Ensuring the virus safety profile of your product: recommendations from the proposed revision to ICH Q5A

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ICH Q5A revision

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A Draft Revision of ICH Q5A is under Public Consultation

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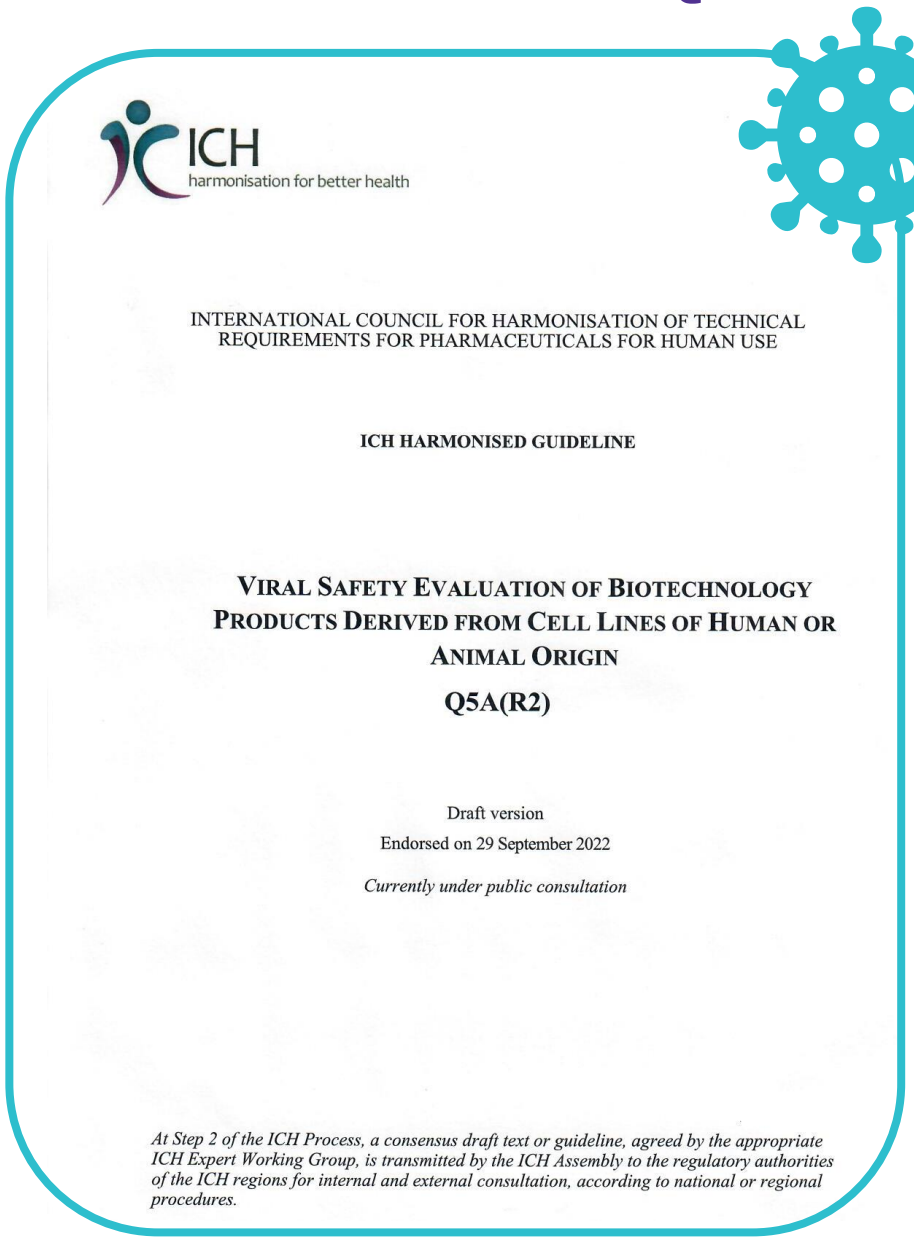

ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

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

Q5A(R2)

Draft version

Endorsed on 29 September 2022

Currently under public consultation

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

The new revision will apply to new classes of products, such as virus-like particles, viral vectored products such as vaccines and gene therapies using novel mammalian and insect-based expression systems

For some of these products, viral clearance may need to be demonstrated. These products may include:

- Adenovirus vectors
- Adeno-associated virus vectors
- Virus-like particles (VLPs)
- Baculovirus-expressed vectors, VLPs, proteins

Revision likely to be finalized late 2023/ early 2024

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Viral Safety Strategy

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Safe sourcing and testing of raw materials

Verify absence of viral contaminants at appropriate stages

Verify capacity of manufacturing process to remove or inactivate potential viral contaminants

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Viral Safety Strategy

BioReliance®

Safe sourcing and testing of raw materials

Verify absence of viral contaminants at appropriate stages

Will this strategy, that has worked so well for traditional biopharmaceuticals, work for novel biological therapies?

Verify capacity of manufacturing process to remove or inactivate potential viral contaminants

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Prevention and Detection of Viral Contamination are in Focus When Removal is not Feasible

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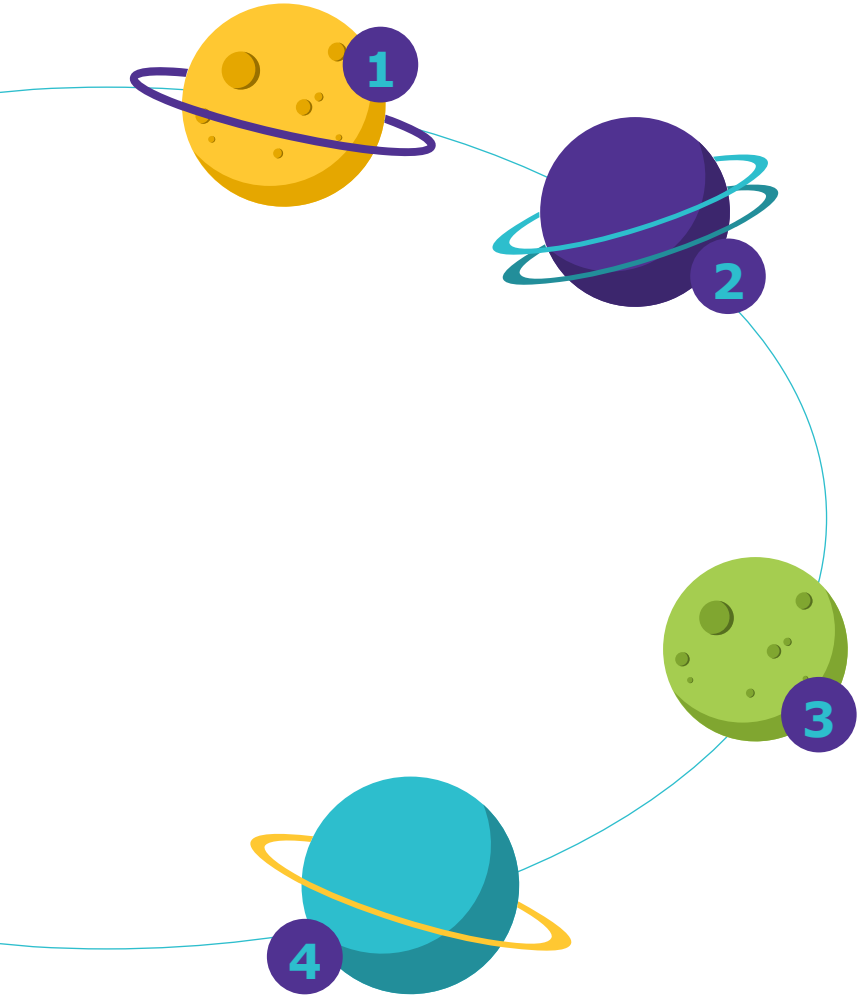

Viral safety assurance strategies are expected to be implemented based on risk and the ability of the vector to withstand clearance methods.

1 Prevention
- Quality of raw/starting materials
- Containment in the facility

2 Detection
- Raw materials
- Entire process

3 Viral clearance required when there is no impact on quality

4 Validation of clearance is required using appropriate model viruses

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Viral Clearance

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Potential for Viral Clearance

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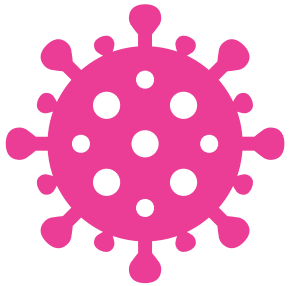
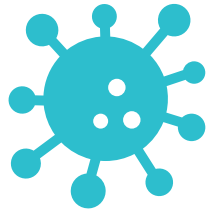
Enveloped Viral Vectors and Cell Therapies

- Inactivation steps will denature enveloped viral vectors or cell therapies
- Due to their large size, viral filtration is not a viable clearance option
- Virus inactivation/removal measures for **culture medium and supplements** is recommended for these products



Non-Enveloped Viral Vectors

- Resistant to inactivation procedures for enveloped viral contaminants
- Potential exists to separate small non-enveloped viral vectors from larger enveloped viral contaminants by large pore virus filtration
- Chromatography steps may contribute to clearance of enveloped viral contaminants



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A Multi-Pronged Approach

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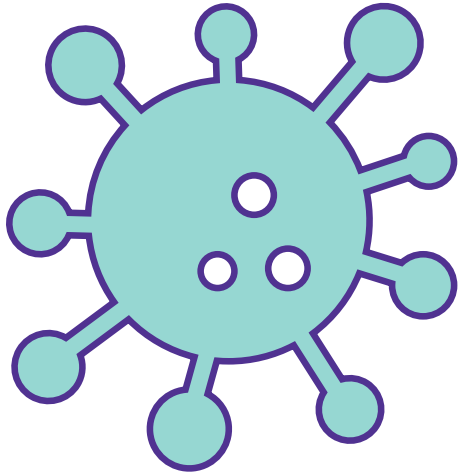
"***When possible, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing or ultraviolet C irradiation can be used as additional virus risk mitigation measures***."

ICH Q5A (R2)



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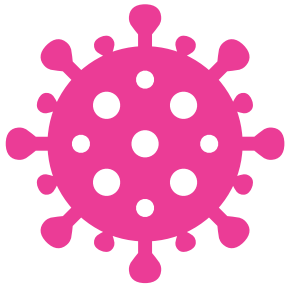

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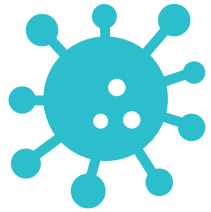

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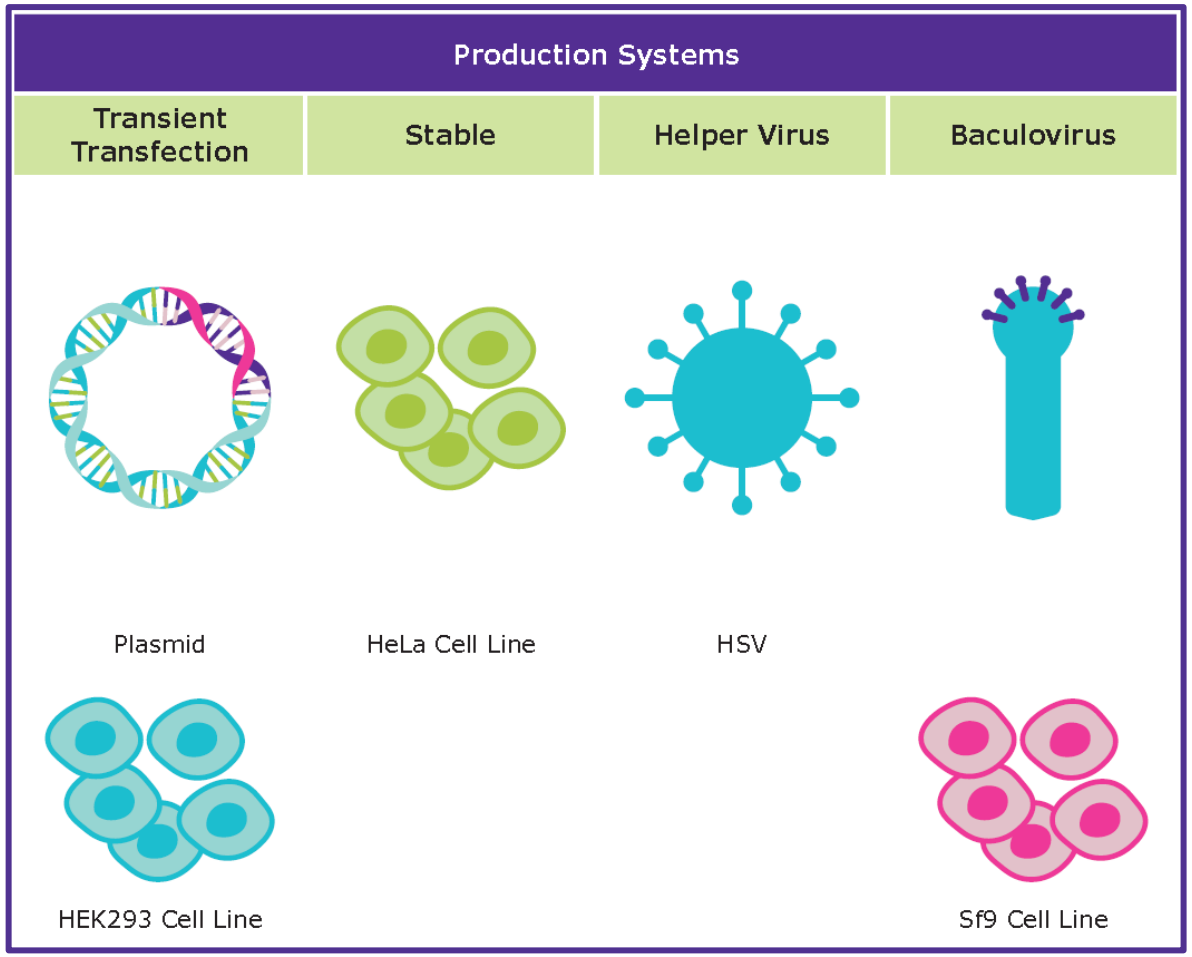

- **Multiple means exist to produce AAV vectors**
- **The panel of viruses used in a viral clearance study depends on potential viral contaminants that may be introduced during production of the virus**

Which viruses do I use for an AAV viral clearance study?

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Virus panel

Virus Panel for AAV Clearance Dependent on Expression System

Considerations for Viral Clearance Studies

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- Proper scale down of steps
- Pre-study
- Determine kinetics of inactivation
- Sanitization of chromatography resins
- Aged resin studies

EMEA/CHMP/BWP/268/95
Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses

ICH Topic Q5A (R2); Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cells Lines of Human or Animal Origin

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Viral Safety at All Stages

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Is a virus or virus-like particle present in the cells or bulk harvest? If yes, then this represents a different risk profile than cells or bulk harvest where no virus can be detected.

Using CHO-derived recombinant proteins as a model, viral clearance data may be expected as part of an IND/IMP application

Are you using a plasmid transfection system, with no animal-derived products, and you determine the risk of viral transmission to be low?

Discuss with regulators. A viral clearance study may not be required before an IND/IMP application.

Note that ICH Q5A outlines the data that should be submitted in marketing applications and registration packages

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Baculovirus expressed virus/virus-like product

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Process Step	Log_{10} Reduction	
	BACV	VSV
Inactivation	≥5.15	≥ 4.41
Chromatography	1.82	3.84
Virus Reduction Filtration (Large Pore)	≥ 4.91	≥ 4.83
Overall Reduction	≥ 11.88	≥ 13.08

BACV expression system small, non-enveloped virus product

BACV expression system small, non-enveloped virus product

Process Step	Log_{10} Reduction	
	BACV	VSV
Inactivation	≥5.05	≥ 4.37
Chromatography	4.13	3.70
Virus Reduction Filtration (Large Pore)	≥ 5.08	≥ 4.65
Overall Reduction	≥ 14.26	≥ 12.72

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Virus/virus-like product expressed in human cell line

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Human cell line Small, non-enveloped virus product

Process Step	Log_{10} Virus Reduction				
	Enveloped Model Viruses			Non-Enveloped Viruses	
	XMuLV	BVDV	PRV	EMC	PPV
Inactivation	≥4.62	4.13	≥5.02	ND	ND
Chromatography	3.84	3.28	3.24	2.19	4.09
Overall Reduction	≥8.46	7.41	≥8.26	2.19	4.09

ND – clearance not evaluated

Process Step	Log_{10} Reduction			
	Enveloped		Non-Enveloped	
	BVDV	PRV	EMC	PPV
Inactivation	≥ 5.63	≥ 5.71	ND	ND
Chromatography	NR	≥ 5.51	NR	1.90
Overall Reduction	≥ 5.63	≥11.22	NR	1.90

Human cell line Small, non-enveloped virus product

ND – clearance not evaluated
NR – no reduction

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How much clearance?

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- The revision of ICH Q5A does not define viral clearance specification for viral vectors, VLPs or other novel products
- AAV can be produced in a variety of systems, and the potential and known contaminants is dependent on the expression system
- It is probable, however, that any expression system that includes a helper virus or an insect virus will be expected to demonstrate the capacity of the manufacturing process to clear in excess of the amount of virus used in that system.

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A Multi-Pronged Approach

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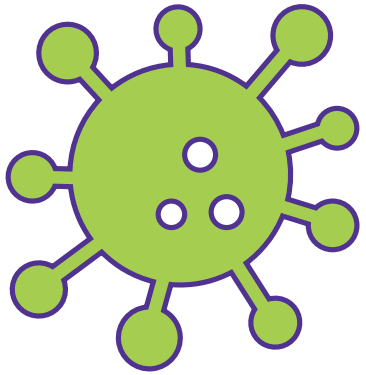
"***Some virus clearance steps used during production of genetically engineered viral vectors and viral vector-derived products may not be as effective as when used for recombinant proteins. In such cases, considerations for further risk reduction (e.g., treatment of raw materials, extensive testing for broad virus detection) should be applied***."

ICH Q5A (R2)



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Viral clearance for non-enveloped viral vectors and VLPs

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- The recent revision of ICH Q5A requires viral clearance studies for non-enveloped viral vectors and virus-like particles
- The design of viral clearance studies for non-enveloped viral vectors is similar to the design of a study for a recombinant protein or monoclonal antibody
- The virus panel used in clearance studies may be unique to each vector product, its expression system and potential contaminants
- Our scientists will help you design a clearance study for your non-enveloped gene therapy vector

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Adventitious Agent Detection using Next Generation Sequencing

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NGS is key to replacement of traditional methods in a comprehensive biosafety testing strategy

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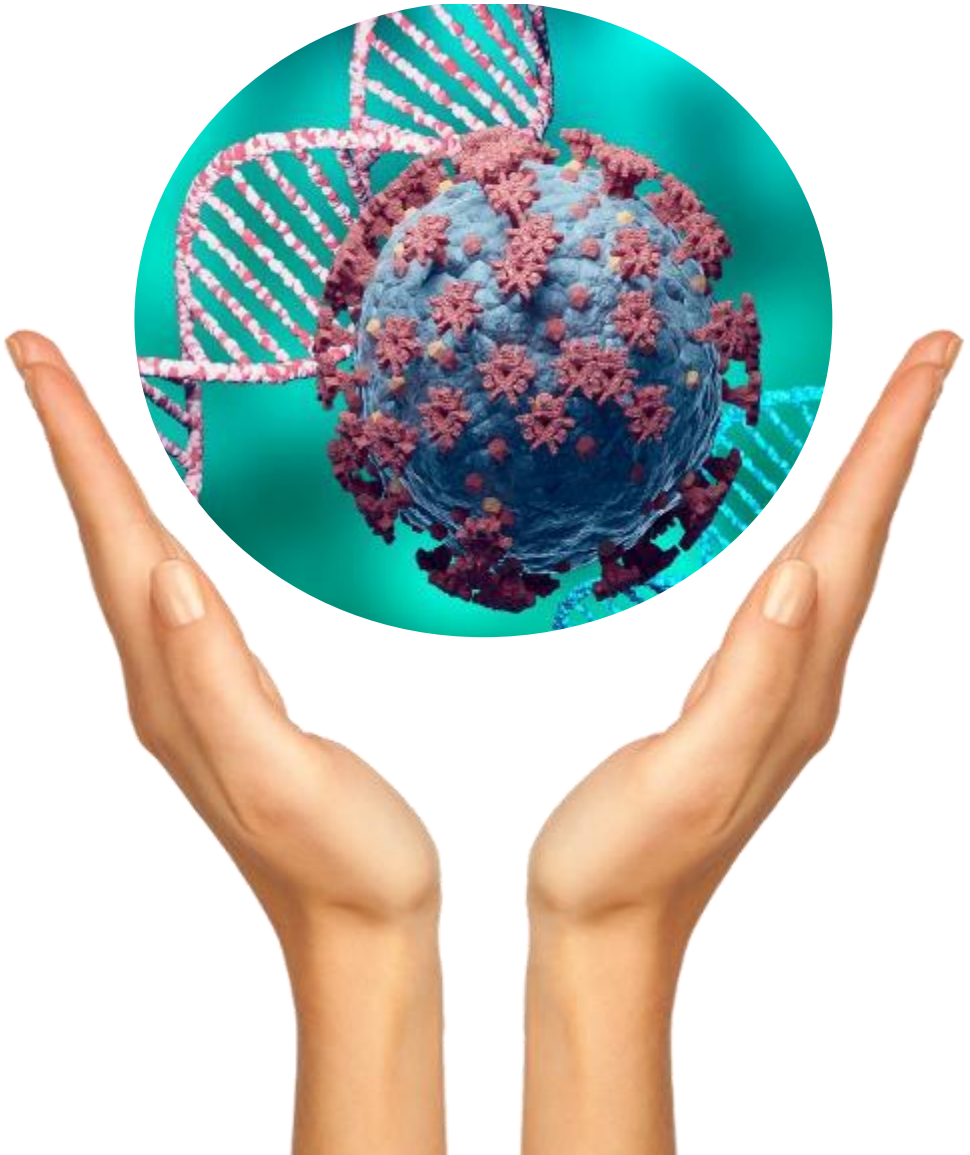
NGS applications as part of a comprehensive biosafety strategy

Characterize and demonstrate the safety of biological products as part of a comprehensive biosafety strategy, including:

- Cell line characterization
- Virus seed characterization
- Virus identification during contamination events
- Raw material testing



NGS as an appropriate replacement for traditional methods

Sequence-agnostic, broad-range virus detection makes NGS an ideal replacement for current virus testing methodology by:

- Removing subjectivity
- Providing a faster alternative
- Supporting 3R principles to replace animal use

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Evolution of NGS over the years

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Early focus on investigations & general curiosity:

What can this technology do?

Fast-tracking COVID19 vaccine development

2005: Roche/454 releases the Genome Sequencer

2010: Illumina releases the HiSeq™ 2000 system

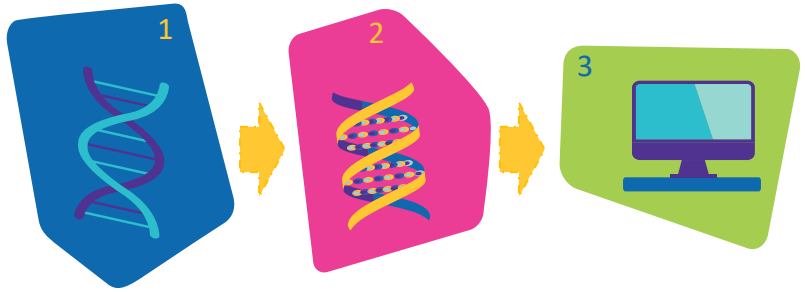
2015: Oxford Nanopore Technologies releases the MiniON™ system

2000 | 2002 | 2004 | 2006 | 2008 | 2010 | 2012 | 2014 | 2016 | 2018 | 2020

2017: Illumina releases the NovaSeq® system

Large-scale, high-throughput, agnostic sequencing with vast utility

Three Basic Steps



Sample Processing | **Sequencing** | **Data Analysis/ Bioinformatics**

Precision medicine

Diagnostics

R&D

Biopharma/ biosafety

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How does NGS contribute to the overall safety profile?

- NGS generates genomic sequence data for all sequences present in a test agent (unbiased method)
- Sequences generated are queried against an appropriate database (generally viral) in a BROAD SCREEN.
- Sequence profile of suspect viral agents are revealed and qualified.
- NGS only identifies the presence of sequence signatures but does not provide information about whether it is infectious.
- Positive hits in NGS typically require follow-up/investigation (e.g. With *in vitro* culture-based methods)
- Can be used on a wide variety of samples and intermediaries:
  - Virus, Cells
  - Bulk harvest
  - Final product

When is it appropriate to use NGS technology?

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- Infectious products or intermediates where a neutralizing antibody is unavailable for culture-based methods
- Cell line screening in lieu of animal studies and *in vivo* testing
- Broad virus screening assay to complement or round out the safety profile of the test agent
- Product release testing as an alternative method to confirm safety profile
- Instances where traditional assays would not be expected to identify a potential contaminant of interest or no traditional assay exists.
- Replacement for *in vivo* and *in vitro* methods (with appropriate qualification/validation testing).
  - Consistent with 3R Principles to reduce animal use
  - Provides faster alternative to traditional testing
  - Consistent with proposed revisions to ICH Q5A

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

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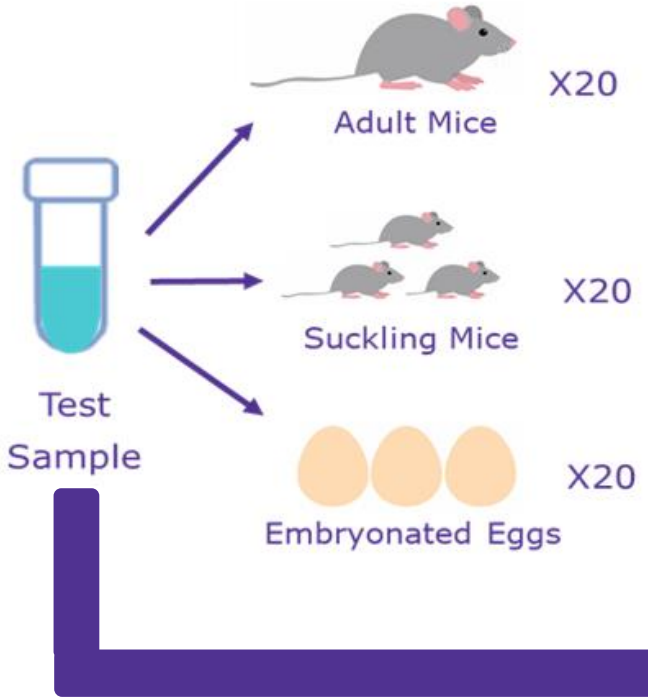
Traditional *in vivo* method

- 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

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Acceptability by Agencies
Adventitious Agent Testing

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- Lots of progress in this area within the past several years (driven by COVID).
- Recent release of proposed revisions to ICHQ5A focus on NGS as a replacement for *in vivo* testing.
- Experience working with clients and regulators on NGS AAT testing strategies for:
  - Drug Substance/Drug product testing
  - In Vivo replacement (e.g. ICH Q5A revisions)
  - In Vitro replacement (in special circumstances)
  - Vaccine products
  - Master cell bank testing
- Industry drive towards NGS
  - Removes subjectivity
  - Faster alternative
  - Consistent with 3R principles to reduce animal model use
- Spiking studies available, as requested by the agencies
- Successful implementation in testing program for preclinical through commercial products

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Assay Overview and Details
Adventitious Agent Testing

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- Provides assessment of "unknown virus" sequences in the client's test sample.
- Queries all sequences generated against proprietary viral database.
- Two main offerings for this
  - AAT for cell lines/crude harvest samples
  - AAT for virus-based samples
- Information provided:
  - Viral sequence hits and qualification of hits
  - Summary of expected sequences (based on test system- e.g. ERV's)
  - Focuses on viruses that impact the test system as well as human health
- Assay are run on the NextSeq2000 to maximize number of reads
- TAT: 42 Days (without forecast)
- Specification: **REPORT RESULTS**
- **What it does not do:** Does not tell you if a virus is infectious (limitation of short read technology)
- **Spiking Studies required for late phase products (aka: PSQ's)**

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The cell-based NGS method addresses all key elements recommended by industry guidance

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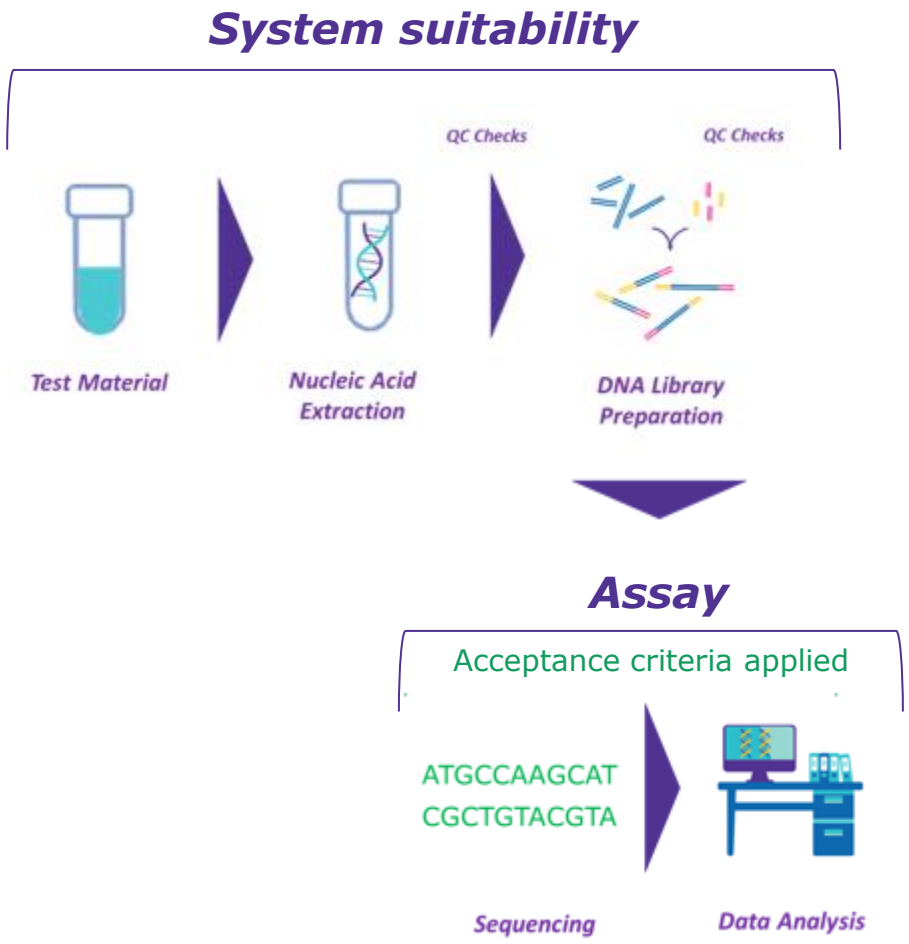

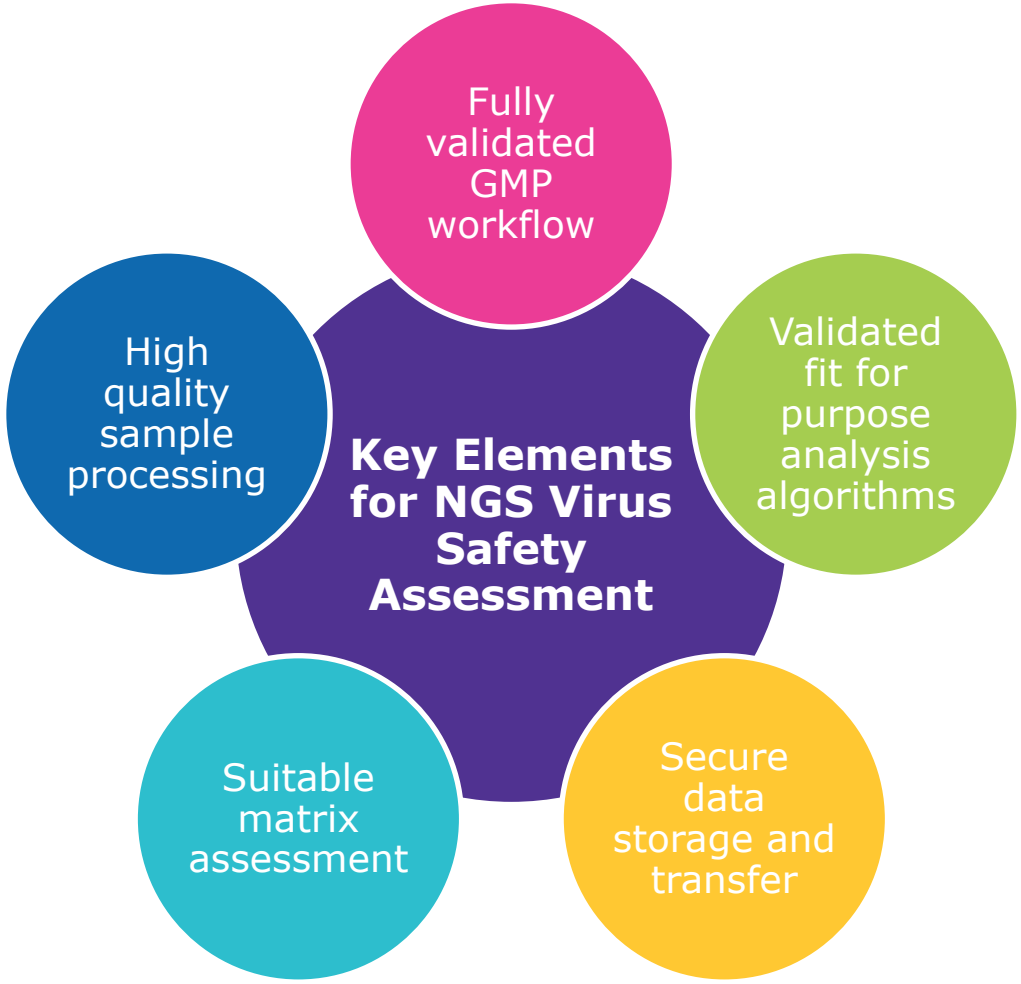

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Data analysis is performed with a robust, validated bioinformatic algorithm and curated database

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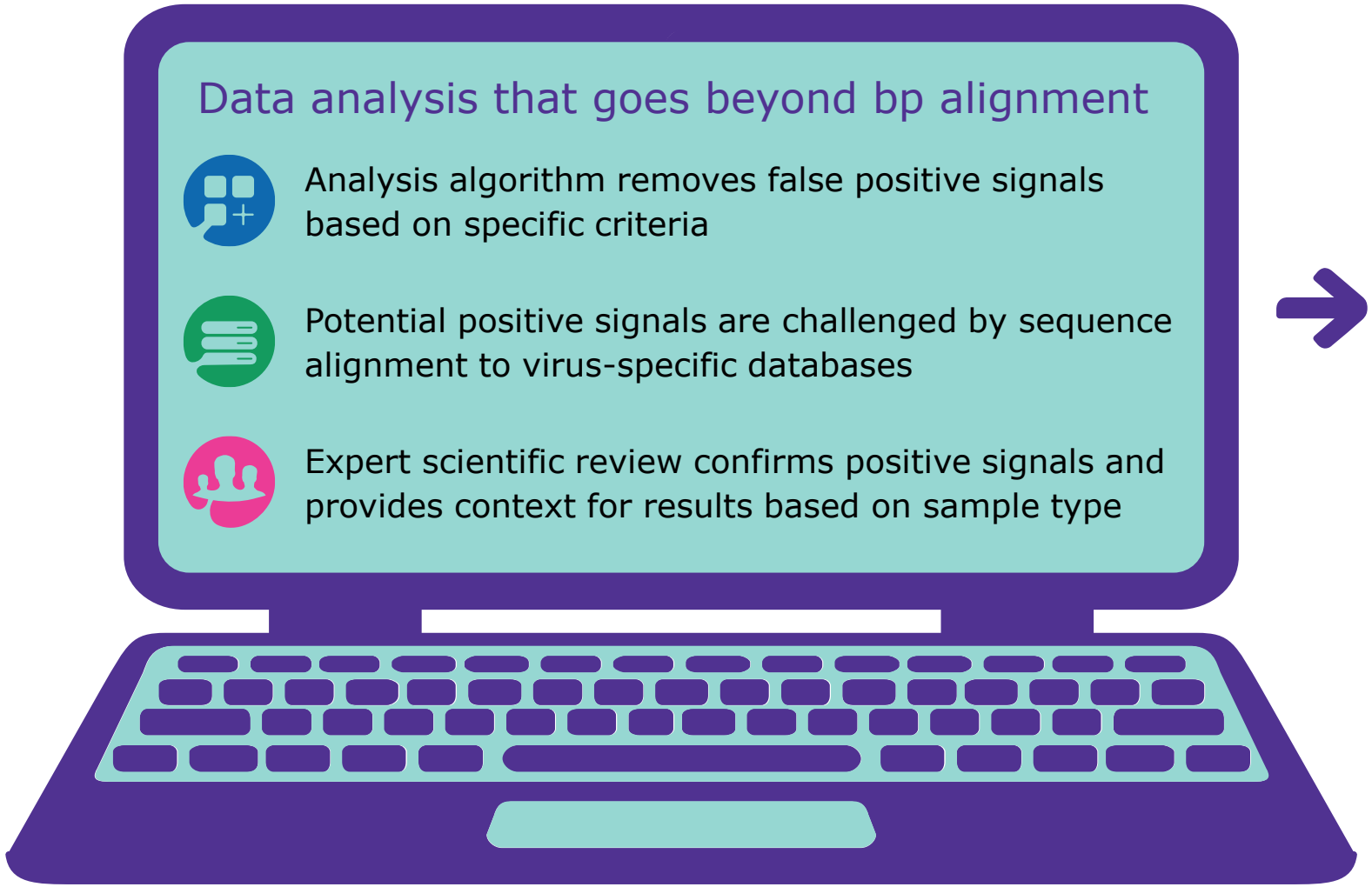

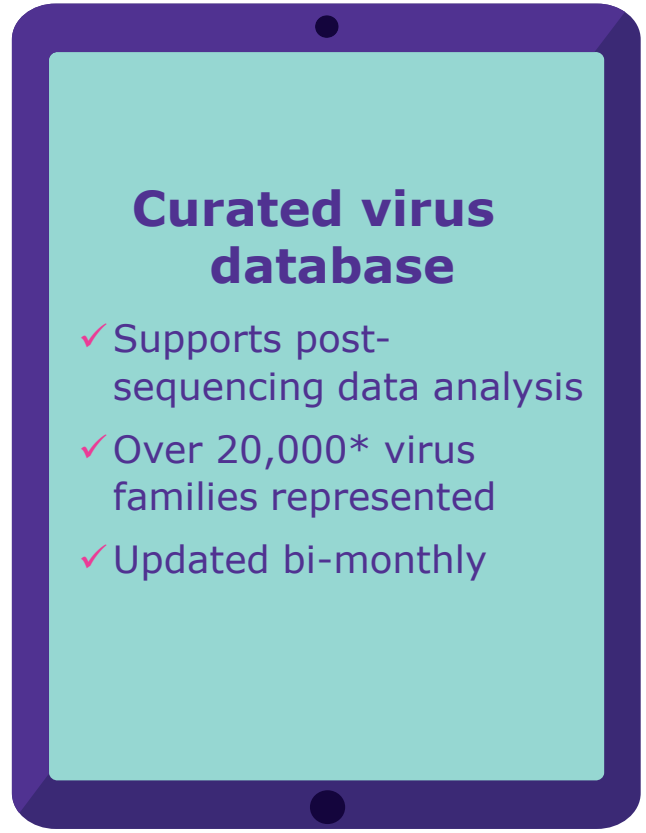

*Depending on classification of sub-families

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Sample results for 293 cells with scientific annotation

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FINAL RESULT:

No exogenous transcripts were detected in the sample based on analysis against viral database

ASSAY NUMBER	SPECIFICATION	RESULT
706522GMP.BSV	Report Result	See Appendix

VIRAL SEQUENCES DETECTED[1]

A transcriptome analysis of the Sponsor's test sample, an HEK293 derived cell line, was conducted. No exogenous transcripts were detected in the sample based on this analysis against 2,371,511 entries within BioReliance's curated viral database.

Human endogenous retroviruses (HERVs; multiple GenBank accession numbers (accnos)) were detected via this analysis, with mapping of the raw sequence reads against select HERV reference genomes (*e.g.* NC_022518 and X82272) revealing nearly 100% sequence coverage with high sequence identity. It is known that chimeric HERV-cellular sequences are a feature of HERVs and this mapping profile is consistent with that observation (Sokol *et al.*, 2016). Furthermore, while expression of HERV sequences is a feature of all human cell lines, no infectious particle has ever been recorded and most sequences are highly defective (Bannert and Kurth, 2004). Therefore, these sequences do not represent "contaminating" viral sequences.

Human adenovirus type 5 sequences were also identified within the test sample. Mapping analysis revealed a near 100% sequence match to the first ~4.3 kb of human adenovirus type 5 (Genbank accno AC_000008). Given the test sample origin, this is expected and consistent with this cell line as the HEK293 cell line was generated by transfection with sheared adenovirus 5 and is known to contain these sequences within chromosome 19 (Graham *et al.*, 1977). Therefore, the presence of these sequences does ***not*** constitute viral contaminants derived from the test sample, but are associated with the test system.

Minor sequence fragments (typically <50-mers) that share some sequence similarity with various v
human herpesviruses) were detected, but upon qualification these were found to correspond to c
repeat sequences (*e.g.* GT repeats, poly T/poly C tracts) or have a high degree of sequence sim
very short regions (typically in the 30-50-
expected cellular sequences and do ***not***

No other viral sequences of significance

*[1]Note that due to the nature of this technology (e.g. short
represent intact, infectious or replicating viruses. These re*

*Sokol M, Jessen KM, and Pedersen FS. Utility of next gen
retroviruses. APMIS. 2016 Jan-Feb;124(1-2):127-39. Doi:1*

Bannert N, and Kurth R. Retroelements and the human genome: new perspectives on an old relation. Proc Natl Acad Sci U.S.A. 2004 Oct 5;101 Suppl 2:14572-9. Epub 2004 Aug 13.

Graham FL, Smiley J, Russell WC, and Nairn R. Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. (1977) J Gen Virol 36: 59-72, doi:10.1099/0022-1317-36-1-59

Explanation of human endogenous retrovirus sequences found, consistent with sequence signatures found in HEK293 cells and do not represent contaminating viral sequences

Explanation of human adenovirus 5 sequences identified, which are known to be contained in HEK293 cells and do not constitute viral contamination

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Validation of NGS Methodology

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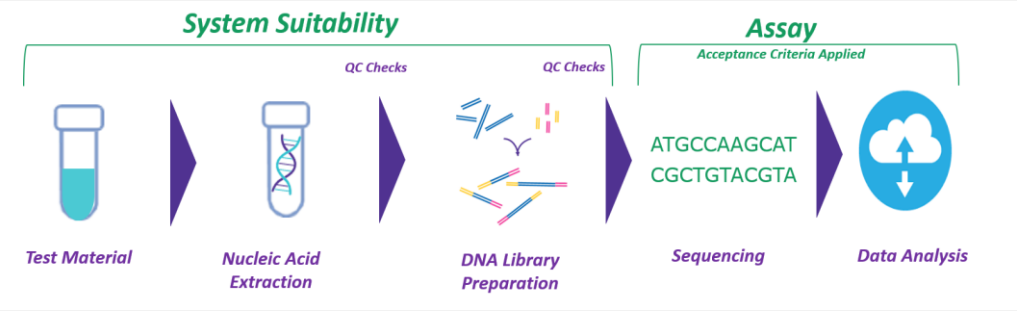
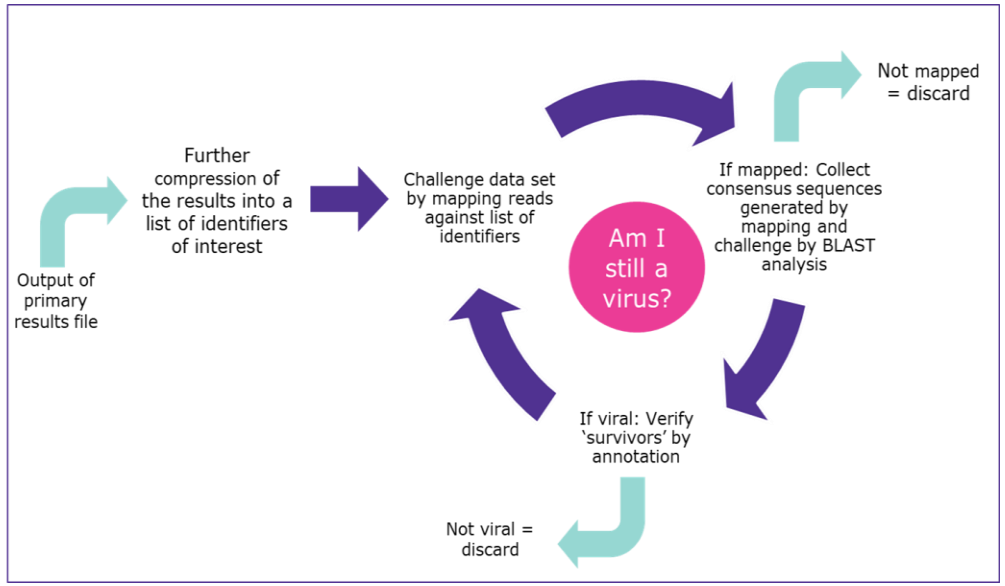


NGS Assay is validated in a Modular Fashion

- Extraction
- Library
- Sequencing
- Bioinformatics



This approach **confirms that each portion of the assay behaves as-expected**.

Limits of detection for client-specific matrices are established through end-to-end analysis with known spikes (per ICH Q5A recommendations).

Full validation package consists of the following:

Generic Validation + Spiking Studies

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Our NGS solution offers robust detection with any cell species

Three (3) RNA viruses from the WHO-recommended reference virus panel were used to assess LOD:

- Respiratory Syncytia Virus (RSV)
- Feline Leukemia Virus (FeLV)
- Reovirus (Reo)

Matrix-specific limit of detection (LOD) for representative cell lines

	Cell Matrix			
	CHO	HeLa	Vero	SF9
LOD*	1.00E+04	1.00E+04	1.00E+04	1.00E+04

*LOD indicates lowest concentration detected for all 3 viruses, expressed as Viral Gene Copies (VGC) / 1.00E+06 cells

All spike-in RNA viruses were detected up to the viral 1e4 dilution in 1e6 cells, indicating an **LOD of 1 VGC / 100 cells**

Cell-based AAT by NGS

- Detection and identification of virus contaminants
- Can be used with any cell bank regardless of cell species
- Transcriptome analysis can detect RNA viruses and mRNA from DNA viruses

What does a Spiking Study need to look like?

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Representative Viruses	
PCV	1e6
REO	1e5
RSV	1e4
FELV	1e3
EBV	1e2

Test Matrix

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

General guidelines:

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

Example of spiking study results

Sample + 1e6 Spikes

EBV	RSV	FLV	PCV	REO	Additional Virus
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
4/4	4/4	4/4	4/4	4/4	4/4

Sample + 1e4 Spikes

EBV	RSV	FLV	PCV	REO	Additional Virus
+ +	+ +	+ +	+ +	+ +	+ +
+ +	+ +	+ +	+ +	+ +	+ +
+ +	+ +	+ +	+ +	- +	+ +
+ +	+ +	+ +	+ +	+ +	+ +
8/8	8/8	8/8	8/8	7/8	8/8

Sample + 1e3 Spikes

EBV	RSV	FLV	PCV	REO	Additional Virus
+ +	+ +	+ +	- -	- -	+ +
+ +	+ +	+ +	+ +	- -	+ +
+ +	+ +	+ +	- -	- -	- +
+ +	+ +	+ +	- -	- -	+ +
8/8	8/8	8/8	2/8	0/8	7/8

Sample + 1e2 Spikes

EBV	RSV	FLV	PCV	REO	Additional Virus
- -	+ -	- -	- -	- -	- -
+ +	+ +	- -	- -	- -	- -
+ -	- -	- +	+ -	- -	- +
+ -	+ +	- +	- -	- -	+ -
4/8	5/8	2/8	1/8	0/8	2/8

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NGS AAT can be incorporated into a comprehensive virus safety strategy for cell banks

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	Checklist of virus safety testing for my cell line	
	Broad Specificity Methods	
	✓ *in vitro* adventitious agent assay	
	✓ ~~*in vivo* adventitious agent assay~~	
	Retroviruses	
	✓ PCR enhanced Reverse Transcriptase	
	✓ Transmission Electron Microscopy	
	✓ Infectivity Assays	
	Species Specific Viruses	
	✓ Virus Specific PCR methods	
	Porcine/Bovine Viruses	
	✓ Culture-based methods (9CFR)	
	✓ Virus Specific PCR Methods	

****NGS AAT Method****

- NGS replaces the *in vivo* adventitious agent test
- Supplements virus safety testing data
- Part of a comprehensive testing package defined by regulatory guidance and risk mitigation assessment

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Select a service provider offering more than just raw data

BioReliance®

Trusted GMP services

- Validated workflows, from sample receipt to final report (per GMP guidelines)
- Assay validation data is part of an FDA Biologics Master File (BMF)

Industry-leading footprint

- Global availability
- Facilities in the US & UK
- Increasing capacity to support growing demand

Secure data, reliable results

- Dependable bioinformatic algorithms
- Dedicated bioinformaticians
- Electronic data delivery
- Secure data management

Expert analysis and QA review

- Expert-delivered data analysis
- QA review & approval
- Detailed reports and raw data available

Support when you need it

- Industry-leading scientific support
- Regulatory support
- Method customization when needed

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Contract Testing Services

Thank you!

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Come see us at our booth or send a message

BioReliance®

Contact Us

SigmaAldrich.com/biosafetytesting

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Thank you!

CGT Testing Services

- Analytical development and product characterization
- Raw and ancillary material testing
- Plasmid DNA characterization
- Cell banking and cell line characterization
- Virus banking and virus bank characterization
- Viral clearance studies
- Drug substance and drug product release testing

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