

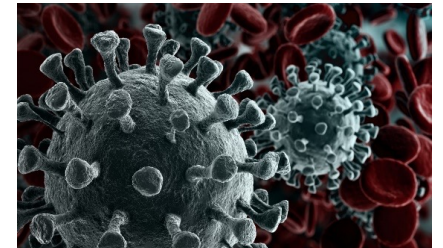
THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



NGS for adventitious viruses: ongoing initiatives and upcoming events



Laurent MALLET, EDQM
HSI webinar



*“Transition to non-animal based vaccine batch release testing.
Policy and regulations theoretical aspects and case studies”*

March 27th, 2024

Outline

- Next Generation Sequencing: What is it?
- Extraneous agents testing for vaccines and viral vectors used as gene therapy products: evolution of the European Pharmacopoeia
- Perspectives on NGS/HTS and elaboration of a new Ph. Eur. Chapter
- Update on the ICHQ5(R2) guideline: focus on NGS introduction
- Upcoming events on NGS
- Key references

Next Generation Sequencing: What is it?

- Also called **High Throughput Sequencing (HTS)** or Massive Parallel Sequencing
- Sequencing of acid nucleics with high throughput, scalability and speed
- Different technologies
 - Short reads, long reads
 - Read length from a hundreds of nucleotides to 50+ Kb
- Application to the detection and identification of viral Extraneous/Adventitious Agents:
 - **Sensitivity**
 - **Breadth of detection**: capability to detect both known and unknown viruses

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- ▶ Extraneous agents testing for vaccines and viral vectors used as gene therapy products: evolution of the European Pharmacopoeia



Extraneous agents testing for vaccines: drivers for change

- **Contamination of a Rotavirus vaccine** by Porcine Circovirus (2010)
 - Victoria *et al.* (Journal of virology): results showed the presence of PCV1 viral sequences using a new high throughput molecular biology method (MPS)
- **Emergence of broad molecular methods** for extraneous agent detection
- **Revised WHO TRS 978 Annex 3** “*Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks*” (adopted in 2010)
 - Risk assessment strategy and new methodologies (e.g. NGS)
- **Convergence with FDA Guidance for Industry** (2010) on testing methodologies
- **3Rs context** in Europe:
 - European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe), EU Directive 2010/63/EU

Extraneous agents testing for vaccines: drivers for change

- **EDQM survey** (2012) with Vaccine Manufacturers and CROs regarding contamination cases over a period of 10 years
- **Publications*** highlighting gaps in compendial tests:
 - Evaluation and comparison of the sensitivity of current testing packages for detection of extraneous agents → poor sensitivity of *in vivo* methods, gaps in testing packages

*J Gombold *et al.* *Systemic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products* (Vaccine) 2014

*R Sheets and P Duncan, in *Vaccine Analysis: Strategies, Principles, and Control*, Springer-Verlag Berlin Heidelberg 2015



of Ph. Eur. requirements!

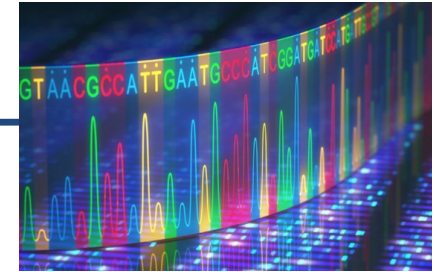
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- ▶ Perspectives on HTS and elaboration of a new Ph. Eur. chapter

Perspectives on HTS



- Ph. Eur. chapters 5.2.3 & 2.6.16 mention HTS and foresee its use as part of the testing strategy for extraneous agents
- However, **no description** of these methods **or any guidance for their validation is provided.**
- The availability of regulatory standards including validation guidelines in the Ph. Eur. will serve as a reference for regulators and manufacturers, while:
 - HTS was recently introduced in the **revised ICH Q5A** guideline (*Viral safety evaluation of biotechnology products*) (*revision R2 adopted in Nov 2023*)
 - A panel of model viruses developed by **FDA** was also recently adopted by WHO as **WHO international reference panel for HTS**

Elaboration of a Ph. Eur. chapter on HTS



- *"High Throughput Sequencing for the detection of extraneous agents in biological products (2.6.41)"*
- Non-binding general chapter
- Content: **description of the technology/methods** and of the HTS workflow, **guidelines for validation of HTS methods**
 - Elaborated by Ph. Eur.'s **HTS Working Party**
(international group of regulators, OMCLs and industry from Europe, US, Canada)
 - Draft chapter has been published for **public consultation in Pharmeuropa!**
<https://pharmeuropa.edqm.eu/home>
 - **Deadline for comments: 30 June 2024**



Elaboration of a Ph. Eur. chapter on HTS – overview

- Draft chapter describes **HTS methodologies** used for the **detection of viral extraneous agents** in biological products including e.g. vaccines, recombinant proteins, viral vectors used for gene therapy, and cell-based preparations for cell therapy
- It outlines the different steps of the **HTS workflow**, the **design of the method**, **analysis approaches**, and the **controls** used in the routine test
- It also provides **guidelines for HTS method validation**, including recommendations for the selection of the spiking material for validation and the evaluation of the relevant performance characteristics for HTS

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- Update on the ICHQ5(R2) guideline: focus on NGS introduction

Update on the ICHQ5(R2) guideline (1/2)

- ICH: VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN Q5A(R2)
 - Adopted the 1st of November 2023. [Link](#)
- Extension of the scope of the guideline:
 - Scope is defined as products that are amenable to viral clearance without negative impact on the product
 - Includes some genetically-engineered viral vectors and viral vector-derived products
 - Now includes viral vector derived products such as virus-like particles (VLPs), protein subunits and nanoparticle-based protein vaccines
- Introduction of Next Generation Sequencing (NGS) for virus detection:
 - Specific opportunities to replace existing methods with targeted or broad (non-targeted) NGS highlighted
 - **Antibody production tests**
 - ***In Vivo* assays**
 - ***In Vitro* assays**

Update on the ICHQ5(R2) guideline (2/2)

- 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 (3.2.5.2):
 - « **Non-targeted NGS can replace the *in vivo* tests** with broad virus detection for unknown or unexpected virus species without a head-to-head comparison.»
 - « NGS (targeted or non-targeted) can replace virus-specific PCR assays and rodent antibody production tests (Section 3.2.4) without a head-to-head comparison.»

Upcoming events on NGS (1/2)

- IABS – DCVMN Meeting: NGS Testing to Replace *In Vivo* Adventitious Testing for Old & New Vaccines
 - 19-20 June 2024, *Brussels, Belgium, on invitation only*
- 2024 PDA Virus Conference: Viral Safety Reloaded - the Finalized ICH Q5A (R2)
 - 26-27 June 2024, *Amsterdam, The Netherlands* [Link](#)
- 2024 PDA Next Generation Sequencing Workshop
 - 28 June 2024, *Amsterdam, The Netherlands* [Link](#)
- ECA European Microbiology Conference, pre-conference workshop, Next Generation Sequencing
 - 25 June 2024, *Munich, Germany* [Link](#)

Upcoming events on NGS (2/2)

- 4th IABS Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animal
 - 3-5 December 2024, Frankfurt, Germany [Link](#)
 - 3rd of December: one-day NGS training workshop

“The 4th NGS meeting will continue to highlight current scientific data and knowledge with focus on NGS implementation for adventitious virus detection in biological products. Discussions will include capabilities of NGS for virus detection in different matrices and validation of the technical and bioinformatics steps involved in NGS for its applications in characterization and safety evaluation of biologics such as vaccines for human and veterinary use, gene and cell therapy products, and biotherapeutics.”



IABS
International Alliance for
Biological Standardisation

Frankfurt, Germany
December, 3-5 2024

**4th Conference on Next Generation Sequencing for
Adventitious Virus Detection in Biologics for Humans
and Animal**

2024 is a key year for globally considering NGS implementation with the recent publication of the revised ICHGSA(R2) guideline and the expected publication of the EDQM/European Pharmacopoeia chapter for comments.

The 4th NGS meeting will continue to highlight current scientific data and knowledge with focus on NGS implementation for adventitious virus detection in biological products. Discussions will include capabilities of NGS for virus detection in different matrices and validation of the technical and bioinformatics steps involved in NGS for its applications in characterization and safety evaluation of biologics such as vaccines for human and veterinary use, gene and cell therapy products, and biotherapeutics.

The meeting will bring together representatives from industry, academia, technology providers, and international regulatory bodies for discussing the current applications of NGS for detection of adventitious viruses in biologics.

A one-day NGS training workshop to be associated to this conference is in preparation and further details will be communicated soon.

Scientific Committee :

- Arifa S. KHAN, FDA-CBER (FDA), U.S.A.
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- Pieter NEELS, IABS Chair, Belgium
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- Noémie DENEYER, GSK, Belgium
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- Siemon NG, Notch Therapeutics Canada
- Yoji SATO, NHIS, Japan
- Michael WALL, Health Canada Canada

www.iabs.org

Key references

- **Draft general chapter 2.6.41 “High-Throughput Sequencing for the detection of viral extraneous agents”** [Link](#)
- **ICH: VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN Q5A(R2)**, adopted the 1st of November 2023. [Link](#)
- **“Report of the third conference on next-generation sequencing for adventitious virus detection in biologics for humans and animals”** A. S. Khan, L. Mallet, J. Blümel, J-P. Cassart, I. Knezevic, S. H.S. Ng, Michael Wall, Miia Jakava-Viljanen, C. Logvinoff, A. Goios, P. Neels, *Biologicals*, 83, 101696. August 2023. [Link](#)
- **“Report of the second international conference on next generation sequencing for adventitious virus detection in biologics for humans and animals”** A. S. Khan, J. Blümel, D. Deforce, M. F. Gruber, C. Jungbäck, I. Knezevic, L. Mallet, D. Mackay, J. Matthijnsens, M. O’Leary, S. Theuns, J. Victoria, P. Neels. *Biologicals*, 67: 94-111, September 2020. [Link](#)
- **“Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection”** R. L. Charlebois, S. Sathiamoorthy, C. Logvinoff, L. Gisonni-Lex, L. Mallet & S. H. S. Ng, *npj Vaccines*, volume 5, Article number: 61, July 2020. [Link](#)
- **“Report of the international conference on next generation sequencing for adventitious virus detection in biologics”** A. S. Khan, L. Benetti, J. Blümel, D. Deforce, W. M. Egan, I. Knezevic, P. R. Krause, L. Mallet, D. Mayer, P. D. Minor, P. Neels, G. Wang, *Biologicals*, 55 , 1–16, 2018. [Link](#)

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- Dr Thuy Bourgeois - EDQM
- Dr Emmanuelle Charton - EDQM
- The ICHQ5A Expert Working Group
- Dr Arifa Khan - US FDA

Thank you for your attention



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