Matrix Effects on Limit of Detection for NGS-based Adventitious Virus Detection Assays

BioReliance®
Contract Testing Services

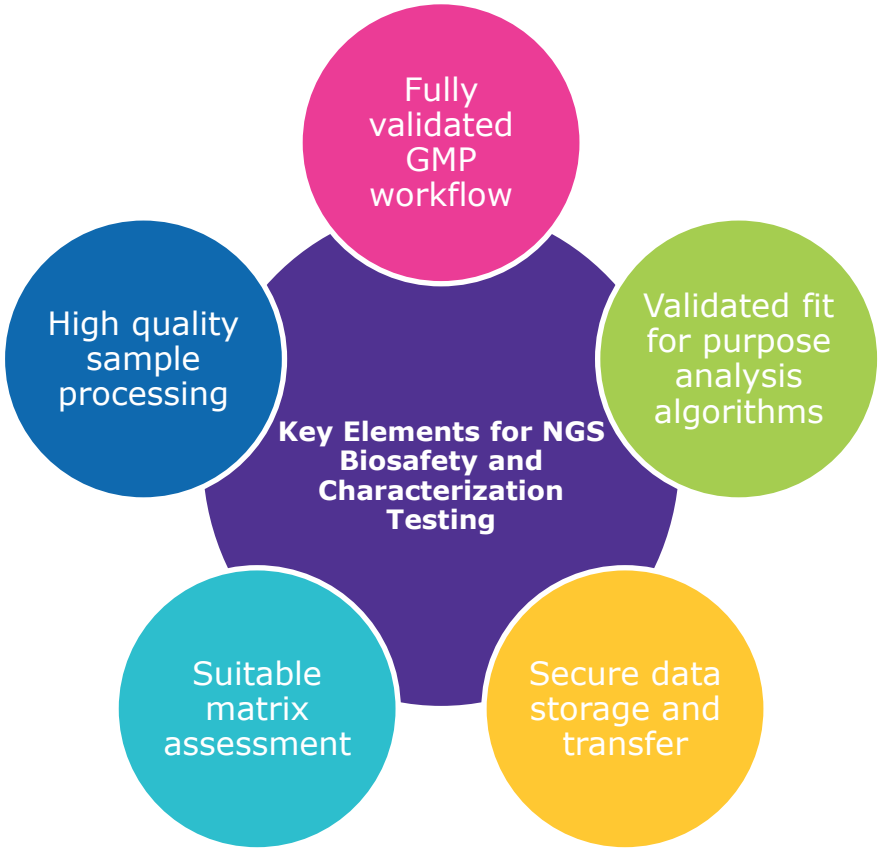
Bradley Hasson
Director of Operations for NGS
Merck

MERCK

The CRO Perspective
A Unique Vantage Point

BioReliance®

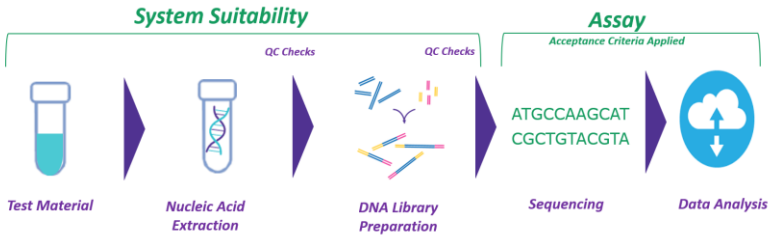
- Work with multiple clients from a cross-section of the industry testing different modalities
- Address regulatory feedback (provided by the client) for multiple applications of the technology
- Have multiple validated methods for different purposes (e.g. Transcriptome vs virome vs genomic approaches) and NGS experts
- **Accumulation of data in multiple products and backgrounds through spiking studies**

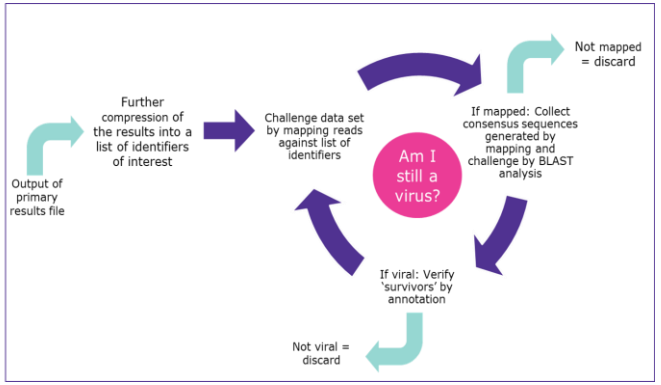


MERCK

NGS methodology
Validation of NGS Methodology and implementation

BioReliance®





NGS Assay is validated in a an

- **Extraction**
- **Library**
- **Sequencing**
- **Bioinformatics**

This approach confirms that each portion of the assay behaves as expected and offers flexibility for updating rapidly changing technology

Limits of detection for client-specific matrices are established through end-to-end analysis using known viruses to achieve ICH Q2 parameters:

Specificity: Conveyed by algorithm, confirmed in validation

LOD: Established for specific matrix

Full validation package consists of the following:

Generic Validation + Spiking Studies

MERCK

NGS Methodology
Spiking Studies- General Overview

BioReliance®

- Representative Reference Viruses are available through BEI Resources from Arifa Khan.
- Viruses qualified by worldwide labs and are standardized to be used in NGS AAT applications
  - Epstein Barr Virus (EBV)
  - Respiratory Syncytial Virus (RSV)
  - Feline Leukemia Virus (FLV)
  - Porcine Circovirus (PCV)
  - Reovirus (REO)
  - OC43 Coronavirus
  - Minute Virus of Mice (MMV)
- Model viruses represent different virus qualities and worst case scenarios.
- Contain different physiochemical properties (ssDNA, dsDNA, ssRNA, RNA, segmented, circular, enveloped, non-enveloped)

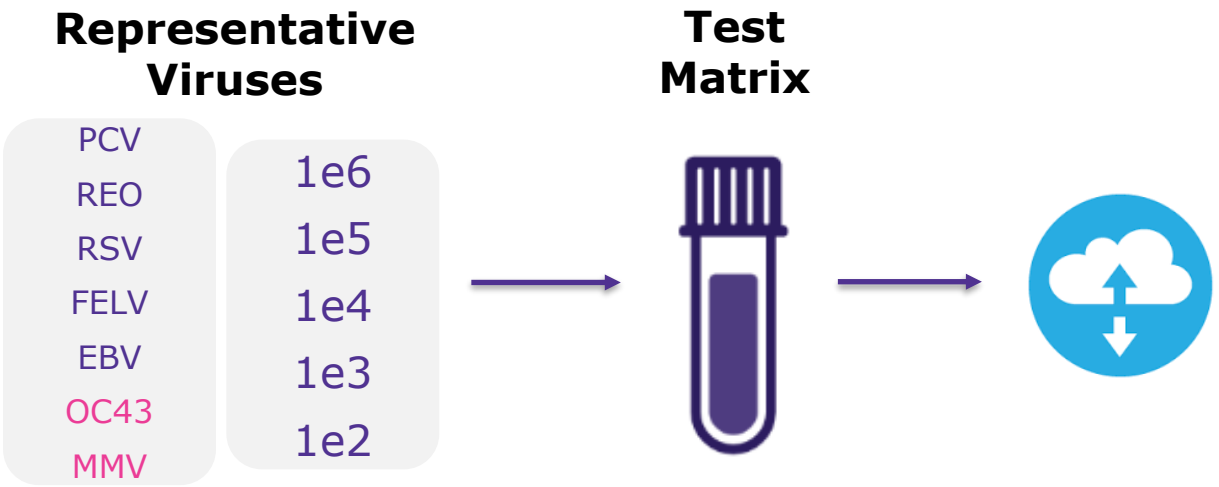
ICH Q5A(R2) Guideline

Suitable reference materials should be used for method qualification and validation to evaluate performance of the different steps involved in the workflow and to demonstrate sensitivity, specificity, and breadth of virus detection. This can include using currently available reference virus reagents/panels which contain viruses of distinct physical (size, enveloped and non-enveloped), chemical (low, medium, and high resistance), and genomic (DNA, RNA, double- and single-stranded, linear, circular) characteristics to evaluate the performance of the entire NGS workflow or specific steps. A comprehensive viral database should be used with diverse viral sequences for broad virus detection. Other types of reference materials may be used to evaluate the specific technical and bioinformatic steps.

MERCK

What does a Spiking Study need to look like?

BioReliance®



General guidelines:

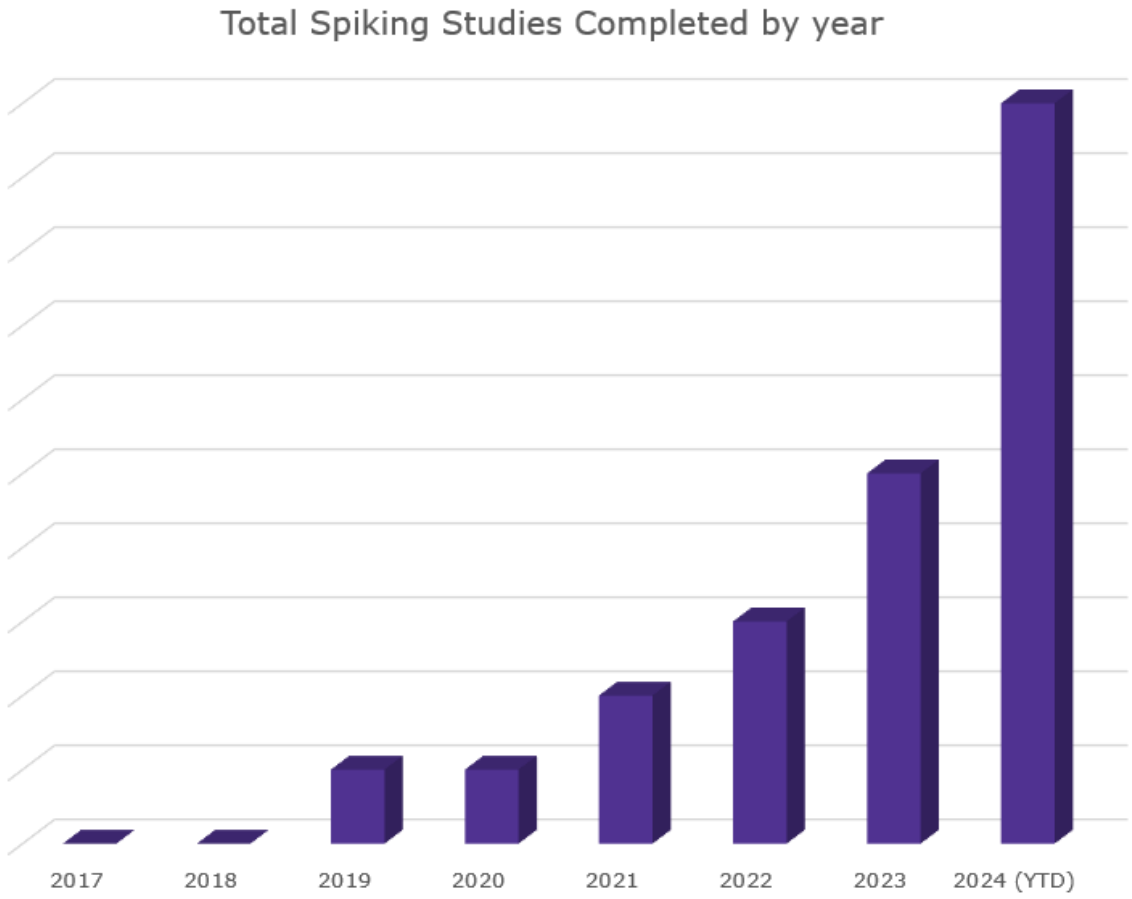
- Use modular validated methodology
- Use client matrix = representative material
- Pre-Study assessment of Nuclease treatment
- 3-5 representative viruses (WHO-CBER panel)
- 3-5 concentrations (depends on matrix and expectations)
- 2-3 replicates at each concentration

Data Analysis (non-targeted)

- Use established criteria for detectability in order to mimic the analysis process.
  - Specificity
  - Homology
  - Fragment Length
  - Number of reads required
- Determine reproducibility via replicates
- Determine required depth of coverage

MERCK

The CRO Perspective
Uptick in Spiking Studies being performed



- Matrix specific LOD determinations are well-supported by ICH Q5A and Ph. EUR 2.6.41 (Draft)
- Performed to demonstrate LOD within the specific sample matrix
- Typical samples seen:
  - Bulk Harvest
  - Master Virus Seed
  - Master Cell Banks

Sample Preparation: Multiple methods depending on sample type

BioReliance®

Transcriptomic Assessment

Viromic/Genomic Assessment

Cell-based Sample

Cell Line (e.g. MCB)

Harvest cells

RNA Extraction

Cell-Free Complex Matrix

Viral Bulk Harvest or DS/DP

Master Virus Seed

Total NA Extraction

(optional Benzonase® endonuclease pre-treatment)

Cell-Free Simple Matrix

Media Samples

mAb Final Products

Total NA Extraction

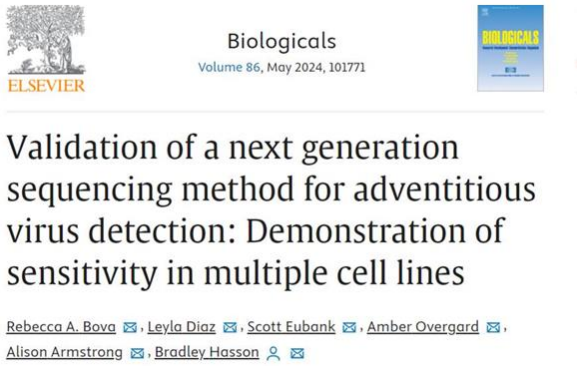
Double-stranded DNA Library Preparation

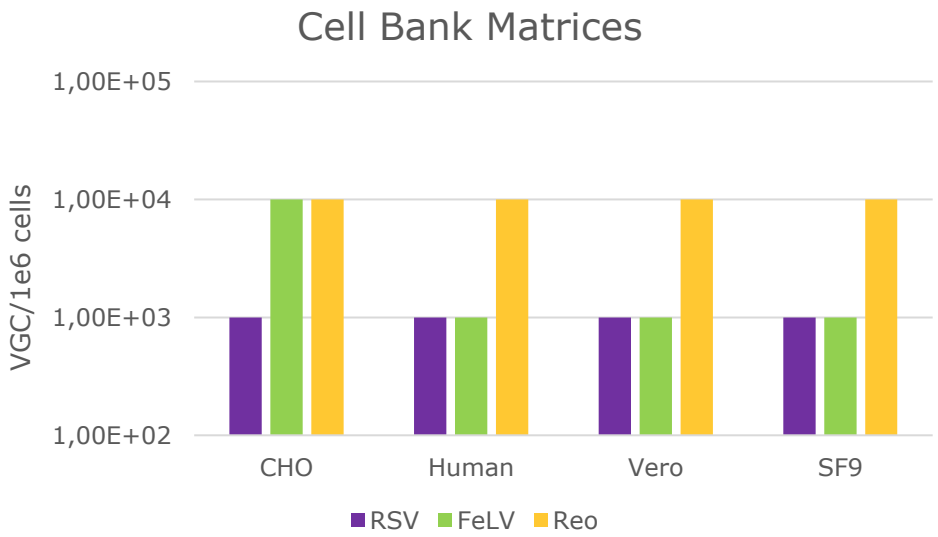
Sequencing

MERCK

Matrix Specific Limit of Detection
Representative Cell Lines

BioReliance®





- **Matrix specific limit of detection was determined using 4 representative cell lines**

- **The cell lines were spiked with different concentrations of 3 RNA viruses from the WHO recommended reference virus panel.**
  - Respiratory Syncytia Virus (RSV)
  - Feline Leukemia Virus (FeLV)
  - Reovirus (Reo)

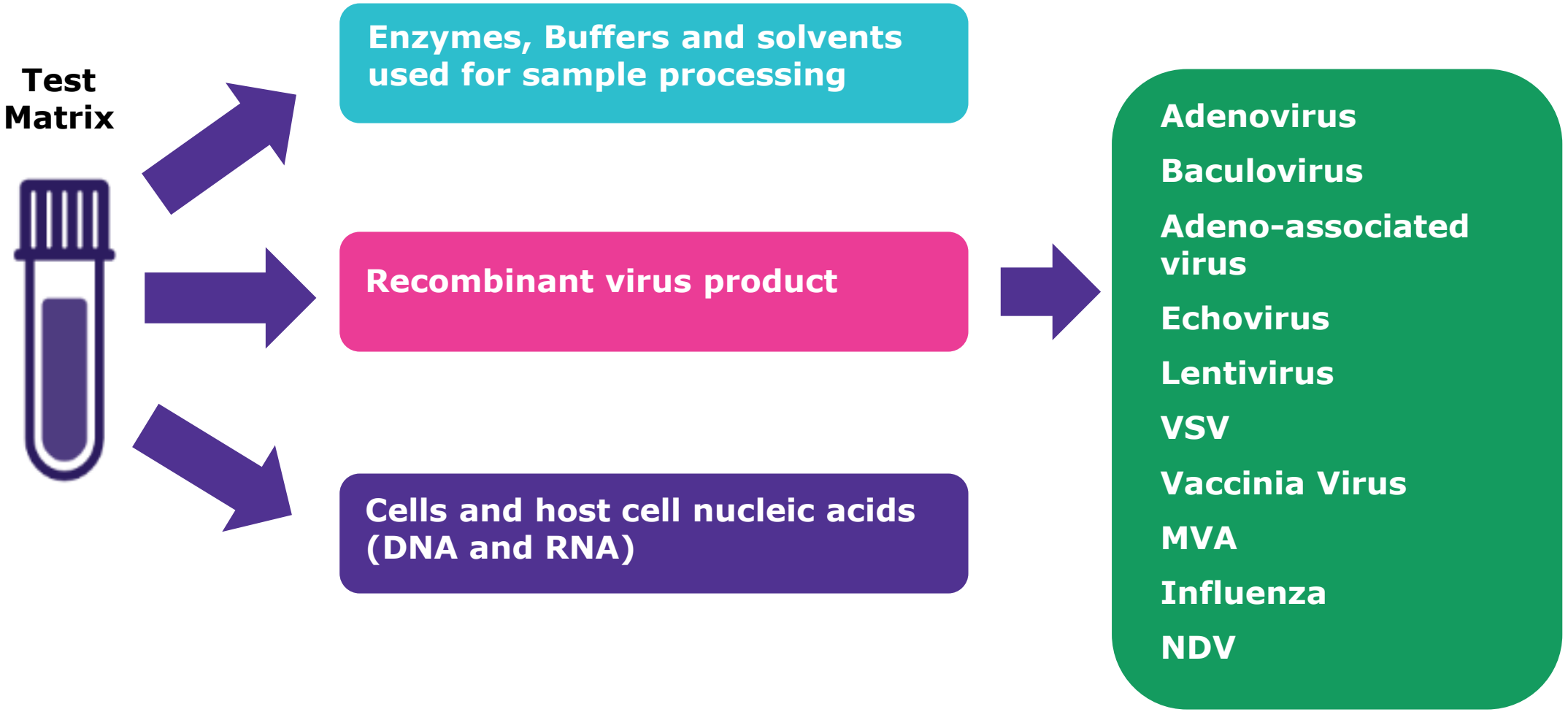
- **Due to the broad specificity nature of the NGS-AAT assay the limit of detection (LOD) of the assay is set as the least sensitive virus tested.**
  - The data indicates the LOD of the NGS-AAT method in all cell lines tested is 1e4 virus genome copies (vgc) per 1e6 cells or 1 vgc/100 cells.

- **LOD verified in client specific cell lines and consistent with generic validation.**

MERCK

Matrix variability

What does a matrix consist of for non cell-bank samples



Matrix Variability
Considerations for different backgrounds

BioReliance®

Things to remember for NGS

1. Agnostic analysis
2. Generally no exponential amplification for agnostic approach, so background nucleic acid content matters greatly in terms of concentration.
3. WHO endorsed NGS reference material was qualified in Adenovirus background only (1e9 Viral Genome Copies/mL)

Specific Challenges

1. Host cell nucleic acids
   - Presence and concentration
2. Viral nucleic acids
   - Presence, genome length, concentration.

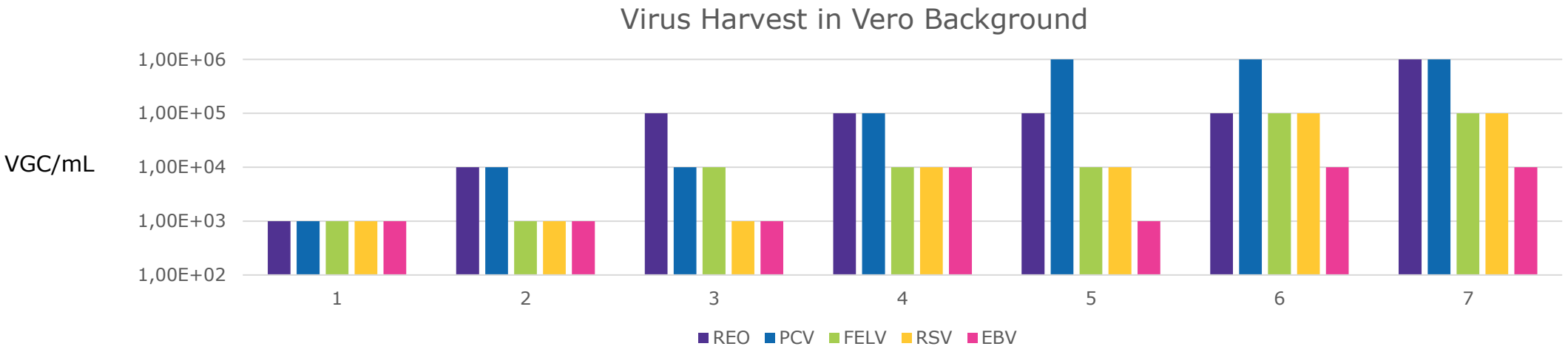
Potential Solutions

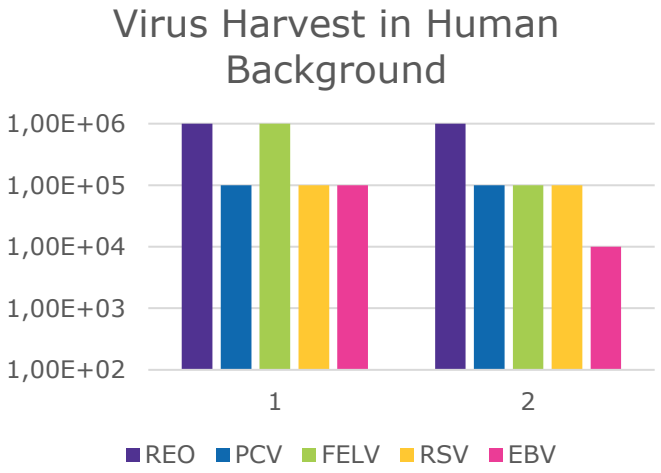
1. Nucleic Acid degradation (e.g. Ribosomal RNA depletion, Dnase, Benzonase® endonuclease)
2. Viral nucleic acid depletion

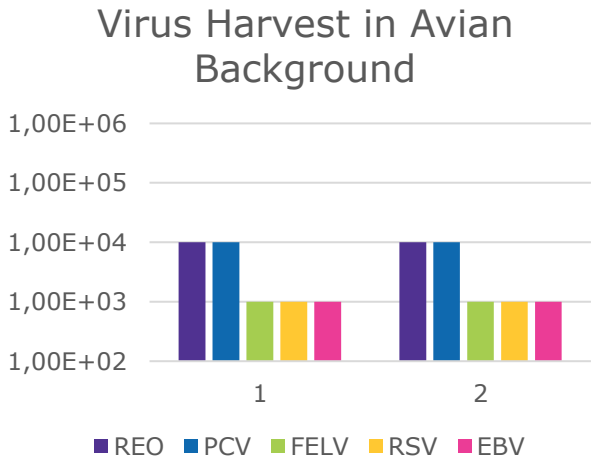
MERCK

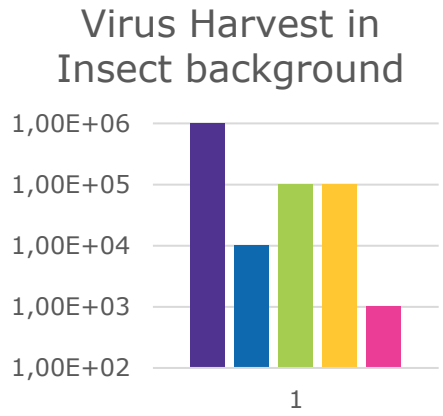
A CRO perspective
LOD may differ depending on matrix

BioReliance®







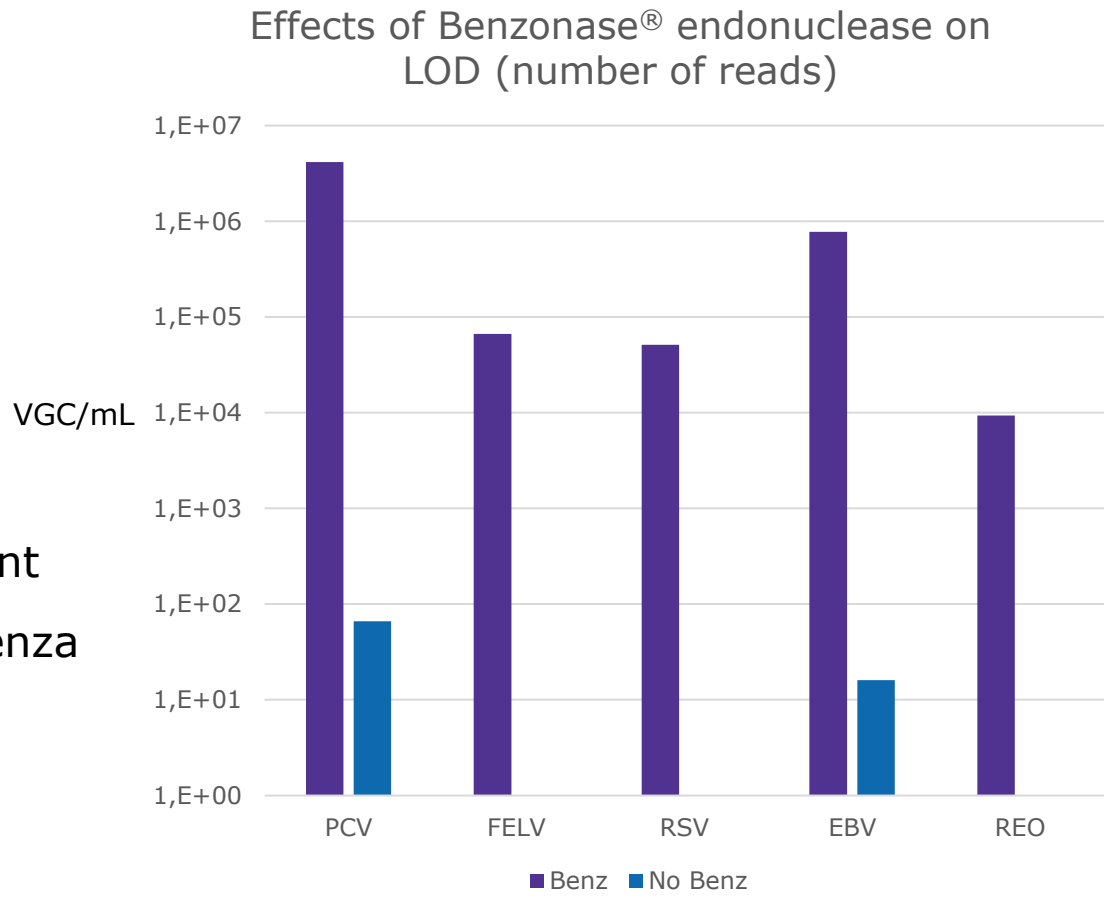


MERCK

BioReliance®

Effects of Benzonase® endonuclease (a case study) in non-viral material

- Issues in US around H5N1 in herds
- Screening of FBS using NGS for specific pathogens only (influenza)
- Demonstrate method suitability using 5 WHO endorsed NGS reference reagents
- Processed:
  - WITH Benzonase® endonuclease pre-treatment
  - NO Benzonase® endonuclease pre-treatment
- Screening focused on 5 virus spikes and Influenza related sequences



MERCK

A CRO Perspective
Summary of Observations

BioReliance®

A CRO Perspective

- Cell lines behave relatively consistently in terms of LOD using representative viruses
- Complex matrices (e.g. bulk harvests, Master virus seeds) may show differences in LOD depending on matrix composition and may require matrix specific processing steps.
  - Key variable seems to be recombinant virus type and concentration
  - "Prior Knowledge" may help predict LOD in certain matrices- A CRO Advantage!
- One representative complex matrix does not currently exist
- Spiking studies using matrix specific representative material will continue to be performed, although this can likely be streamlined through LOD verification procedures instead of full spiking studies

Key Questions:

- Is there a minimum acceptable LOD?
- What would a true infection look like at the point of testing?
  - Is this assessed on a product by product basis?

MERCK

Acknowledgements

Leyla Diaz

McKenzie Landgraf

Alison Armstrong

Global NGS Team

Visit our Posters

Rebecca Bova

Amber Overgard

BioReliance®

Contract Testing Services