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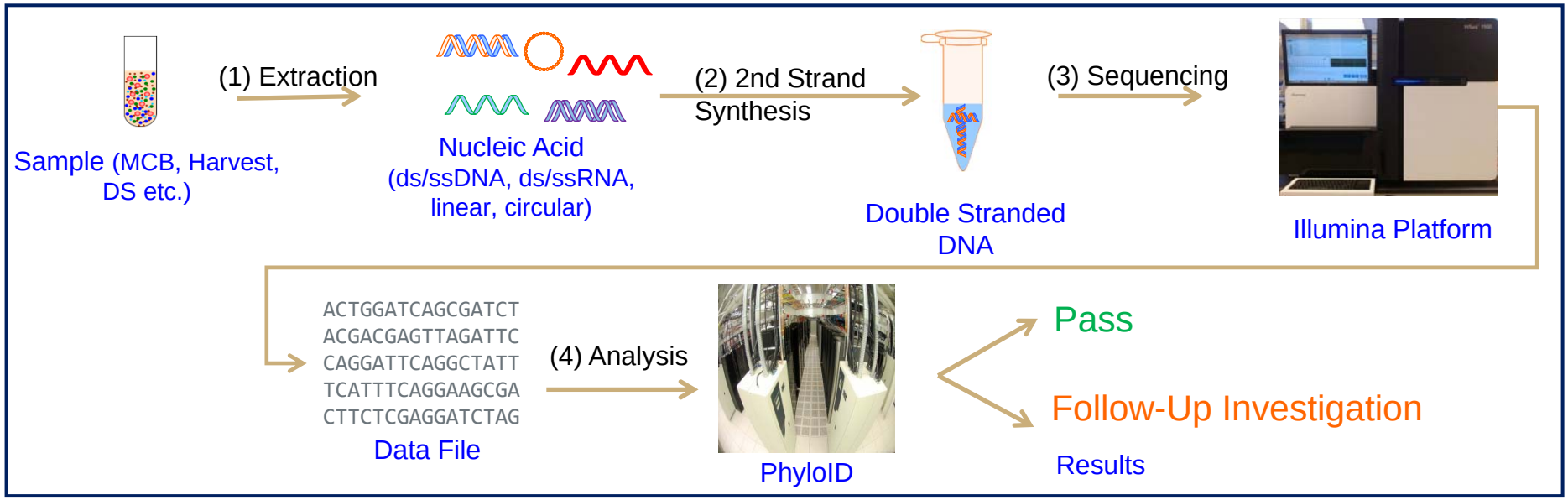
# Potential Controls for an NGS Adventitious Virus Detection Assay

# Outline

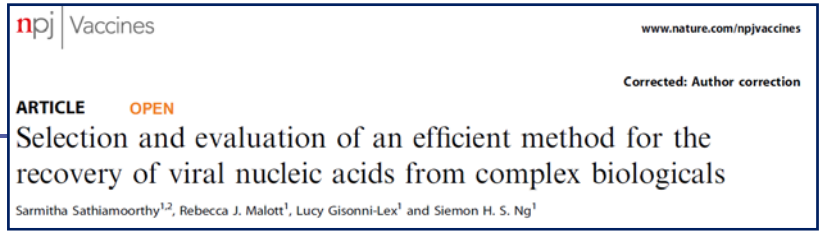
- **Overview of Sanofi Pasteur's adventitious virus detection by NGS assay**
- **Controls within the assay**
- **Panel of model viruses**
- **AVDTIG Subgroup A perspective on controls**
- **Summary**

# Overview of Sanofi Pasteur's Adventitious Virus Test

- **Designed to test any type of biological material (cell banks, viral seeds, crude harvests, drug substances, drug products)**
  - A whole-genome approach that takes advantage of very deep sequencing



# Sample Extraction



npj | Vaccines

www.nature.com/npjvaccines

Corrected: Author correction

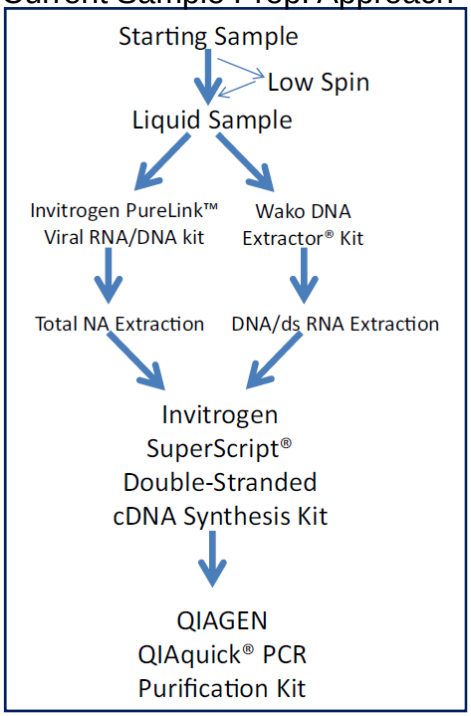
ARTICLE OPEN

Selection and evaluation of an efficient method for the recovery of viral nucleic acids from complex biologicals

Sarmitha Sathiamoorthy[1,2], Rebecca J. Malott[1], Lucy Gisonni-Lex[1] and Siemon H. S. Ng[1]

- **Two extraction arms that are done in parallel**
  - Based on comparisons between 11 different extraction kits/methods using EBV, RSV, Reo and FeLV as model viruses
  - Enhance the recovery of dsRNA virus
  - Includes an optional low-speed centrifugation to remove cell debris but no nuclease treatment
- **Sequencing library preparation using the Nextera XT**

Current Sample Prep. Approach



# Illumina HiSeq1500 Sequencing System

- **The advantage is deep sequencing from a single biological sample**
  - Read length: 150 nt
  - Average # of Read: 400-500 M (paired-end reads)
- **Validated the Illumina HiSeq1500 in 2016**
  - HiSeq 1500 sequencer, Illumina cBot and two computers
  - Network attached storage and remote data storage in a data center

# PhyloID Analysis

PDA Journal of Pharmaceutical Science and Technology

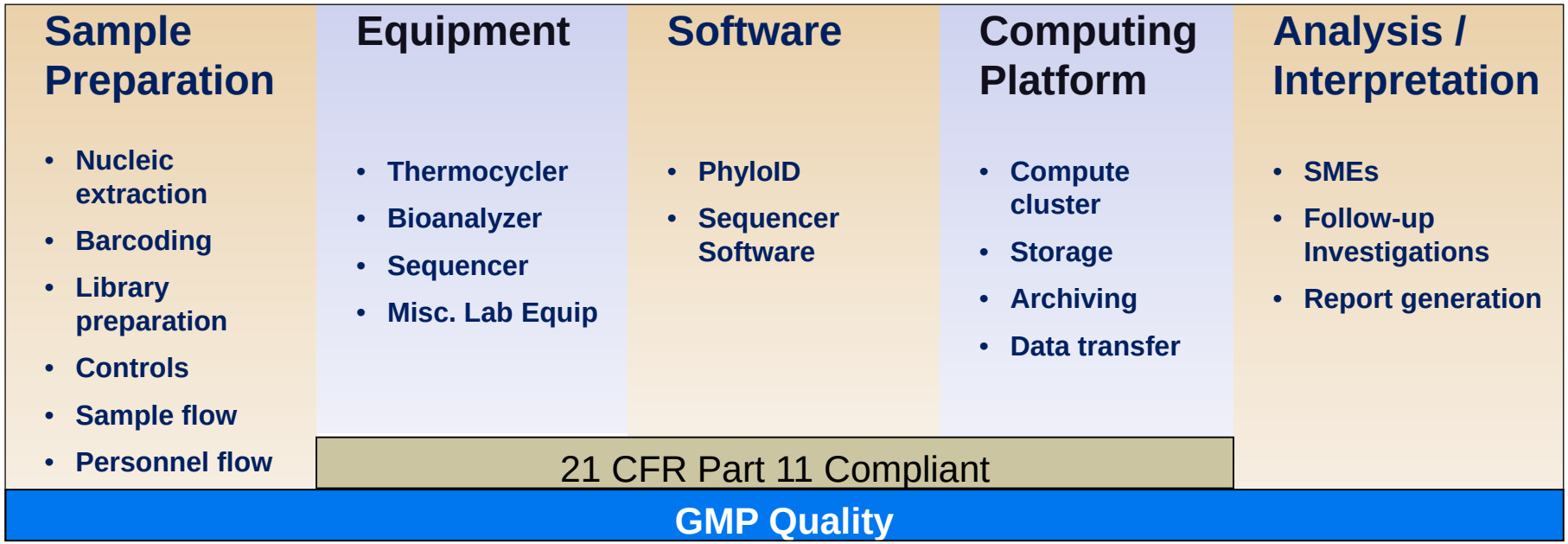
**Cataloguing the Taxonomic Origins of Sequences from a Heterogeneous Sample Using Phylogenomics: Applications in Adventitious Agent Detection**

Robert L. Charlebois, Siemon H. S. Ng, Lucy Gisonni-Lex, et al.

*PDA J Pharm Sci and Tech* **2014**, *68* 602-618
Access the most recent version at doi:10.5731/pdajpst.2014.01023

- **PhyloID™- an automated analysis pipeline for adventitious agent detection with NGS data**
  - Specifically designed for analyzing large sequence datasets for adventitious agent detection
  - A sequence is identified based on its phylogenomic distance to a known set of reference sequences. Both the match strength and the degree of fit can indicate confidence in the assignment
- **Reference genomes are based on RefSeq database**
- **Software and hardware were validated in 2017**

# Sanofi Pasteur's Validation Approach

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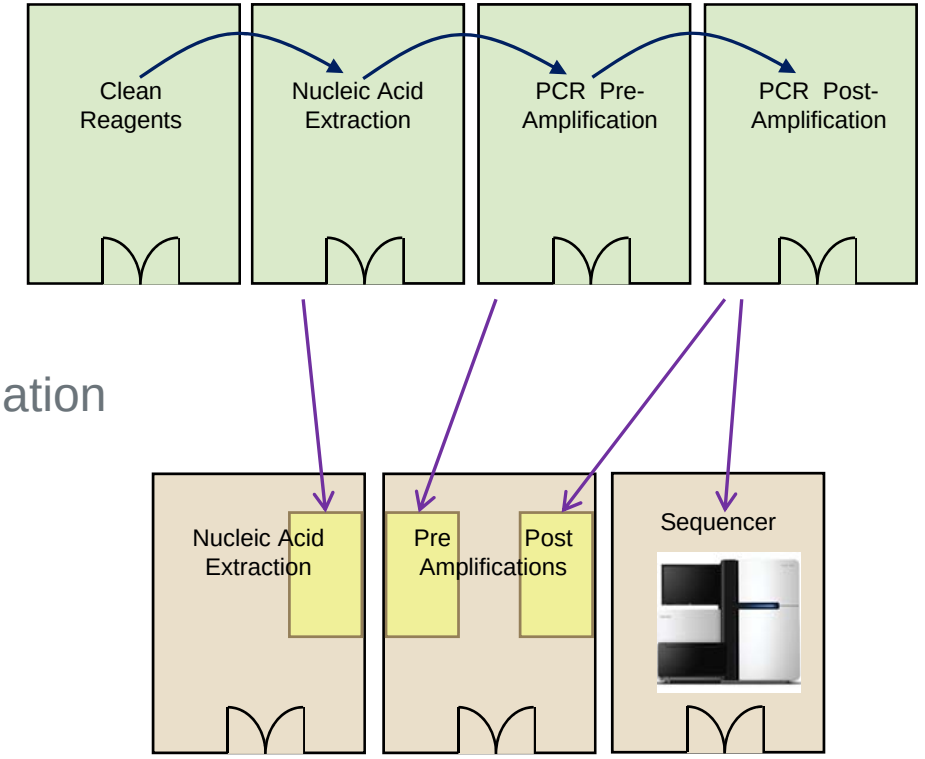
# Assay Controls and QC Steps

# Controls

- **To minimize potential contaminations from the environment**
- **QC steps are important check-points in ensuring performance of the assay**
  - Quantity and integrity of the starting material
  - Sequencing library assessment (size and quantity)
- **Assessment of sequencing reads to remove low quality data**

# Flow

- **Personnel flow similar to that of how PCR rooms are setup for a PCR assay**
  - Differential airflow to minimize contamination
  - All work are done in dedicated BSC



# Sample Preparation Controls

- **Each sample are spiked with two types of pseudo-viral particles containing DNA and RNA separately prior to nucleic acid extraction**
  - Designed with sequences that are not expected to be in any samples or the environment
  - System control that monitors the extraction efficiency, cDNA synthesis, library generation and sequencing
  - The number of reads matching the expected DNA and RNA sequences are identified by PhyloID, tracked and use as validity criteria.
- **Each sample is dual-indexed so that each sample can be easily identified and distinguished from previous samples**
  - All testing samples are run in individually lanes or flow cells and are not multiplexed
- **Quantify and sized the nucleic acids using the Agilent BioAnalyzer 2100**
  - cDNA synthesis
  - Sequencing library

# Sequencing Control / PhyloID Data Analysis

- **A pre-made dual-indexed sequencing library is added to every sequencing run as a sequencing control**
  - Requires a minimum number of reads to ensure that the test meets the necessary sensitivity
  - Minimum average read quality
- **PhyloID**
  - Adaptor trimming
  - Filters out low quality reads to removes sequencing/PCR errors that might lead to mis-identifications.
    - Helps improve the robustness of the assembly step

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# Panel of Model Viruses

# Model Viruses

- **Model viruses are critical to assessing the performance of an NGS adventitious virus detection assay**
- **NIH published a study in 2014 (Gombold *et al.*,) that linked *in vivo* and *in vitro* tests for adventitious virus detection and demonstrated that the *in vivo* tests are not as sensitive**
  - 16 viruses across 9 viral families, representative of potential contaminants that could be introduced during vaccine production
  - Includes human and animal viruses from a variety of families, both RNA and DNA genomes as well as enveloped and non-enveloped viruses
- **This panel can be used to link NGS data to the published *in vivo and in vitro* data**
  - Difficult to justify repeating the *in vivo* study because of the ethical consideration and the 3R initiatives to replace, reduce and refine the use of animals.

- Adenovirus 5
- Adenovirus 41
- Simian CMV
- HSV 1 (MacIntyre)
- Simian Virus 40
- BVDV (NY-1)
- Influenza A (A/PR/8/34)
- Measles virus (Edmonston)
- Mumps virus (Enders)
- Bovine Parainfluenzavirus Type 3
- Coxsackievirus A16
- Coxsackievirus B3
- Echovirus 11 (Gregory)
- Rhinovirus 2
- Vesicular Stomatitis Virus (Indiana)
- Rubella virus (M-33)

# Summary of NIH study

Vaccine 32 (2014) 2916–2926

Contents lists available at ScienceDirect

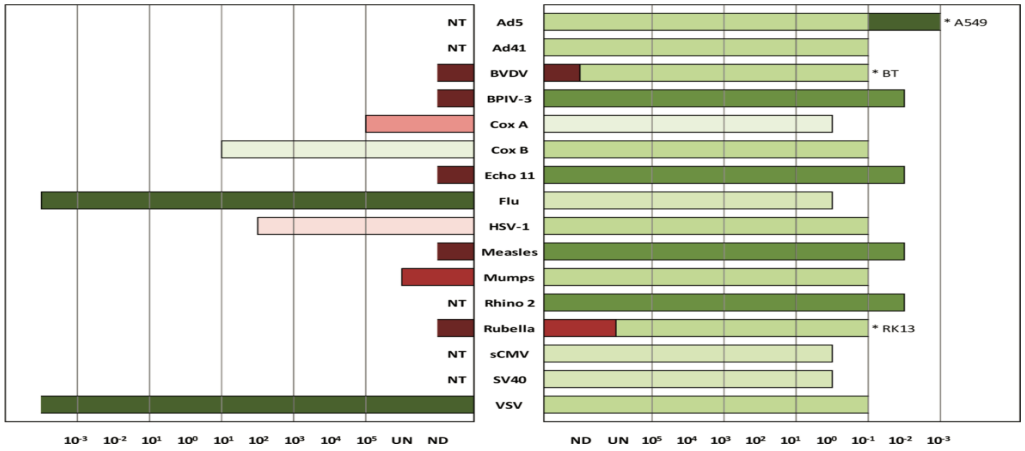
Vaccine

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☆

James Gombold[a], Stephen Karakasidis[a], Paula Niksa[b], John Podczasy[a], Kitti Neumann[a], James Richardson[c], Nandini Sane[c], Renita Johnson-Leva[c], Valerie Randolph[d], Jerald Sadoff[e], Phillip Minor[f], Alexander Schmidt[g], Paul Duncan[b], Rebecca L. Sheets[i,*]

*In vivo* *In vitro*



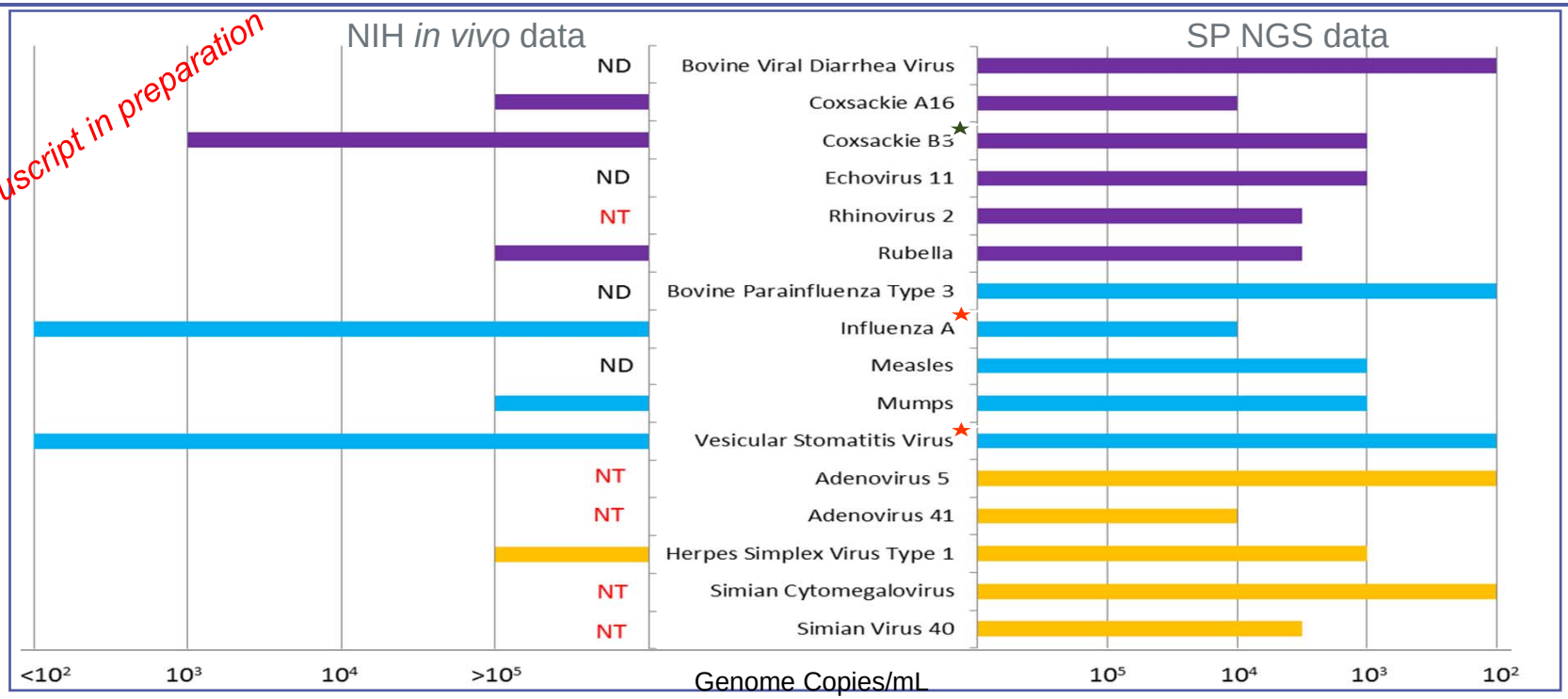

| Viral Family | Virus | Strain | Enveloped | Viral Genome | Genome Size (Kb) | Production Cell Line | Cell Line For Titration Assay |
|---|---|---|---|---|---|---|---|
| *Adenoviridae* | Adenovirus 5 | Adenoid 75 | No | dsDNA | 36 | A549 | A549 |
| | Adenovirus 41 | N/A | No | dsDNA | 34 | HEK 293 | HEK 293 |
| *Flaviviridae* | Bovine Viral Diarrhea Virus | NY-1 | Yes | ssRNA (+ve) | 12.4 | BT | BT |
| *Herpesviridae* | Herpes Simplex Virus Type 1 | MacIntyre | Yes | dsDNA | 150 | Vero | Vero |
| | Simian Cytomegalovirus | CS6 | Yes | dsDNA | 221 | MRC-5 | MRC-5 |
| *Orthomyxoviridae* | Influenza A | A/PR/8/34 (H1N1) | Yes | 8 ssRNA (-ve) | 12.5 | MDCK | MDCK |
| *Paramyxoviridae* | Mumps | Enders | Yes | ssRNA (-ve) | 15.4 | Vero | Vero |
| | Bovine Parainfluenza Type 3 | N/A | Yes | ssRNA (-ve) | 15.5 | Vero | Vero |
| | Measles | Edmonston | Yes | ssRNA (-ve) | 15.9 | Vero | Vero |
| *Picornaviridae* | Coxsackie A16 | N/A | No | ssRNA (+ve) | 7.4 | Vero | Vero |
| | Coxsackie B3 | N/A | No | ssRNA (+ve) | 7.4 | LLC-MK2 | LLC-MK2 |
| | Echovirus 11 | Gregory | No | ssRNA (+ve) | 7.4 | LLC-MK2 | LLC-MK2 |
| | Rhinovirus 2 | HGP | No | ssRNA (+ve) | 7.1 | HeLa | HeLa |
| *Polyomaviridae* | Simian Virus 40 | Pa-57 | No | dsDNA | 5.2 | Vero | Vero |
| *Rhabdoviridae* | Vesicular Stomatitis Virus | Indiana | Yes | ssRNA (-ve) | 11.2 | Vero | Vero |
| *Togaviridae* | Rubella | M-33 | Yes | ssRNA (+ve) | 9.7 | BSC-1 | RK-13 |

# Equivalent Viral Stocks

- **Produced a set of equivalent viral stocks**
  - Followed the NIH protocols for virus propagation and titration (as close as possible)
    - Same-sourced cells, media formulations, multiplicity of infection (MOI), infection time, and harvest conditions
    - For some viruses, MOI, infection time and harvest conditions were modified
  - Determined the titer and genome copies for both SP stocks and the NIH stocks
  - **Viruses were provided by the National Institute of Allergy and Infectious Diseases (via Rebecca Sheets)**

- **Used this panel to assess the performance of NGS for adventitious virus detection**

# Comparison of Sensitivity between NGS and *In Vivo* Tests




**NT = not tested; ND = not detected;**
**Viral genome types: dsDNA; ssRNA (–ve); ssRNA (+ve)**

★ *In vivo* test show better sensitivity than NGS (and *in vitro* test)

★ Equivalent between *in vivo* and NGS tests

# Characterizations of Model Viruses

- **Representation from different viral properties**
  - DNA / RNA
  - Single-/Double-stranded
  - Size
  - Segmented/Circular
  - Some differences in detection sensitivity have been observed for very similar viruses (but additional work is needed)
- **Titer**
  - Limit the number of freeze-thaw
- **Genome copies**
  - NGS is a nucleic acid based test
- **Culturing conditions (e.g., serum and host cells) and purification**
- **Sequence information**
  - How much data is sufficient?
  - What about lab-specific / kit-specific artifacts (do you combine the data or look for the intersect from different labs)?
- **Link to in vivo data as we are considering NGS as a replacement for in vivo assays**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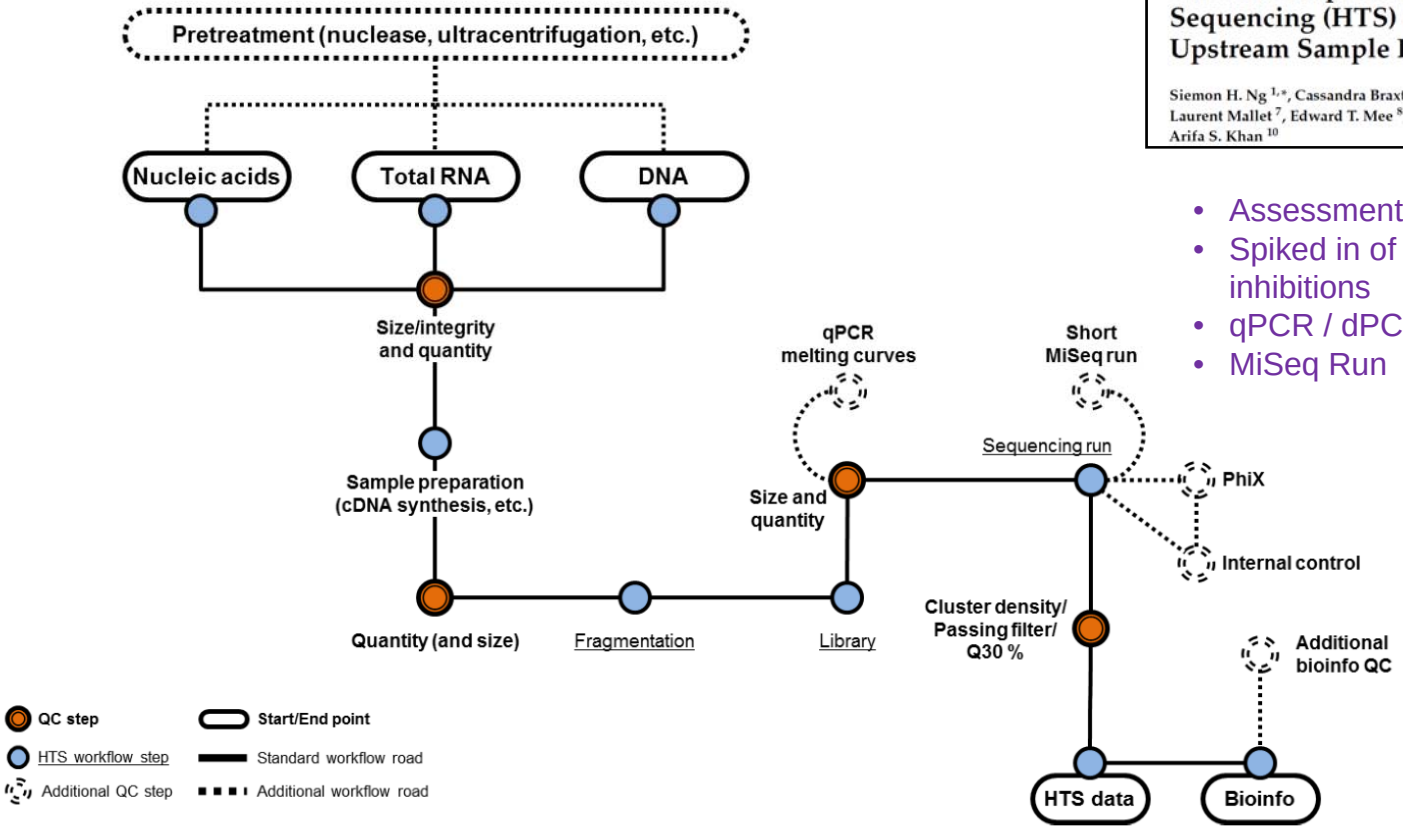
# Controls (on behalf of Subgroup A of AVDTIG)

# AVDTIG Subgroup A Perspective




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]

- Assessment of RNA and/or DNA integrity
- Spiked in of RNA controls to monitor inhibitions
- qPCR / dPCR as QC steps
- MiSeq Run

# Summary

- **QC and controls are important in ensuring consistency of a NGS assay**
  - Different rooms/workflow
  - Controls for extraction, libraries preparation and sequencing
  - Assessing quantity and integrity
- **Need for negative control?**
- **A panel of well-characterized viruses is key to understanding the performance of the assay**

# Thank You

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