BioReliance®
Contract Testing Services

Implementing NGS for adventitious virus detection in a live virus vaccine

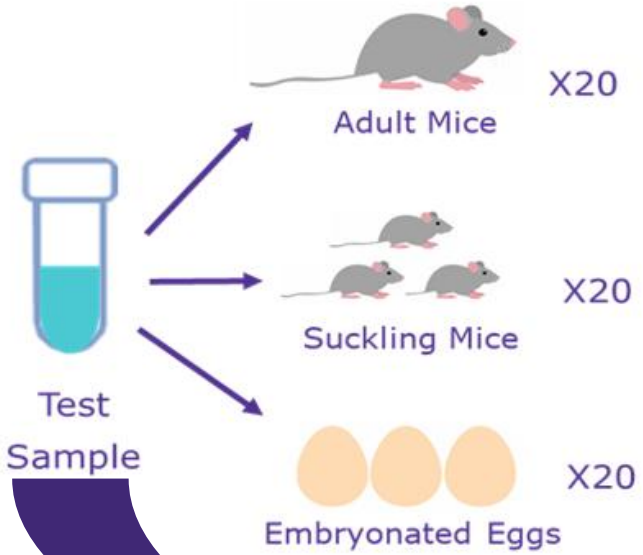
Leyla Diaz
November 10, 2026

NGS as an appropriate replacement for the *in vivo* adventitious virus test

BioReliance®

Traditional *in vivo* method

- Well-established method utilizing animals to detect adventitious virus in a biological material
- Viral contaminant indicated by the impact of infection on the host animal
- No direct identification of a contaminant without further testing by *in vitro* or molecular methodologies



NGS Alternative

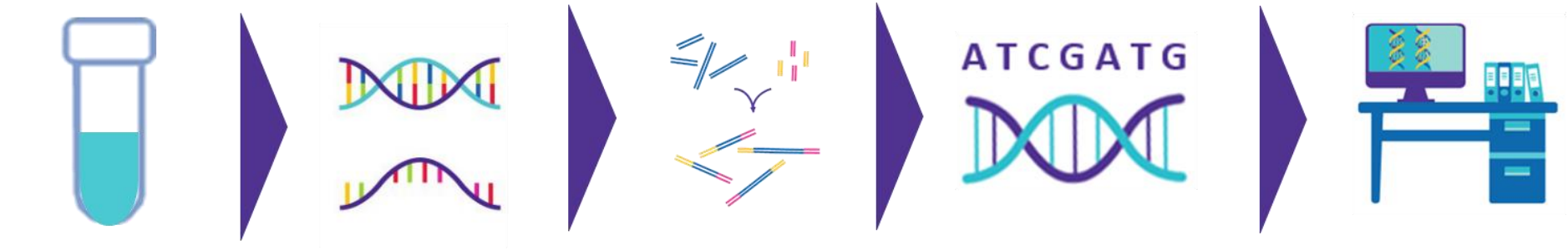
- ✓ Broad spectrum virus detection
- ✓ Ability to demonstrate comparable or better virus detection
- ✓ Reduction in use of animals for virus detection methods

Suitability of *in vivo* replacement supported by:

- Peer-reviewed studies demonstrating ability of other methods to provide more reliable virus detection
- Industry trend to reduce the number of animals used in testing
- Supported by recommendations in ICH Q5A (R2)

BioReliance®

NGS Method Overview



Broad Detection Coverage

NGS allows for simultaneous detection of a wide range of viruses without the need of prior knowledge of specific agents.

Sequences all nucleic acid in the test sample to detect both RNA and DNA viruses

NGS method validation

- Demonstrate that the method is suitable for its intended purpose considering the sample type and testing approach.
- Modular validation confirms that each step of the method behaves as expected.
  - Provides gating criteria to move to next step
  - Allows for flexibility of updating individual steps without the need to revalidate the entire process.
- Virus detection methods are qualitative limit tests (ICH Q2)
  - Specificity and sensitivity assessment

 *Nucleic Acid Extraction*	Minimum quantity and quality for nucleic acid yields
 *DNA Library Preparation*	Acceptable library yield and fragment size range
 *Sequencing*	Quality score for sequencing data and cluster density (if applicable)
 *Data Analysis*	Confirmation of complete analysis run with no errors

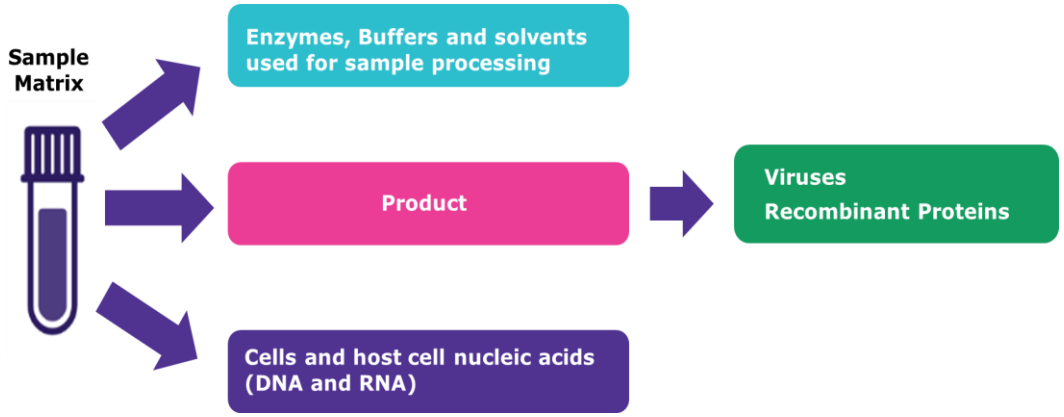
Demonstration of sensitivity and specificity

Assay specificity evaluated by:

- Recovery of positive controls
- Recovery of reference virus spikes from spiked samples
- No recovery of virus spikes from unspiked samples

Assay sensitivity – Limit of Detection (LOD)

- LOD depends on the test article matrix
- Assessments of LOD are accomplished through spiking studies
  - A spiking study is performed in an end-to-end analysis in the test matrix to demonstrate the limit of detection
  - Reference viruses used in spiking study to demonstrate LOD across multiple virus types



Spiking Material

BioReliance®

- Ph. Eur. 2.6.41 Recommends the use of model virus spikes or relevant infected cell models
- Spike viruses should represent viral diversity
  - Structure (enveloped/non-enveloped)
  - Nucleic acid content (DNA, RNA, single or double stranded)
  - Size
  - Morphology
  - Chemical resistance
- Virus stocks should be well characterized
- WHO International Reference Panel for Adventitious Virus detection is recommended as the minimum model virus panel.
  - Additional viruses can be added based on a risk assessment

Virus name	Genome	ss/ds	Genome Size (kb)	Particle size (nm)	Env.	Chemical Resistance
Epstein Barr Virus (**EBV**)	DNA	ds	172	120-180	Yes	Low/Medium
Mammalian orthoreovirus type 1 (**REO**)	RNA	ds	23.6	80	No	Medium/High
Human respiratory syncitial virus (**RSV**)	RNA	ss	15	150-200	Yes	Low/Medium
Feline Leukemia Virus (**FeLV**)	RNA	ss	8.5	80-100	Yes	Low
Porcine Circovirus (**PCV**)	DNA	ss	1.8	16-18	No	High

Replacement of *in vivo* virus detection method

BioReliance®

- **Sample:** Bulk harvest material for quadrivalent influenza vaccine
  - Four separate influenza strains per lot
  - Manufactured using SPF eggs
    - Allantoic fluid matrix
- **Traditional testing**: *in vivo* virus testing for every lot specified in Ph. Eur. 2.6.16.
- **Alternative approach**: Validate a suitable NGS-based alternative.

Validation strategy:

- Leverage generic modular validation.
- Use reference virus panel to demonstrate
  - Specificity
  - Sensitivity
- Each strain is spiked with all 6 viruses in duplicate at different genome copy level
- Criteria for detection
  - 1 virus specific hit
  - 7/8 replicates



Spiking Study Results

BioReliance®

Sample + 1e4 spikes					
EBV	RSV	FeLV	PCV	Reo	BVDV
+ +	+ +	+ +	+ +	+ +	+ +
+ +	+ +	+ +	+ +	+ +	+ +
+ +	+ +	+ +	+ +	- +	+ +
+ +	+ +	+ +	+ +	+ +	+ +
8/8	8/8	8/8	8/8	7/8	8/8

All viruses detected in 7/8 replicates

Sample + 1e3 spikes					
EBV	RSV	FeLV	PCV	Reo	BVDV
+ +	+ +	+ +	- -	- -	+ +
+ +	+ +	+ +	+ +	- -	+ +
+ +	+ +	+ +	- -	- -	- +
+ +	+ +	+ +	- -	- -	+ +
8/8	8/8	8/8	2/8	0/8	7/8

EBV, RSV, FeLV and BVDV detected in 7/8 replicates
PCV and Reo not detected in 7/8 replicates

Sample + 1e2 spikes					
EBV	RSV	FeLV	PCV	Reo	BVDV
- -	+ -	- -	- -	- -	- -
+ +	+ +	- -	- -	- -	- -
+ -	- -	- +	+ -	- -	- +
+ -	+ +	- +	- -	- -	+ -
4/8	5/8	2/8	2/8	0/8	2/8

All viruses detected in 7/8 replicates

Sample + 1e6 spikes					
EBV	RSV	FeLV	PCV	Reo	BVDV
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
4/4	4/4	4/4	4/4	4/4	4/4

Positive Control

BioReliance®

Spiking Study Results Summary

Sample Tested	EBV	RSV	FeLV	PCV	Reo	BVDV
Sample +1e6 spike	+	+	+	+	+	+
Sample +1e4 spike	+	+	+	+	+	+
Sample +1e3 spike	+	+	+	-	-	+
Sample +1e2 spike	-	-	-	-	-	-
Sample + no spike	-	-	-	-	-	-

	Genome copy per mL	TCID50 equivalent per mL
Epstein Barr Virus	1000	29.73
Respiratory Syncytial Virus	1000	1.1
Feline Leukemia Virus	1000	0.43
Porcine Circovirus	10000	0.44
Reovirus	10000	12727.27
Bovine Viral Diarrhea Virus	1000	8.86

- Results showed:
  - Detectability of EBV, RSV, FeLV and BVDV at 1,000 gc/mL
  - Detectability of PCV and Reo at 10,000 gc/mL

BioReliance®

Conclusions

- NGS was able to detect representative virus spikes at a suitable limit of detection, in-line with previous matrices published through worldwide studies between 1e2 and 1e4 Viral genome copies per mL
- Higher Limit of Detection in REO and PCV is not unexpected given the nature of those 2 viruses
  - Reo- Double-stranded RNA virus
  - PCV- Small single-stranded DNA virus
- NGS-based virus detection testing was implemented for bulk harvest testing of vaccine batches
  - Used routinely as part of lot release program since 2023
- Advantages of using NGS vs animal-based method
  - Reduced assay turn around time
  - Reduction in invalid assays
  - Reduced investigations for unconfirmed out of specification results

Questions?

BioReliance®

Contract Testing Services

Leyla Diaz

Technical Consultant

Leyla.Diaz@milliporesigma.com

The vibrant M, Blazar, Aptegra, BioReliance are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
©2024 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

