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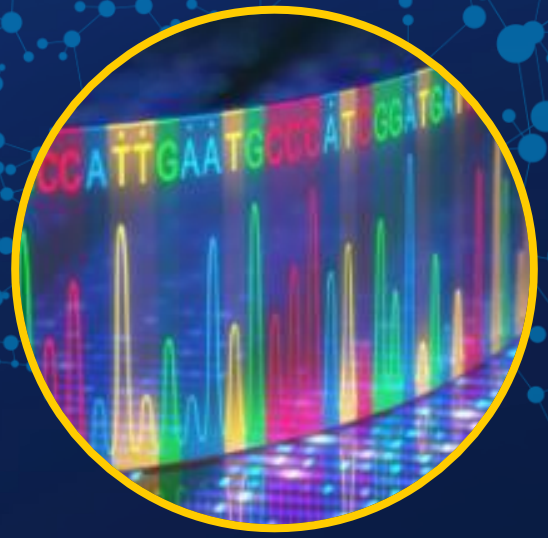
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HTS/NGS for Adventitious Agents: A Regulatory Overview



AFSA/IABS Webinar, Reference Materials for HTS/NGS-Based Adventitious Virus Detection, 10 November 2026

Laurent Mallet, Council of Europe, EDQM

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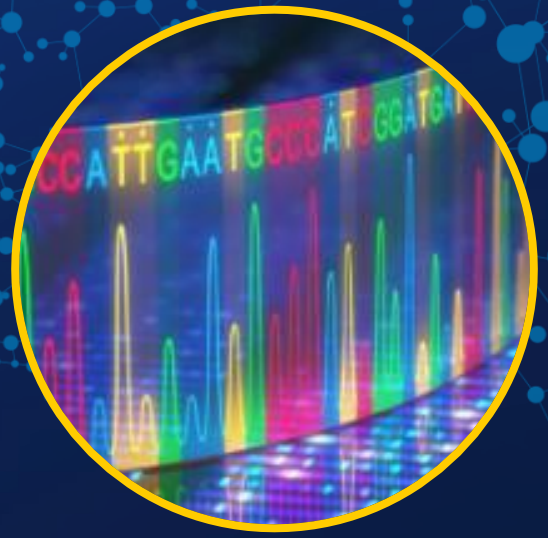
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Outline of the Presentation

- ★WHO recommendations and guidelines
- ★ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
- ★EDQM – European Pharmacopoeia
- ★Indian Pharmacopoeia
- ★Chinese Pharmacopoeia
- ★Other regulatory documents
- ★Conclusion

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WHO recommendations and guidelines



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WHO recommendations/guidelines (1/5)

- **Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks**, Annex 3, TRS No 978: Link

  Adopted by WHO ECBS in October 2010

  Scope: all biological products for human use

  First regulatory recommendations to mention HTS: *"New and sensitive molecular methods with broad detection capabilities are being developed. These are not yet in routine use but, as they become widely available and validated, they will play an increasing role in the evaluation of cell substrates. The sensitivity of these methods, as well as their breadth of detection, should be considered when evaluating their applicability. One of the advantages of some of these new methods is that they have the potential to discover new viruses. These new approaches involve either degenerate PCR for whole virus families or random-priming methods, which do not depend on a known sequence. Analysis of the resulting amplicons has employed sequencing, hybridization to oligonucleotide arrays, and mass spectrometry (103–105)…*

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WHO recommendations/guidelines (2/5)

- **Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks**, Annex 3, TRS No 978: Link
  - … *The new generation of massively parallel sequencing (MPS) methods may have particular utility. They can be applied to detect virions following nuclease treatment to remove cellular DNA and unencapsidated genomes. In this mode, MPS has been used to discover new viruses in serum and other tissues and has revealed the contamination of human vaccines by porcine circovirus (103, 106–110). MPS can also be employed to screen cell substrates for both latent and lytic viruses by sequencing the transcriptome. In this mode, enormous quantities of data are generated and robust bioinformatic methods are required to detect viral sequences by either positive selection against viral databases or negative selection to remove cellular sequences (103, 110, 111). Care is required to exclude false "hits" to viruses due to recognition of transduced cellular sequences present in some viral genomes, or due to viral genes like virokines that have a close homology to cellular genes (103, 105, 111). It is probable that application of methods of this type will be expected or required by regulatory agencies in future. At present the methods have not been evaluated for sensitivity and specificity and should be thought of as powerful investigational tools that can reveal issues that can be explored by more established methods. »*

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WHO recommendations/guidelines (3/5)

- Several WHO recommendations for vaccines published after TRS978 Annex 3 mentionned a similar wording:
  - Example, **TRS 979 Annex 2: Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated)** adopted at WHO ECBS in October 2011

    "New molecular methods with broad detection capabilities are being developed for adventitious agent detection. These methods include: (i) degenerate nucleic acid amplification techniques for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (ii) nucleic acid amplification techniques with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or digital subtraction of expressed sequences; and (iii) high throughput sequencing. These methods may be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and agreement from the NRA."

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WHO recommendations/guidelines (4/5)

- **Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine, Annex 2, TRS No 993** Link
  - Guidance document adopted at WHO ECBS in October 2014
  - Scope: vaccines for human use
  - Objective: *"provide guidance to regulators on the scientific principles of risk evaluation when a signal for a potential adventitious agent or novel endogenous agent is detected in an already licensed or registered vaccine"*
  - *"The main areas to consider in a risk evaluation performed by a regulatory agency relate to the following questions:*
    - *How was the signal detected?*
    - *What is already known about the product concerned?*
    - *Where was the signal detected?*
    - *What exactly was detected?"*

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WHO recommendations/guidelines (5/5)

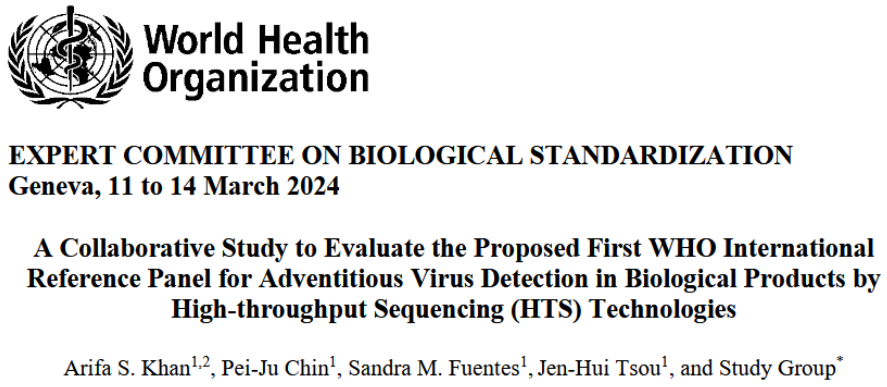
- **Guidelines on the replacement or removal of animal tests for the quality control of biological products**
  - Adopted at the WHO ECBS in October 2025
  - Scope: all biological products for human use
  - Objective: *"These WHO Guidelines provide guidance to biological product developers, manufacturers and regulators on a range of scientific and regulatory considerations with regard to the replacement or removal of animal tests for the quality control of biological products."*
  - Chapter 5 on Adventitious agent testing:

"With the adoption of the ICH Q5A(R2) Harmonised Guideline in November 2023 (48) and the publication of the new general chapter entitled "High-throughput sequencing for the detection of viral extraneous agents (2.6.41)" in the European Pharmacopoeia in October 2025 (49), there is broad international recognition that all in vivo adventitious agent assays and antibody production tests can be replaced with molecular methods such as polymerase chain reaction (PCR) and high-throughput sequencing (HTS) based on either targeted or non-targeted detection (50, 51). The use of these alternative methods is recommended."

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WHO International Reference Panel

- Prepared/co-ordinated by US FDA (Dr Arifa Khan)
- Adopted at WHO ECBS in March 2024
- 1st WHO International Virus Panel for adventitious virus detection by HTS
- 7 viruses: The virus panel includes Epstein-Barr virus (EBV; also known as human herpesvirus 4), mammalian orthoreovirus type 1 (REO), human respiratory syncytial virus (RSV), feline leukemia virus (FeLV), porcine circovirus type 1 (PCV1), human betacoronavirus OC43 (hCoV), and minute virus of mice (MVM).



World Health Organization

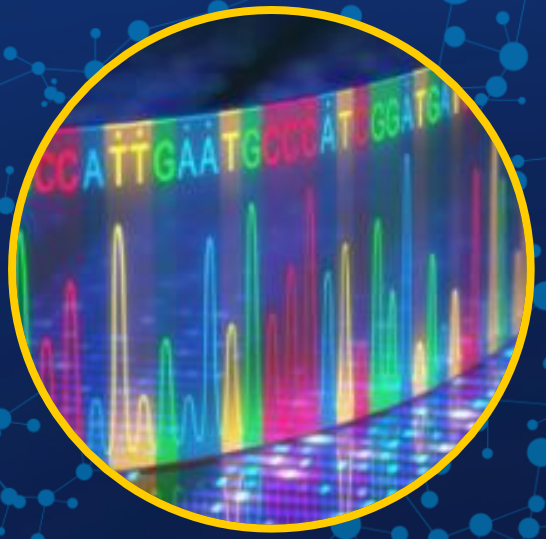
EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 11 to 14 March 2024

A Collaborative Study to Evaluate the Proposed First WHO International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing (HTS) Technologies

Arifa S. Khan[1,2], Pei-Ju Chin[1], Sandra M. Fuentes[1], Jen-Hui Tsou[1], and Study Group[*]

Link to WHO/BS/2024.2471

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ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

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ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (1/3)

- Published in November 2023 Link
  - ICH Q5A(R2) Step 4 presentation: Link
  - ICH Q51(R2) training material: Link
- Scope: all biological products for human use
- Introduces Next Generation Sequencing (NGS) for virus detection in this revision
- Major updates:
  - Encourages use of new alternative tests (includes Next Generation Sequencing (NGS) and Polymerase Chain Reaction (PCR))
  - Clarifies head-to-head comparison of NGS with existing methods is not recommended
  - Describes that NGS is considered a limit test (ICH Q2)
- Specific opportunities to replace existing methods with targeted or broad (non-targeted) NGS highlighted
  - Antibody production tests
  - In Vivo assays
  - In Vitro assays

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ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (2/3)

- Focus on in vivo assays (3.2.3):
  - «Non-targeted NGS is encouraged as a replacement for *in vivo* assays due to its breadth and sensitivity of virus detection and the limitations of the *in vivo* assays. Furthermore, this promotes the global initiative to replace, reduce, and refine the use of animal testing»

- Focus on Tests for specific viruses (3.2.4):
  - «Examples of such tests are the Mouse Antibody Production (MAP) test, Rat Antibody Production (RAP) test, and Hamster Antibody Production (HAP) test. The viruses currently screened for in the antibody production assays are discussed in Table 3. NAT such as PCR assays or targeted or non-targeted NGS or other molecular methods can be used for replacing the animal assays described in Table 3, without head-to-head comparison.»

- Focus on Next Generation Sequencing (3.2.5):
  - « Non targeted NGS can be used to replace the in vivo assays and supplement or replace the in vitro cell culture assays, without a head-to-head comparison as long as the method is demonstrated to be suitable for its intended purpose. A head-to-head comparison is not recommended due to the different end points of the assay systems and limitations of the breadth of virus detection by the in vitro and in vivo methods compared to the enhanced capability of NGS for broad virus detection of known and unknown viruses. The results of the in vitro and in vivo assays rely on virus replication and biological properties for detection in the specific target system which limits the breadth of detection. Replacement of in vivo assays by NGS also meets the intent of the global objective to replace, reduce, and refine the use of animals for testing. »

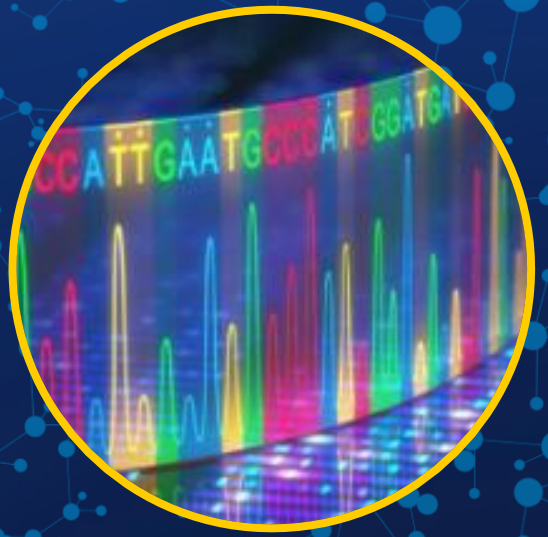
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ICH Q5A(R2): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (3/3)

- Reference materials:
  - « 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. »

- Validation:
  - « For any NGS method used, validation/qualification should be provided to support its intended use for the application. This includes method validation and matrix-specific verification when used as a replacement method. When used as a supplementary method, this includes method qualification and matrix-specific verification. Method validation requires predefined performance criteria while method qualification only evaluates the performance characteristics of the method. NGS is used as a limit test and as such, the performance characteristics (specificity/breadth of detection and sensitivity) for method validation/qualification should consider the principles of ICH Q2.

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Overview of HTS in the Ph. Eur.

General chapter	*Cell substrates for the production of vaccines for human use (5.2.3) **	*Tests for extraneous agents in viral vaccines for human use (2.6.16) **	*Management of extraneous agents in immunological veterinary medicinal products (5.2.5)*	*Principles for the detection of extraneous viruses in immunological veterinary medicinal products using culture methods (2.6.37)*	*Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines (5.2.14)*
Test and method	Tests for extraneous agents: HTS cited - as an alternative to *in vivo* tests or - as a supplement/alternative to *in vitro* cell culture tests			HTS is part of the listed methods	Test for extraneous agents: Novel molecular methods: HTS cited as potential substitute *for* in *vivo* methods

* These general chapters are themselves referenced in other texts (e.g. *Vaccines for human use (0153), Gene therapy medicinal products for human use (3186)*)



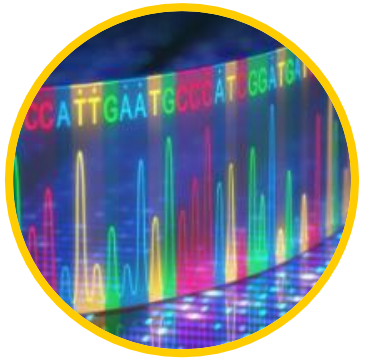
★ General chapter *5.2.14*: Pharmeuropa 37.3 (July 2025)

★ General chapters *5.2.3* & *2.6.16*: Pharmeuropa 38.4 (October 2026)

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Ph. Eur. General Chapter 2.6.41 « High-throughput sequencing for the detection of viral extraneous agents

- **Publication of the EDQM Ph. Eur. Chapter 2.6.41** *High-throughput sequencing for the detection of viral extraneous agents* in Ph. Eur. Issue 12.2 (October 2025). The chapter entered into force on 1 April 2026.
  - Objective: Facilitate the implementation of HTS for the detection of viral extraneous agents in biological products
    - Description of HTS methodologies
    - Guidelines for method validation
  - Scope: All biological products (human & veterinary)
  - Ph. Eur.: https://pheur-online.edqm.eu/content/20641/en/current/
- **Webinar** took place in September 2026 and the recording is available on-demand:
  - **From text to practice**: high-throughput sequencing for the detection of extraneous agents under Ph. Eur. general chapter 2.6.41
  - The webinar guided participants through the chapter, beginning with a general introduction and an overview of its scope. This was followed by a detailed overview of the HTS workflow, including the controls used in routine testing. Key aspects of method validation are addressed, with recommendations for selecting spiking materials.



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General chapter 5.2.14 Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines

Detection of viral extraneous agents by high-throughput sequencing

Detection of viral extraneous agents in cell banks, seed lots and cell culture harvests can be conducted using a panel of *in vivo* and *in vitro* methods at different stages of the manufacturing process. 32

High-throughput sequencing (HTS, also known as next-generation sequencing) refers to sensitive molecular biology methods with broad virus-detection capabilities that are increasingly used to test for viral extraneous agents in biological products. The use of broad molecular methods such as HTS has highlighted gaps in testing strategies that rely on conventional cell culture and *in vivo* methods by identifying previously undetected viral contaminants in the final product, in the cell banks from which it was produced and at intermediate manufacturing stages. 33

HTS (using a non-targeted analysis) may be used as a substitute for *in vivo* tests and to mitigate the risk of contamination by viral extraneous agents. For this purpose, the HTS method must be demonstrated to be suitable for its intended purpose and validated as described in general chapter *2.6.41. High-throughput sequencing for the detection of viral extraneous agents.* 34

HTS and *in vivo* tests use different end-points. HTS detects viral genomes, in contrast to *in vivo* methods, which are based on observations of the effects viruses have on experimental animals. Moreover, the breadth of virus detection by *in vivo* methods is limited compared to the enhanced capability of HTS for broad detection of known and unknown viruses. The results of *in vivo* tests rely on the replication and biological properties of extraneous viruses for their detection in the specific animal model, which limits the breadth of detection. 35

Therefore, when assessing the suitability of an HTS method to replace an *in vivo* method, a head-to-head comparison of the HTS method against the *in vivo* method may not be justified. HTS validation data (sensitivity and breadth of detection) may however be compared with existing data for the *in vivo* test obtained using a panel of equivalent model viruses, i.e. well-characterised model viruses equivalent to the virus stocks used in prior studies that evaluated the performance of the *in vivo* test. 36

Version published in Pharmeuropa 37.3 (July 2025)

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General chapter 2.6.41 (*Introduction*)



Implemented on 1 April 2026
(published Ph. Eur. Issue 12.2)

Built-in **flexibility** to accommodate diverse HTS technologies and workflows

- ★ HTS can be used as a **replacement/substitution of *in vivo* tests** and **NAT methods**, as well as **replacing** or **supplementing *in vitro* cell culture tests**
- ★ HTS is applicable across **diverse biological products** (e.g. vaccines for human and veterinary use, ATMPs) and **manufacturing stages** (e.g. cell banks, virus seeds, unprocessed bulks)
- ★ HTS is **especially relevant for new cell lines** (detection of known and unknown viruses) and **virus seeds or harvests** (avoiding interference experienced in conventional tests due to lack of neutralisation of the vaccine/vector virus or sample toxicity)
- ★ 2.6.41 acknowledges the **various HTS technologies** (short and long-read sequencing technologies) and **approaches** (targeted and non-targeted) available

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General chapter 2.6.41: content and structure



1. INTRODUCTION AND SCOPE

3. HTS METHOD VALIDATION

3-1. General considerations for validation
3-2. Selection of spiking material for validation
3-3. HTS method validation (generic method validation)
3-4. Product-specific validation



2. DESCRIPTION OF METHODS

2-1. General considerations
2-2. Sample pre-treatment
2-3. Extraction of nucleic acids
2-4. Post-nucleic acid extraction treatment
2-5. Library preparation
2-6. Sequencing
2-7. Bioinformatics analysis
2-8. Scientific evaluation of the results
2-9. Follow-up investigation
2-10. Controls in the routine test



4. TARGETED HTS

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3-2. Selection of spiking materials for validation

A: Genomics or Viromics Approach

The use of model viruses as spike is relevant for the viromics and genomics approaches […]
→Use full virus particles

The use of nucleic acids as spiking material is not recommended

The reference materials are viruses representing viral diversity in terms of structure (enveloped, non-enveloped), nucleic acid type (RNA or DNA genome, single-stranded or double-stranded genome, linear or circular or segmented), genome size, virus morphology and chemical resistance.

These reference model virus stocks are characterised for genome copy number per millilitre, infectious titre, viral genome sequence(s) including any variants, and any additional expected background signals (e.g. from production substrates).

A risk assessment taking into consideration the sample type, the approach and the intended use of the test can be conducted to select the most relevant spiking material

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*"The **WHO International Reference Panel** for Adventitious Virus Detection in Biological Products by High-Throughput Sequencing provides diverse virus families (EBV, FeLV, RSV, PCV, Reovirus, MVM and HCoV-OC43) and is **recommended** as a suitable well-characterised panel of model viruses for validation representing the minimum requirement of viral diversity."*

World Health Organization

WHO/BS/2024.2471

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 11 to 14 March 2024

A Collaborative Study to Evaluate the Proposed First WHO International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing (HTS) Technologies

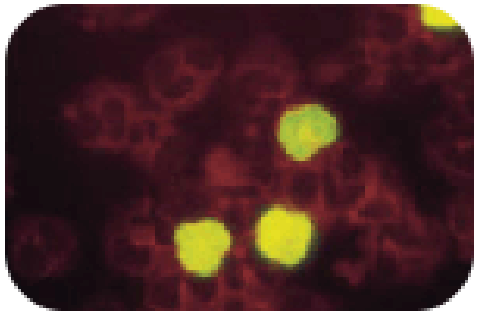
Arifa S. Khan[1,2], Pei-Ju Chin[1], Sandra M. Fuentes[1], Jen-Hui Tsou[1], and Study Group[*]

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B: Transcriptomics approach

virus-infected cells



or

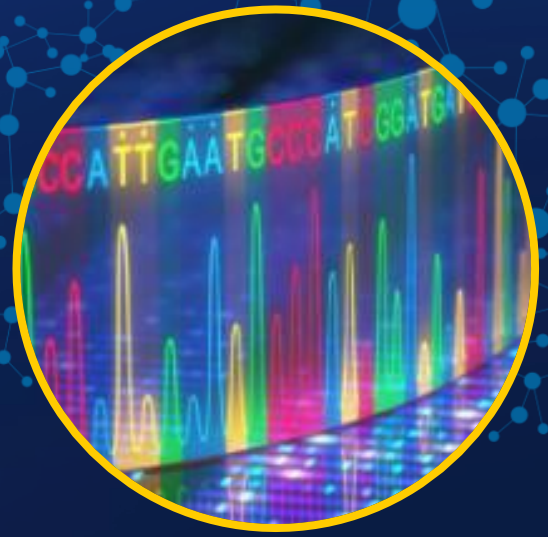
synthetic RNAs including polyadenylated and non-polyadenylated molecules



- There is no WHO reference panel for transcriptomics approach
- Do not use the WHO virus particles as a direct spike for validation of transcriptomics approach
- Consider viruses with various transcripts (e.g poly A and non-polyA)

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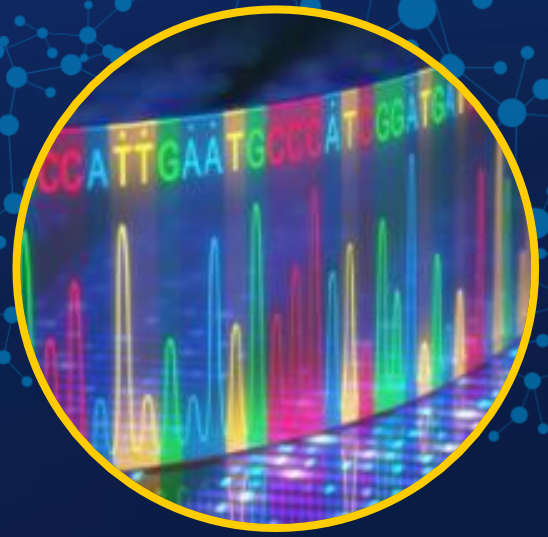
Indian Pharmacopoeia

- **Chapter 2.2.35 « Detection of Viral Extraneous Agents Using High-Throughput Sequencing (HTS)**
  - Published in 2025
  - Scope: all biological products
  - Very similar to Ph. Eur. Chapter 2.6.41 (covers both description of the methods and validation)
  - Mentions the WHO international Reference panel

- **Other chapters:**
  - **2.7.2. Cell Substrates for the Production of Vaccines for Human Use:** mentions HTS, very similar to Ph. Eur. 5.2.3 general chapter
  - **2.7.3. Test for Extraneous Agents in Viral Vaccines for Human Use:** mentions HTS, very similar to Ph. Eur. 2.6.16 General Chapter
  - **2.7.20. Substitution of in-vivo Method(s) by in-vitro Method(s) for the Quality Control of Vaccines:** dedicated section on detection of viral extraneous agents by novel molecular methods, very similar to Ph. Eur. 5.2.14 general chapter

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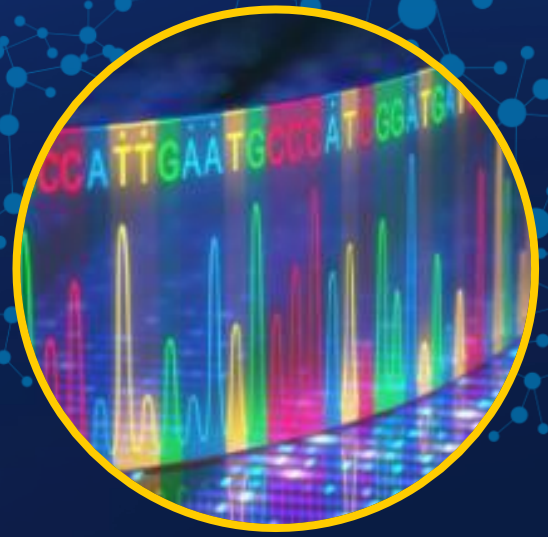
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Chinese Pharmacopoeia

- 2025 Edition
- Integration of NGS into its requirements for the quality control of animal cell substrates
- **0234 Requirements for Preparation of Animal Cell Substrates Used for Production of Biologics**
  - Scope: all biological products
  - New paragraph on Molecular Biology Methods including NGS
  - Broad-spectrum NGS method mentions to replace in vivo assays or supplement or replace in vitro assays (e.g., there is a lack of virus-susceptible cells, or the sample interferes with the detection or has toxicity).
  - Virus reference material characteristics described: very similar to ICH Q5A(R2)
  - Validation/Verification: very similar to ICH Q5A(R2):
    - With method validation and sample suitability confirmation included when NGS is used as an alternative method
    - With method verification and sample suitability confirmation included when NGS is used a supplementary method

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Other regulatory documents



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Other regulatory documents

- ★ Korea/MFDS:
  - **Guideline for the Management of Cell Substrates Used in the Production of Biopharmaceuticals** (published in September 2025) Link
  - Cross reference to ICH Q5A(R2) for the selection of appropriate testing methods for adventitious agents
- ★ Japan/PMDA
  - **Points to Consider for Replacement of Conventional Tests for Viruses with Next Generation Sequencing (NGS) Assays (Early Consideration)** (published in January 2026) Link
  - Cross reference to ICH Q5A(R2) and mention of the WHO International Reference Panel

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Conclusion

- HTS/NGS fully integrated in the regulatory landscape (Global, Regional, National) with a good regulatory convergence
- Detailled technology description available in Ph. Eur. Chapter 2.6.41
- Validation approach mentions in several documents (ICH Q5A(R2), Ph. Eur...) and validation guidelines available in Ph. Eur. Chapter 2.6.41
- Substitution/Replacement of *in vivo* methods by HTS/NGS (without head-to-head comparison) mentions in several documents (ICH Q5A(R2), Ph. Eur., Indian Ph....)
- Reference virus panel characteristics described and well-harmonised across the various documents
- WHO International Reference Panel recommended for validation purpose

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