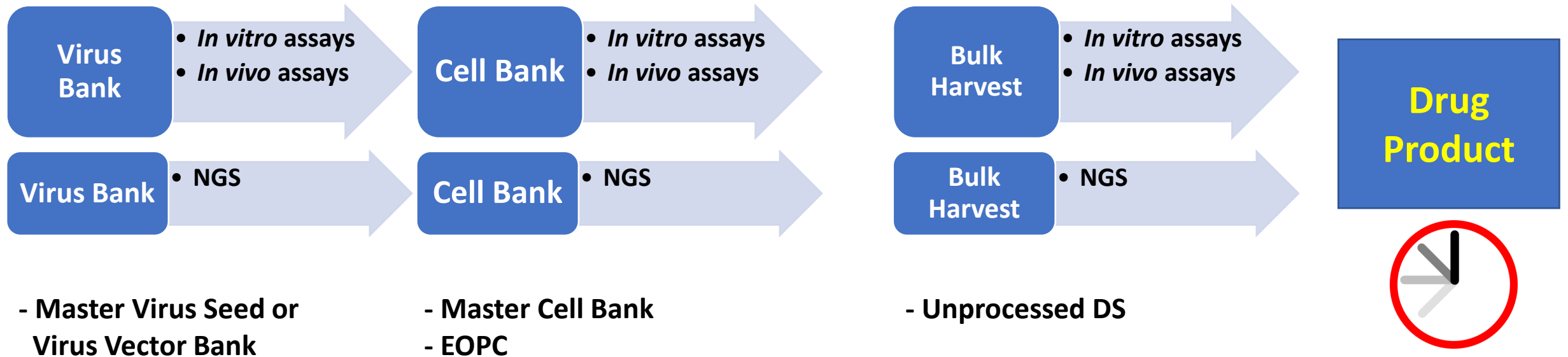
Acceleration of Ongoing Efforts Facilitated Rapid Development of SARS-CoV-2 Vaccines

- > Vaccine platforms
- > Manufacturing processes
- > Diagnostic assays
- > New technologies for viral safety testing

Next Generation Sequencing
(High-throughput Sequencing)



Increased Efficiency of Product Development by NGS



- ❖ NGS can be a **“One-Stop-Shop”** as an alternative assay for the current AV testing
- ❖ The production timeline may be accelerated by **6-9 weeks (current estimations)**
 - Based on the sample type and bioinformatics analysis pipeline (*targeted vs agnostic, known vs unknown viruses, low-vs high-level of virus*)
 - Ongoing efforts on optimization of bioinformatics (pipelines and databases)

Introducing New Assays for Regulatory Applications

❑ Assay qualification and/or validation

- Development of relevant controls (*positive and negative*)
- Determination of sensitivity and specificity
- Demonstration of precision (*reproducibility and repeatability*)
- Evaluate assay robustness (*change of assay conditions and reagents*)
- Demonstrate the reliability of the assays (e.g. interference of sample matrix to the assay's intended use)
- More details on the validation of analytical assays and statistical analyses are described in ICH Q2(R1)

❑ Availability of assay (establishment of method in-house or through CROs)

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

- OVRR has been receiving NGS submissions for using NGS as a broad adventitious virus test
- 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 -> Substitution of one or more conventional virus detection assays

NGS Review and Ongoing Efforts in OVRR

- NGS may be considered for supplementing or substituting the conventional assays for adventitious virus detection based upon justification for suitability and fit for purpose
- NGS data is considered on a case-by-case basis
 - Efforts are directed toward standardization and validation of NGS
- Within OVRR we highly recommend that sponsors request a technical working group discussion related to the use of NGS and their product characterization
 - Non-regulatory meeting to discuss “plans” for use of NGS
 - Reach consensus prior to initiating lengthy, expensive studies
- In-house efforts for broad implementation of NGS
 - Development of viral standards and pipelines for adventitious virus detection
 - Generation of databases that are complete and correctly annotated to facilitate virus-specific detection
- Continue international discussions to harmonization of requirements for NGS

Acknowledgements

- Khan Lab*
 - Pei-Ju Chin
 - Trent Bosma
 - Sandra Fuentes
 - Hailun Ma
 - Andrea Kennard
 - Nicholas Mattson
 - *Norman Goodacre*
 - *Subhiksha Nandakumar*
 - *Aisha Al-janahi*
 - *Eunhae Hannah Bae*
- FDA/CDRH (HPC Team)
 - Mike Mikailov; Fu-Jyh Luo
- FDA/OVRR
 - Robin Levis; Marion Gruber
- Univ. Delaware
 - Shawn Polson
 - Karol Miaskiewicz
 - Jaysheel Bhavsar
 - Kelvin Lee
- Advanced Virus Detection Technologies Interest Group (AVDTIG)
 - Subgroup Leaders & IG Members
 - Subgroup AB: Siemon Ng and Jean-pol Cassart
- *NCBI / NIH*
 - *Rodney Brister*

Thank you!