General Presentation
General Overview of the NGS

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- Carine Logvinoff is a Sanofi employee and may hold shares and/or stock options in the company
- Siemon Ng is a Notch Therapeutics employee and a former employee of Sanofi and may hold shares and/or stock options in both companies
- The content and views expressed in this presentation are those of the individual experts participating to the IABS NGS conference and should not be construed to represent the views or policy of any organization, regulatory authority, or governmental agency.
- All criteria, tools or ways of working described must be considered as “considerations” only and should not be taken as recommendations, mandatory use or regulatory views.

Viral Contamination Events in Biologicals and Viral Safety Principles

- Contaminations could have many negative effects :

  Illness or injury due to a contaminated product
  Shortage of drugs or vaccines
  Manufacturing facilities shut down
  Consumer distrust

- Lessons learned from past contaminations :

  Safer Practises
  Banking System
  Characterisation of starting materials
  Raw materials treatments and testing
  Improved testing package for adventitious virus

VIRAL SAFETY

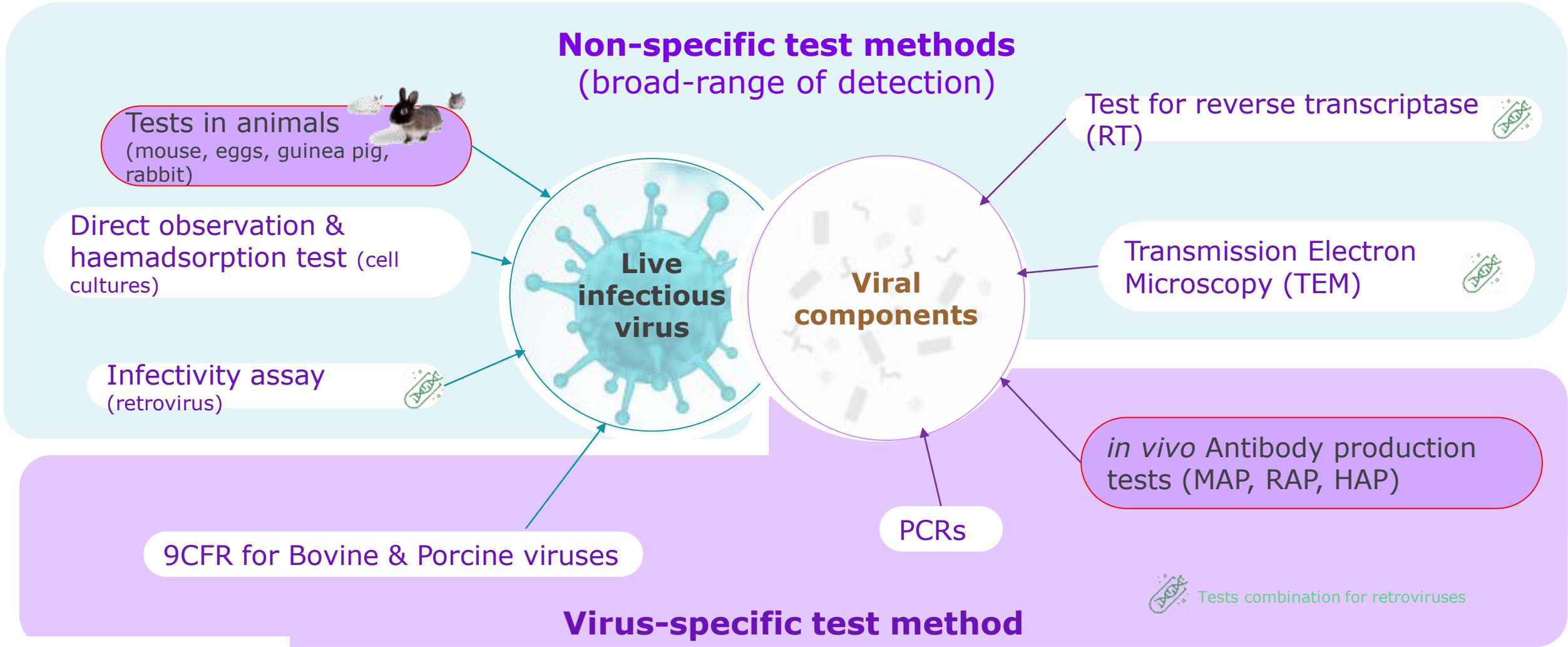
Selecting and testing cell lines and other raw materials, including media components, for the absence of undesirable infectious viruses

Assessing the capacity of the production processes to clear infectious viruses;

Testing the product at appropriate steps of production **for the absence of contaminating infectious viruses**

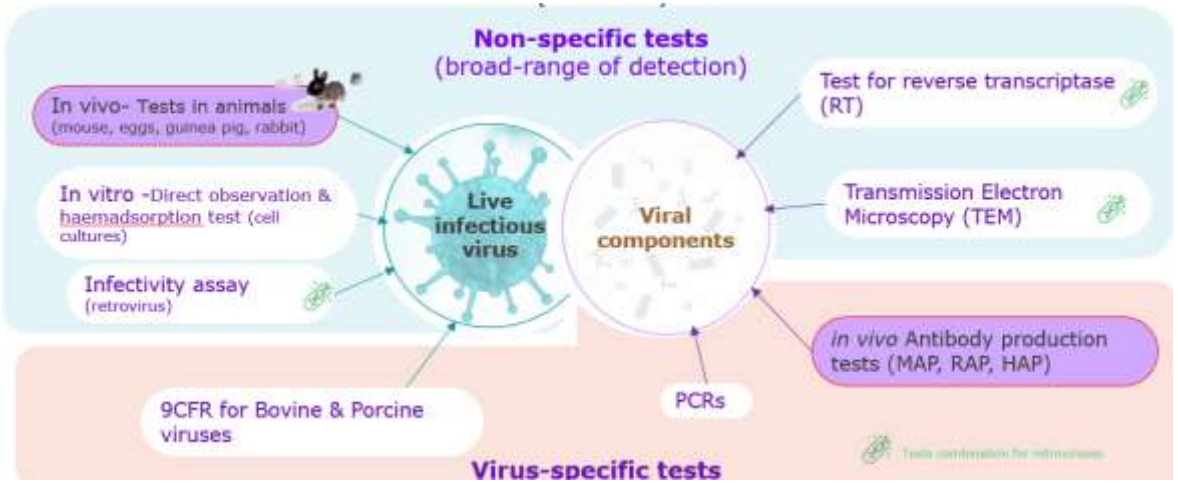
How to Detect Viral Contaminants

- "Traditional" test methods [before 2010]



Some Limitations of the Traditional Testing Package

- For non-specific tests



Test	***Test Read out***	*To be detected, the contaminant virus has …*
In vivo – Tests in animals	Compromised animal model health	to replicate in the animal model & to alter animal health
In vitro - Direct observation & haemadsorption (HAD) test	CPE and/ or HAD on indicator cells	to replicate on indicator cells & to induce CPE or HAD

Vaccine 32 (2014) 2916–2926

Contents lists available at ScienceDirect

Vaccine

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

Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products☆

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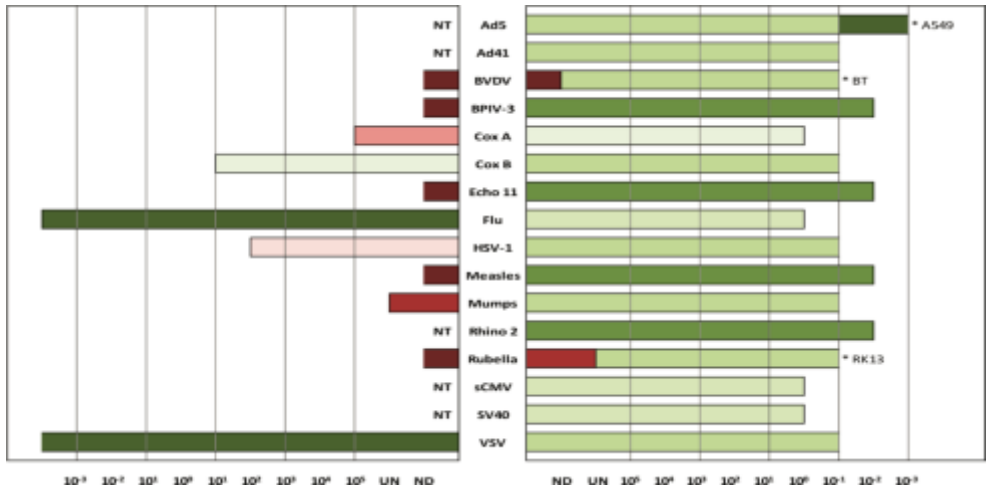
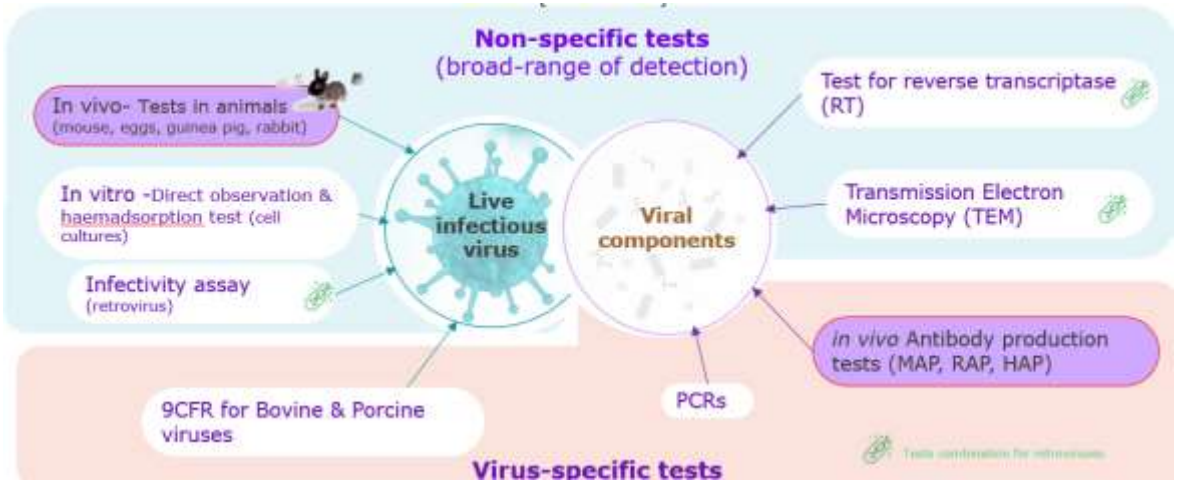


Some Limitations of the Traditional Testing Package

- For virus specific tests

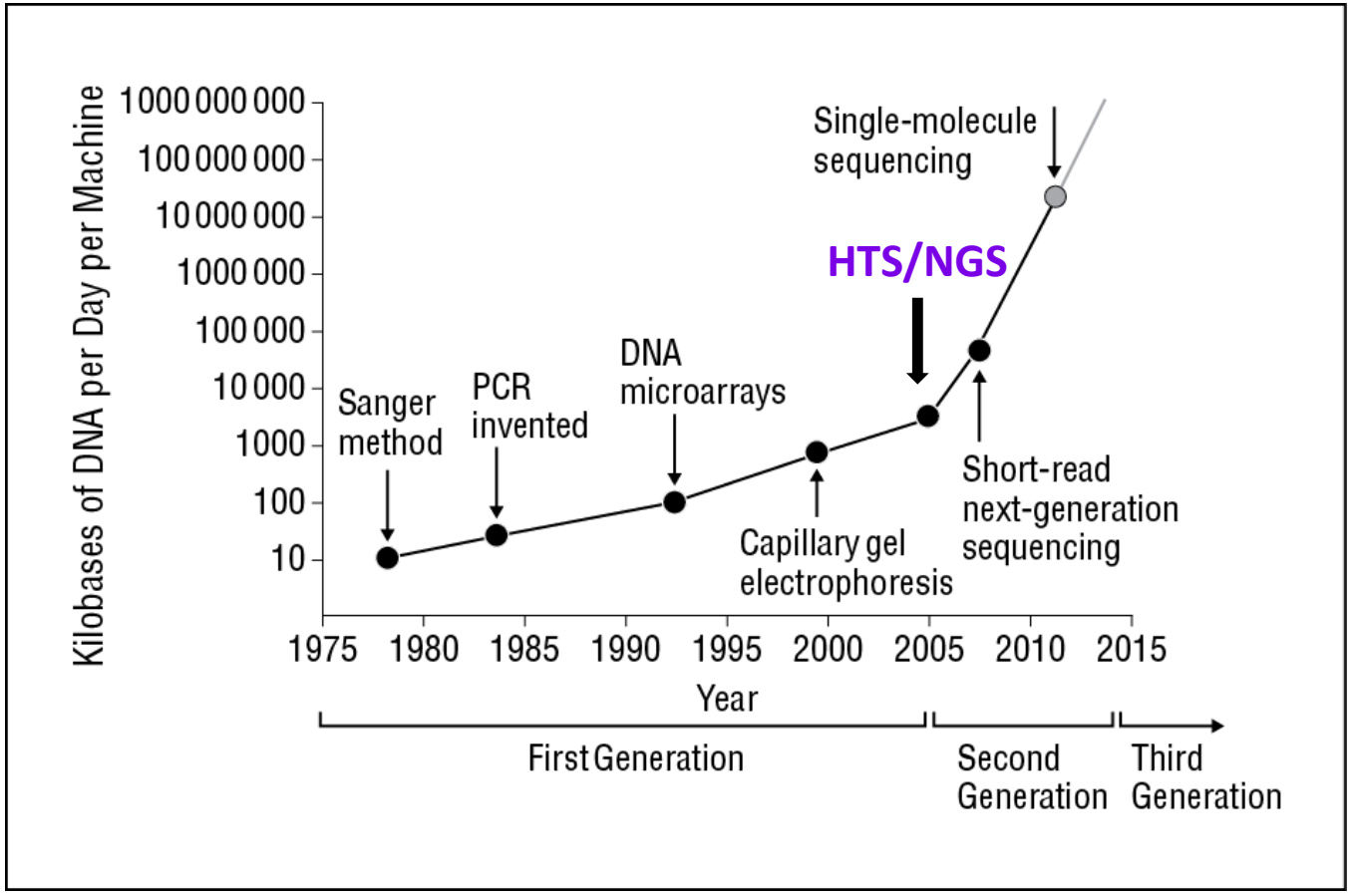


- Need to select **which virus** to specifically look for

=> Viral Risk Assessment is to be conducted to define the adventitious testing package, and the specific testings

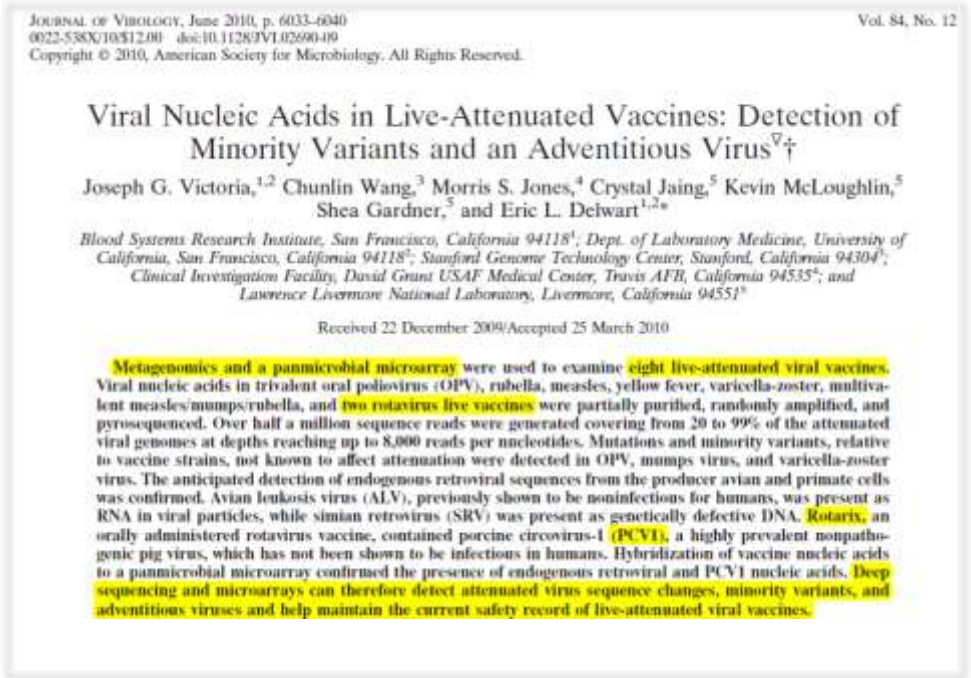
- Viral variants are to be considered, especially when designing a specific test such as PCR

Emergence of High Throughput / Next Generation Sequencing Technologies



JAMA Neurol. 2013;70(6):696-702. doi:10.1001/jamaneurol.2013.2068

A *Noisy* Entry of NGS for Adventitious Virus in the Vaccine World



JOURNAL OF VIROLOGY, June 2010, p. 6033–6040 Vol. 84, No. 12
0022-538X/10/$12.00 doi:10.1128/JVI.02690-09

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] and Eric L. Delwart[1,2]*

Blood Systems Research Institute, San Francisco, California 94118[1]; Dept. of Laboratory Medicine, University of California, San Francisco, California 94118[2]; Stanford Genome Technology Center, Stanford, California 94304[3]; Clinical Investigation Facility, David Grant USAF Medical Center, Travis AFB, California 94535[4]; and Lawrence Livermore National Laboratory, Livermore, California 94551[5]

Received 22 December 2009/Accepted 25 March 2010

Metagenomics and a panmicrobial microarray were used to examine eight live-attenuated viral vaccines. Viral nucleic acids in trivalent oral poliovirus (OPV), rubella, measles, yellow fever, varicella-zoster, multivalent measles/mumps/rubella, and **two rotavirus live vaccines** were partially purified, randomly amplified, and pyrosequenced. Over half a million sequence reads were generated covering from 20 to 99% of the attenuated viral genomes at depths reaching up to 8,000 reads per nucleotides. Mutations and minority variants, relative to vaccine strains, not known to affect attenuation were detected in OPV, mumps virus, and varicella-zoster virus. The anticipated detection of endogenous retroviral sequences from the producer avian and primate cells was confirmed. Avian leukosis virus (ALV), previously shown to be noninfectious for humans, was present as RNA in viral particles, while simian retrovirus (SRV) was present as genetically defective DNA. **Rotarix**, an orally administered rotavirus vaccine, contained porcine circovirus-1 **(PCV1)**, a highly prevalent nonpathogenic pig virus, which has not been shown to be infectious in humans. Hybridization of vaccine nucleic acids to a panmicrobial microarray confirmed the presence of endogenous retroviral and PCV1 nucleic acids. **Deep sequencing and microarrays can therefore detect attenuated virus sequence changes, minority variants, and adventitious viruses and help maintain the current safety record of live-attenuated viral vaccines.**

- March 2010 : HTS revealed the presence of Circovirus in the final product of a commercial vaccine.
- May 2010 : VRBPAC Meeting
- Aug 2010 : FDA letter to licensed vaccine manufacturers

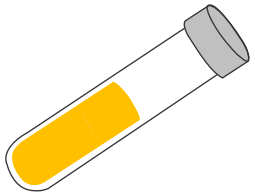
> Please describe any plans you may have to implement additional adventitious agent testing methods as part of your manufacturing process as these methods become available including, but not limited to, screening for PCV and PCV DNA as well as any additional in-process testing for adventitious agents that you may have recently added, but not reported to the agency. In this regard, please consider any animal derived materials (e.g., culture medium, albumin, enzymes, lipids, etc) and the point at which they are used in your product manufacture, any adventitious agent related quality control testing performed by the material vendor or done in-house, and any applicable viral clearance or inactivation steps provided by your manufacturing process.

- The conventional testing package could miss viral contamination.
- Next Generation Sequencing (NGS) has the potential to identify both known and unknown adventitious viruses by sequencing all the nucleic acid within a sample without needing prior knowledge of the contaminating agents whatever the virus.

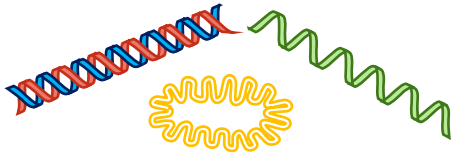
General NGS Workflow

- Next Generation Sequencing is a technology capable of sequencing all the nucleic acid extracted from a biological sample and to detect any potential viral contaminations.

Sample



Nucleic Acid Extraction



Sequencing Library preparation

Sequencing

Bioinformatics Analysis

Follow-up analysis

Considered Uses of NGS for Adventitious Virus Detection

Uses of Next Generation Sequencing (NGS) 	Investigational Tool	Characterization / Screening studies (de-risking)	Specification test for release	Specification test for release & **compendial test substitution**
Tested Stage	NGS as « read out » at any stage	preMCB, preMSL, raw materials, vaccine substrates (e.g. eggs)	MCB, WCB, ECB MSL, WSL (DS)	
Analytical Quality Environment	Research only	Fit for purpose (qualification)	GMP ICH validation package	GMP ICH validation package + **Comparison bridging study**

NGS as Specification Test in Substitution of *in Vivo* Tests

01/2018:50214

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

... The implementation of such new molecular methods as substitutes for in vivo methods requires ***a comparison of the specificity (breadth of detection) and the sensitivity of the new and existing methods****. For this purpose,* ***an appropriate panel of representative, well-characterised model viruses*** *should be used to assess the ability of the new method to detect viruses that are (or are not) detected by the in vivo methods, and to determine if the sensitivity is at least equivalent to the sensitivity of the in vivo methods. ...*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Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products☆

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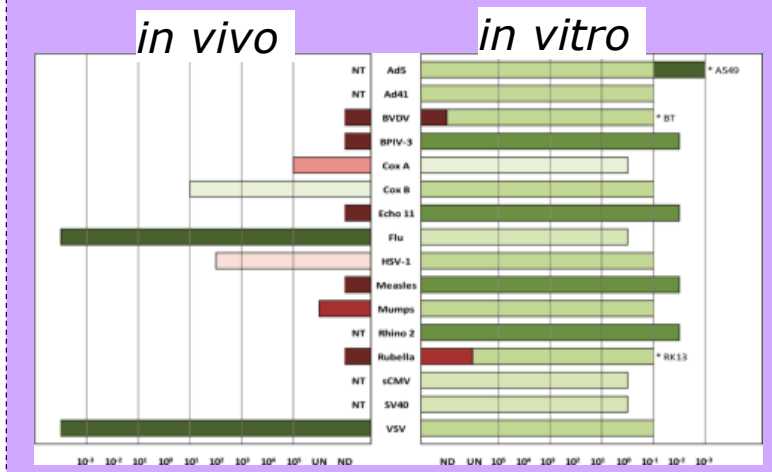
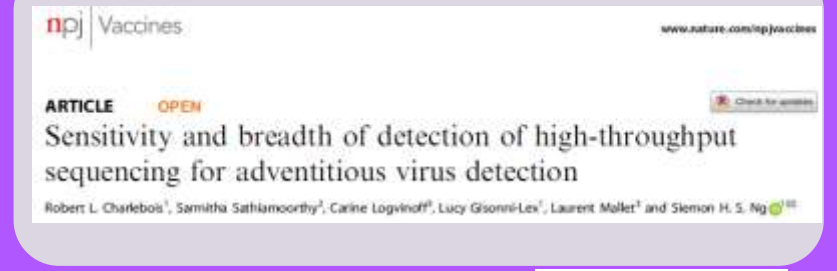
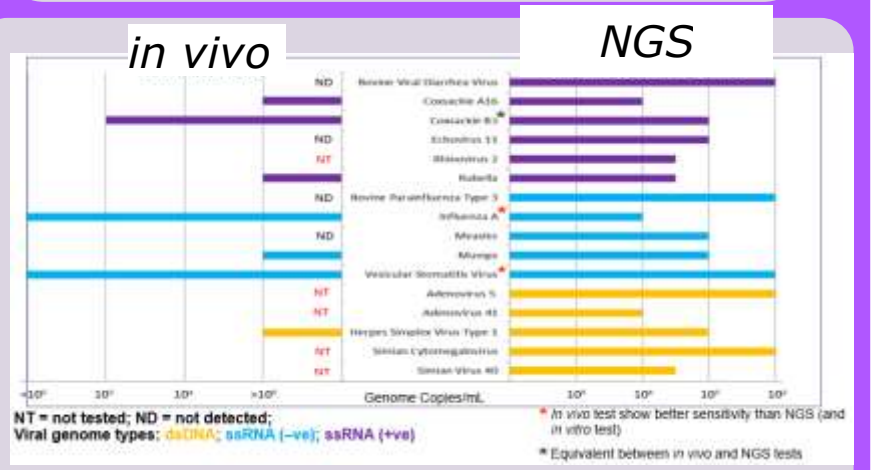


NIH assessed the sensitivity of *in vivo* vs. *in vitro* tests by using a panel of 16 viruses across 9 viral families (Gombold *et al*, Vaccine, 2014)


npj Vaccines
www.nature.com/npjvaccines
ARTICLE OPEN

Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection

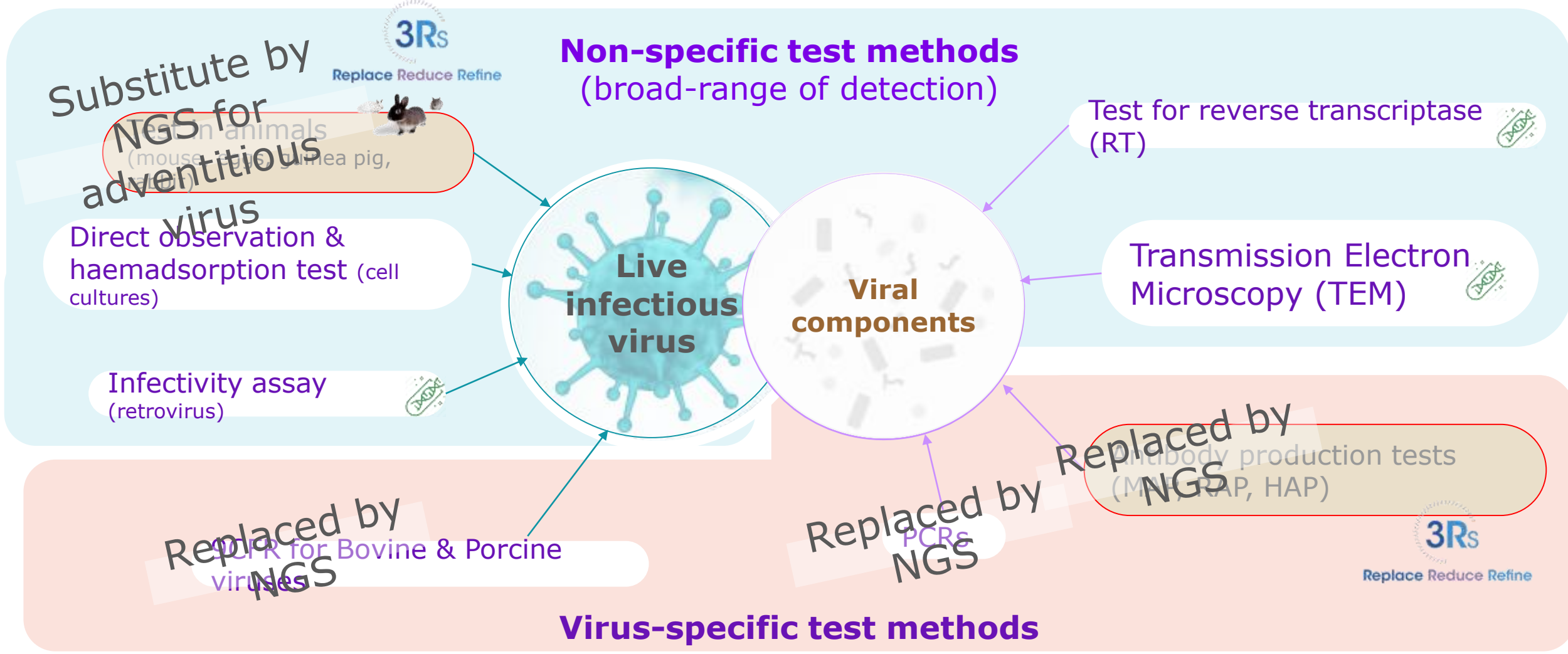
Robert L. Charlebois[1], Samitha Sathiamoorthy[2], Carine Logvinoff[3], Lucy Gisonni-Lex[1], Laurent Mallet[3] and Siemon H. S. Ng[3]



Use of the same panel of viruses to compare NGS to *in vivo* data (Charlebois *et al*, npj vaccine, 2020) => This data package supports the substitution of *in vivo* test by NGS for adv virus detection test, as recommanded within Ph.Eur. 5.2.14

NGS streamlines the adventitious virus testing package

A "current" test methods package



Regulatory Environment Evolution

- **WHO**

  Technical Report Series on Cell substrates, Yellow Fever vaccine, Dengue Vaccine, IPV (since 2012)

  "[…]high-throughput sequencing[…]to supplement existing methods, or as alternatives methods to both in vivo and in vitro tests , after appropriate validation and approval by NRA"

- **FDA**

  PDA/FDA Advanced Virus Detection Technologies Interest Group (AVDTIG/ AVDTWG) (since 2012)

- **ICH** Q5A (R2) : Viral Safety Evaluation Of Biotechnology Products (Nov 2023)

  "3.2.5.2 Next Generation Sequencing […] can replace the in vivo tests […] without a head-to-head comparison […] may also be used without a head-to-head comparison to supplement or replace the in vitro cell culture assays"

- **Chinese Pharmacopea** (2025)

  Requirements for Preparation and Quality Control of Animal Cell Substrates Used for Production of Biologics (draft)- aligned with ICHQ5A (R2)

- **European Pharmacopoeia**

  Notion of Viral Risk Assessment to build the adv. virus testing package , *and "HTS may be used either as an alternative to in vivo tests and specific NAT or as a supplement/alternative to in vitro culture tests"*

  - Ph. Eur. Chapter 5.2.3: "Cell Substrates for the production of vaccines for human use" (2017)
  - Ph. Eur. Chapter 2.6.16: "Tests for extraneous agents in viral vaccines for human use" (2017)
  - Ph. Eur. Chapter 2.6.41: "High Throughput Sequencing for the detection of viral extraneous agents" (draft 2024)

new

NGS for Adv. Virus Detection to substitute *In vivo* test

Yesterday…

Specification test for release & compendial test substitution

MCB, WCB, ECB
MSL, WSL (DS)

Ph. Eur 5.2.3
Ph. Eur. 2.6.16
Ph. Eur 5.2.14

GMP ICH validation package + **Comparison bridging study**

ICH HARMONISED GUIDELINE

VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN

Q5A(R2)

3.2.3 In Vivo Assays

NGS is encouraged as a replacement for *in vivo* assays because of the breadth of viruses it detects and because its use promotes the global objective to replace, reduce, and refine the use of animal testing. Use of NGS to replace *in vivo* assays may be justified by submitting a validation package. Based on risk assessment and on the overall testing strategy, the use of the

Charlebois *et al*, NPJ Vaccine 2020


Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection

Beurdeley-Fehlbaum *et al*, Vaccine 2023

….*et al*, 2024/2025

… Today

Specification test for release & compendial test substitution

MCB, WCB, ECB
MSL, WSL (DS)

ICH Q5A (R2)
Ph. Eur. 2.6.41
Ph. Eur 5.2.3
Ph. Eur. 2.6.16

GMP
ICH validation package
w/o head to head comparaison

(AVDTWG)

Formation of FDA/Industry led interest Group – PDA sponsored : **Advanced Virus Detection Technologies Interest Group** (AVDTIG)

World Health Organization

International Reference Standards for Adv Virus Detection in Biological Products by NGS (CBER-5)

World Health Organization

First World Health Organization International Reference Panel for Adventitious Virus Detection in Biological Products by HTS

2010 | 2012 | 2017 | 2020 | 2022 | 2023 | 2024

HTS revealed Porcine Circovirus in a commercial vaccine (Victoria *et al* , 2010)

edqm

Ph Eur revision / creation

5.2.3 Cell Substrates for the production of vaccines for human use
2.6.16 Tests for extraneous agents in viral vaccines for human use
5.2.4 Substitution of in vivo method(s)…

WP 2.6.41

edqm

Ph Eur 2.6.41
New in Pharmeuropa (04/24)
2.6.41 High Throughput Sequencing for the detection of viral extraneous agents

World Health Organization

New molecular methods with broad detection capabilities for the detection of adv. agents introduced in WHO TRS revision (LAV YF, Dengue, IPV…)
"These methods may be used in the future.[…], as alternative to both in vivo and in vitro tests"

IABS

Next Generation Sequencing for Adventitious Virus Detection in Biologics(2017)

IABS

2nd Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals (2019)

ICH

Q5A(R2) : Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

IABS

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

IABS

4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals (2024)

What is NGS?

Key Advantages of NGS

- Next Generation Sequencing is a technology capable of sequencing all the nucleic acid extracted from a biological sample and to detect any potential viral contaminations. 10,000 to 10 billion+ reads simultaneously
- Potential to identify both known and unknown adventitious viruses by sequencing all the nucleic acid within a sample without needing prior knowledge of the contaminating agents whatever the virus.
- Large amount of sequence data can provide higher sensitivity detection compared to other methods
- Potentially faster turnaround time than in vivo assays

Victoria et al, 2010 J of Virology

JOURNAL OF VIROLOGY, June 2010, p. 6033–6040 Vol. 84, No. 12
0022-538X/10/$12.00 doi:10.1128/JVI.02690-09

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

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Received 22 December 2009/Accepted 25 March 2010

Ma *et al*, 2014 J of Virology

JVI
Journals.ASM.org

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

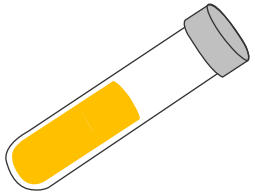
Hailun Ma, Teresa A. Galvin, Dustin R. Glasner,* Syed Shaheduzzaman, Arifa S. Khan

Laboratory of Retroviruses, Division of Viral Products, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, Maryland, USA

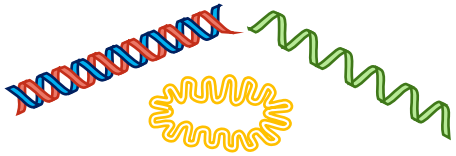
General NGS Workflow

- Each steps also have specific consideration when NGS is use for adventitious agent detection

Sample



Nucleic Acid Extraction



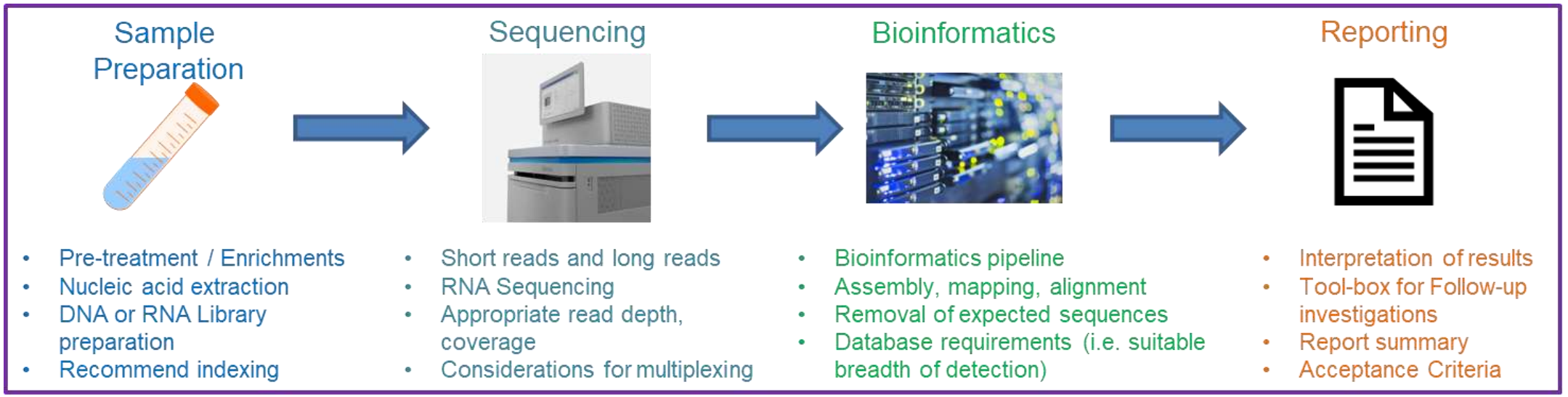
Sequencing Library preparation

Sequencing

Bioinformatics Analysis

Follow-up analysis

Areas of Key Considerations for Ad. Virus detection using NGS

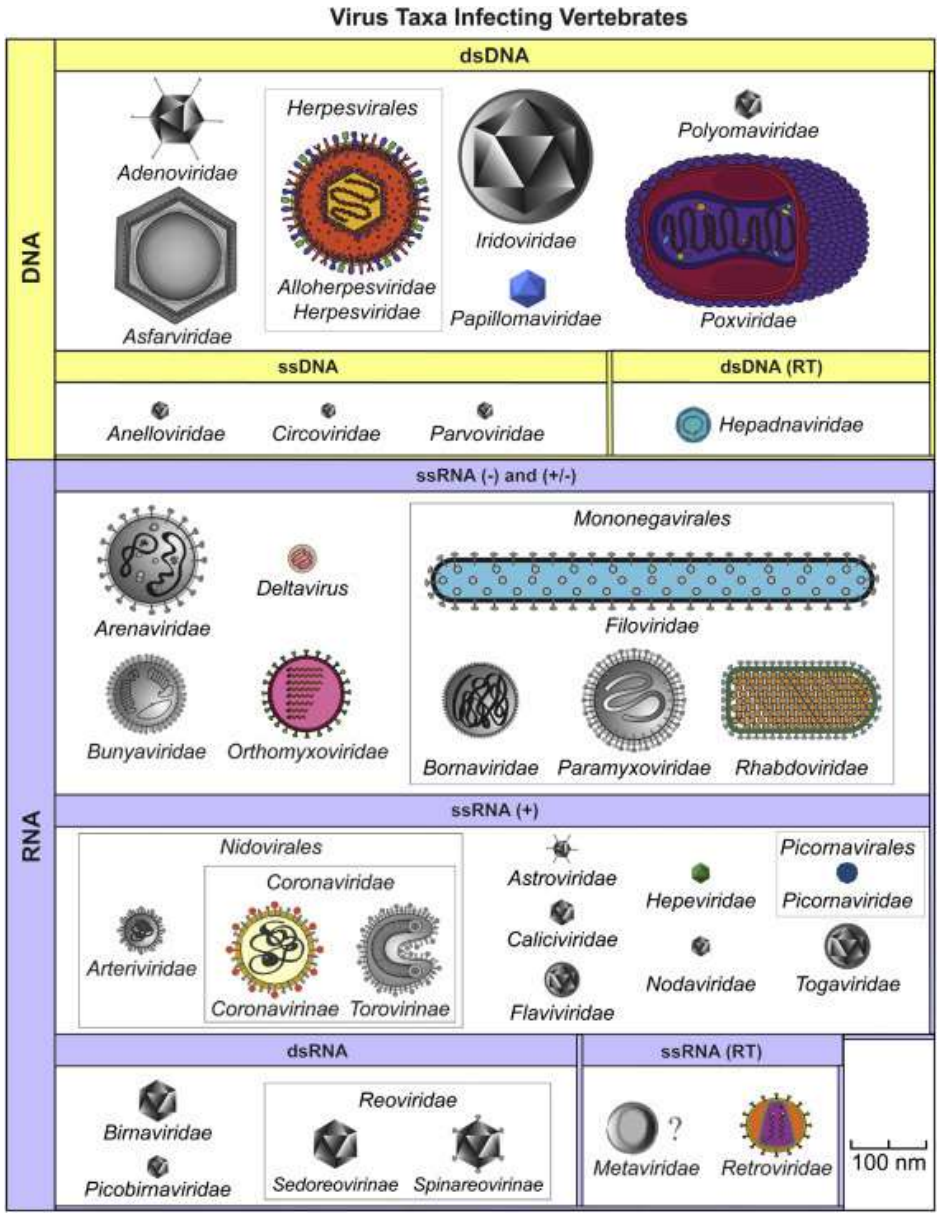


Sample Preparation

- Viruses are very diverse in their genomic and physical properties

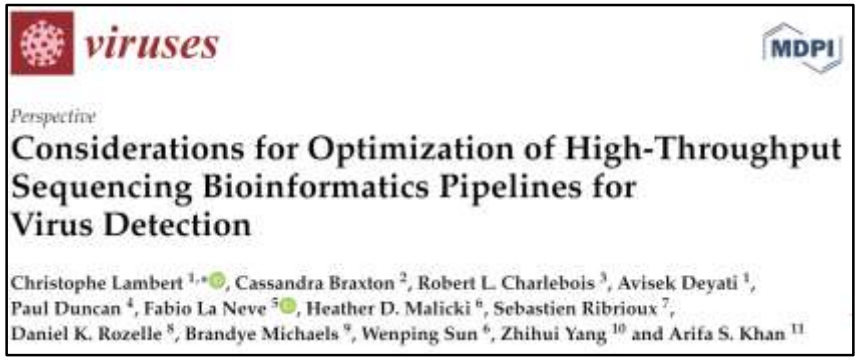
Properties	Variability
Genome nucleic acid	ssDNA, dsDNA, ssRNA, dsRNA
Genome structure	Linear, circularized, segmented
Genome size	~3kb to 300kb +
Viral capsid	icosahedral or helical
Viral envelop	Enveloped or non-Enveloped

- Efficient recovery of all nucleic acids
- Generation of high-quality dsDNA or RNA as input material



Virus Structure and Classification
Essential Human Virology, 01 Jan 2016, :19-29

Sequencing and Bioinformatics



- Selection of an appropriate sequencing platforms
  - Read Length, # of Reads, Accuracy
  - Influence the downstream bioinformatics pipeline and upstream sample preparation

- Suitable computational algorithm is important to ensure accurate identification of viral sequence from the sequencing results.
  - Some common elements
    - QC of sequencing data
    - De novo assembly to reduce dataset
    - Identification of viruses using a reference Database
    - Host removal
    - Coverage and depth
    - Counter screening

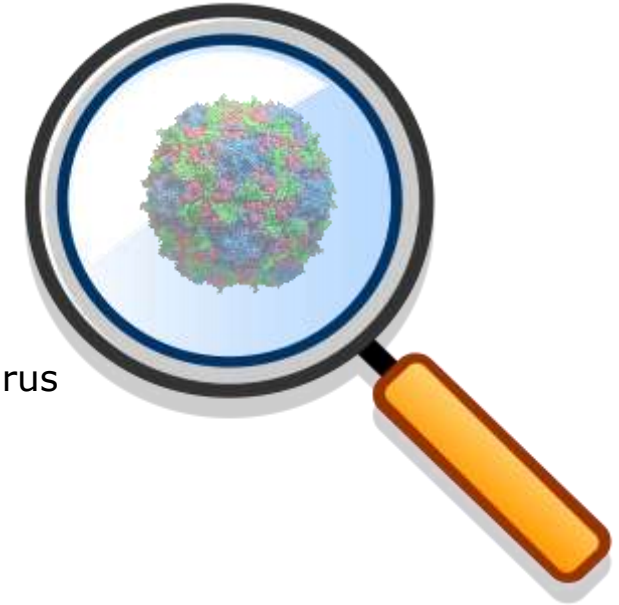
Short-Read vs Long Read NGS platforms

	Short Read	Long Read
Platforms	Illumina Ion Torrent	Oxford Nanopore PacBio
Read length	<400bp	15-20kb (could be optimized to be much higher)
Throughput	Generally higher throughput (cost per base is lower)	Lower number of reads (with much longer read length)
Other considerations	Amplification Step needed	Direct sequencing of the nucleic acid

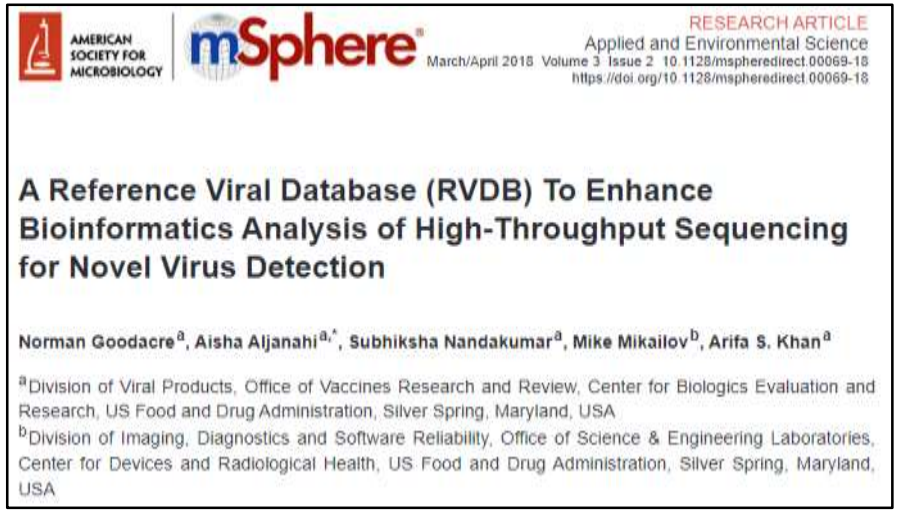
Identification of a positive hit

- Considerations for positive identification
  - Depth of coverage of the identified hit
  - Genome coverage
  - Sequence Identity

- These parameters can influence how confident a positive hit is to the identified virus



Reference Database



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RESEARCH ARTICLE
Applied and Environmental Science
March/April 2018 Volume 3 Issue 2 10.1128/mspheredirect.00069-18
https://doi.org/10.1128/mspheredirect.00069-18

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]

[a]Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, USA
[b]Division of Imaging, Diagnostics and Software Reliability, Office of Science & Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, Silver Spring, Maryland, USA

- Considerations for Database
  - Accurate identification (missed or misidentified if database is incomplete)
  - GenBank sequences not always accurate. Some sequence are mis-annotated, contain extraneous sequences, incomplete, or redundant

- For example: RVDB (Reference Virus Database)
  - Generated based on semantic datamining of GenBank. (i.e. using specific and control ontology) followed by manual curation
  - Includes all viral, virus-related, and virus-like sequences, excluding bacteriophages
  - Duplicated or related sequences were computationally clustered to reduce redundancy
  - Downloadable in various formats for users
  - Version 29.0 with 10000605 unique viruses

Follow-up and Reporting of Results

- Similar to PCR and other molecular techniques, a positive hit by NGS doesn’t necessary indicate an infectious virus.
  - Examples of laboratory follow-up
    - Long-Range PCR, PCR/qPCR, Infectivity, TEM, ELISA, Reverse Transcriptase assay

Controls

- Provide important check-points in ensuring performance of the assay
- Assess the integrity of the starting material and subsequent steps
- Verify the integrity, size, concentration of sequencing library
- System in place to detect cross-contamination or minimize potential contaminations from the environment
- Evaluating sensitivity and useful for assay qualification / validation

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; limit to 1 order per year.

Virus	Genome type	Genome size	Particle size	Envelope	Chemical resistance
Reovirus (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	5.1kb	26 nm	No	HIgh
Human coronavirus (HCoV-OC43)	RNA; single-strand; Linear	30.7kb	80-120nm	Yes	Low

* Additionally 5 of the viruses are available as CBER NGS Virus Reagents (BEI NR-59622) for NGS development

Advance Virus Detection Technologies Working Group

AVDTWG – Objectives

The Advanced Virus Detection Technologies Working Group was initiated as a Users Group in 2012, and the focus has been on high throughput sequencing technologies for broad virus detection.

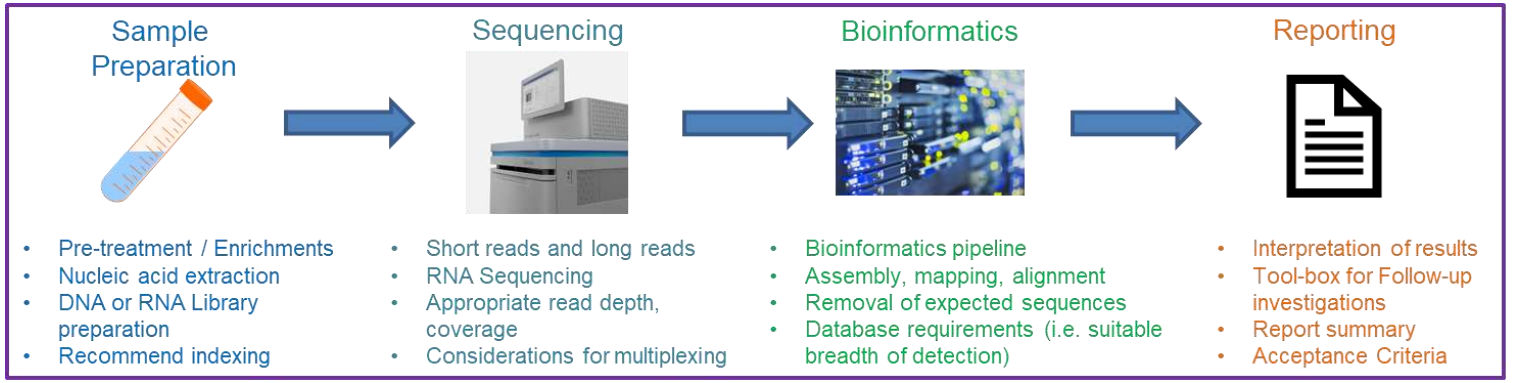
- Provide an **informal, scientific forum** for discussions, knowledge exchange, and scientific collaborations among scientists from different organizations
- Develop **consensus views**
- Promote and manage WG **collaborative studies**
- Publish **best practices, perspective and position papers** for considerations of NGS applications in biologics

More than 240 participants from over >60 organizations

- Regulatory agencies, Government agencies, Industries, Service providers, Technology developers, Academics
- Co-chairs: Arifa Khan (FDA), Siemon Ng (Notch Therapeutics) Noémie Deneyer (GSK), Ken Keno 河野さん (National Institute of Health Sciences)

AVDTWG Subgroups

Multiple key challenges requiring additional scientific data



2012

Subgroup A

Sample selection/ preparation/processing

Subgroup B

Virus standards and reference materials

Subgroup C

Complete and correctly annotated, virus reference database

Subgroup D

Bioinformatics pipelines analysis

Subgroup E

Follow-up strategies to confirm the identity of a "hit"

2024

Subgroup AB

Subgroup C

Subgroup DE

AVDTWG – Collaborative Spiking Studies

- Members of the group and the scientific community for performance evaluation and standardization of NGS
  - Evaluate reference standards and bioinformatics tools
  - Compare and recommend experimental protocols

Spiking study n°1 HeLa cell line
(2013-2016)

Spiking study n°2A Virus in Cell background
Started 2017

Spiking study n°2B Viral seed/Viral vector background
Started 2016

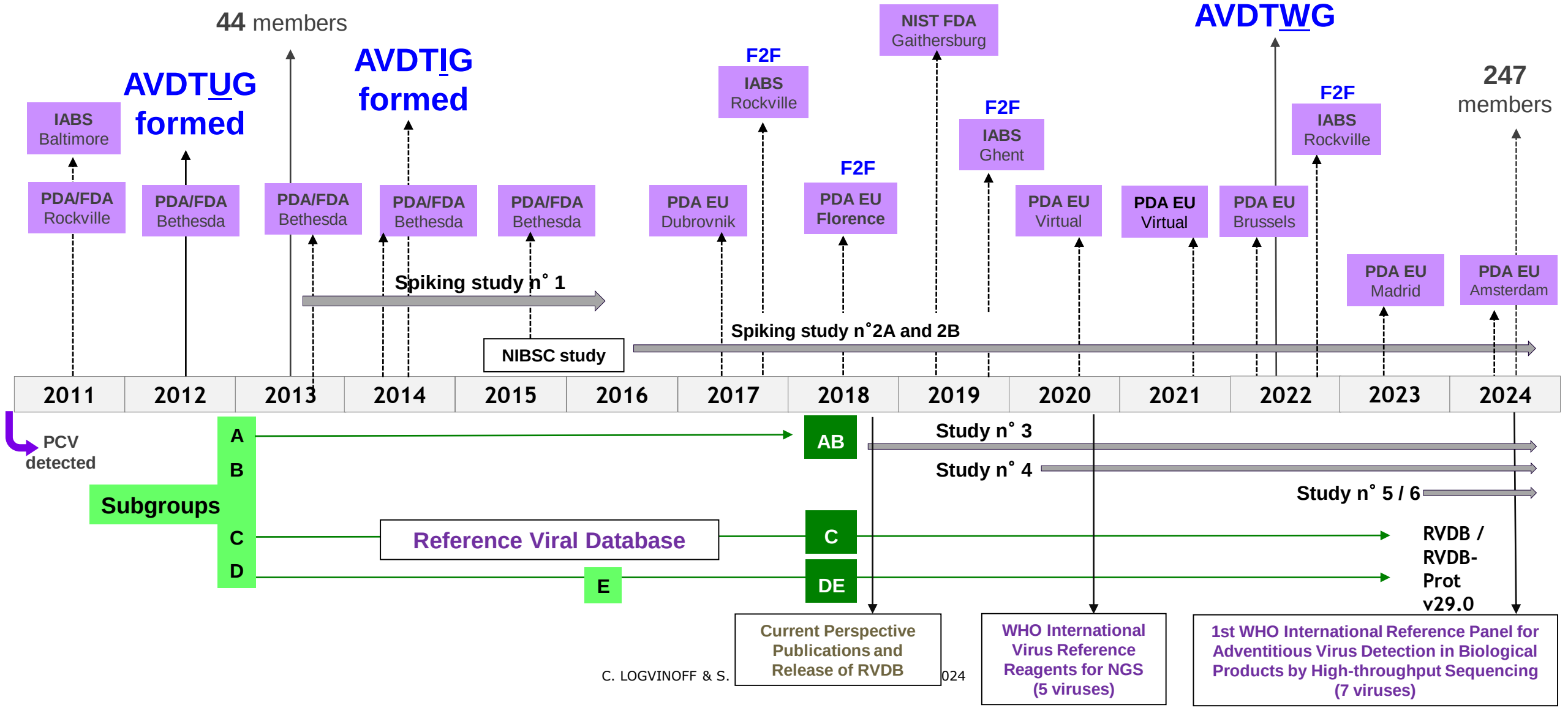
Spiking study n°3 Transcriptomics
Started 2019

Spiking study n°4 Long reads – Viral seed/Viral vector background
Started 2020

Spiking study n°5 Long reads – Unprocessed Bulk
Started 2023

Spiking study n°6 Long reads – Transcriptomics/Cell bank
Started 2023

AVDTWG – History



Achievements and Contributions of AVDTWG participants


Biologicals
Volume 55, September 2018, Pages 1-16

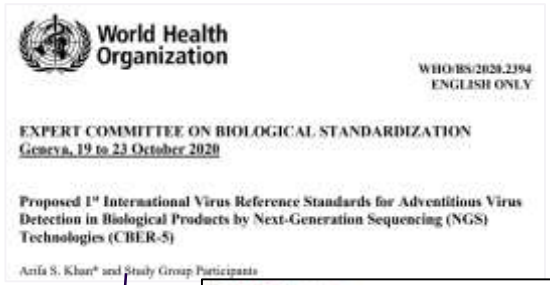
Report of the international conference on next generation sequencing for adventitious virus detection in biologicals ☆

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


Biologicals

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

Arifa S. Khan, Johannes Blümel, Dieter Deforce, Marion F. Gruber, Carmen Jungbäck, Ivana Knezevic, Laurent Mallet, David Mackay, Jelle Matthijnssens, Maureen O'Leary, Sebastian Thrane, Joseph Victoria, Pieter Neels


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


mSphere

RESEARCH ARTICLE
Applied and Environmental Science

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

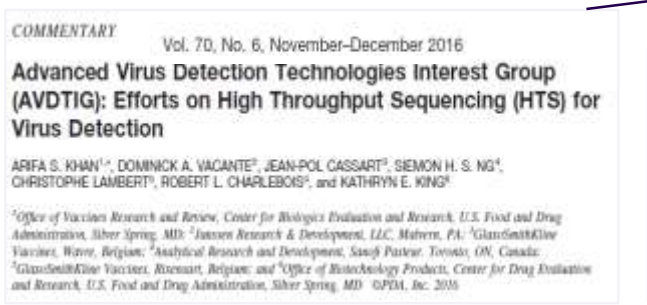

F1000Research

DATA NOTE

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

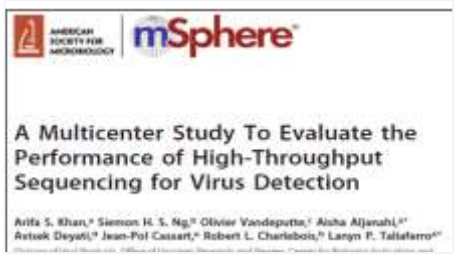
Thomas Bigot, Sarah Temmam, Philippe Pérot, Marc Eloit

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, DOMINICK A. VACANTE, JEAN-POL CASSART, SIEMON H. S. NG, CHRISTOPHE LAMBERT, ROBERT L. CHARLEBOIS, and KATHRYN E. KING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A Multicenter Study To Evaluate the Performance of High-Throughput Sequencing for Virus Detection


viruses
MDPI

Perspective

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


viruses
MDPI

Perspective

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

Contact for AVDTWG Membership

- Co-chairs:
  - Arifa Khan (FDA): arifa.khan@fda.hhs.gov
  - Siemon Ng (Notch Therapeutics): sng@notchtx.com
  - Noémie Deneyer (GSK): noemie.x.deneyer@gsk.com
  - Ken Keno 河野さん (National Institute of Health Sciences): kenkono@nihs.go.jp

- PDA Coordinator:
  - Josh Eaton: eaton@pda.org

Summary

- NGS can be a powerful tool for the detection of adventitious viruses in Biologics
  - NGS is an agnostic method detecting a broader range of adventitious virus not necessarily detected by compendial tests, as the detection (and identification) of the adventitious virus is made through its nucleic acids.

- There are multiple key considerations :
  - Each step has specific considerations as part of the overall NGS assay design
  - Multidisciplinary expertise (molecular biologist , bioinformatician, virologist…)

- Adventitious virus testing package can be streamlined, with 3R considerations:
  - NGS for adventitious virus detection can be used to substitute/replace multiple tests such as *in vivo* test, specific tests such by PCRs, HAP/MAP/RAP tests, 9CFR for bovine and porcine viruses , and potentially *in vitro* test.

- Product viral safety is strengthened

- There is global regulatory alignment on the potential uses of NGS , especially in replacement of *in vivo* testing. Given the available data, head-to-head comparison is not required anymore.

Thank You