International Conference, 19-21 September 2022

Collaboration, Innovation and Scientific Excellence: the European Pharmacopoeia 11th Edition

Session 5: Supporting microbiological and viral safety

Moderator: Lukas Bruckner,
Swiss Delegation of the Ph. Eur. Commission

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

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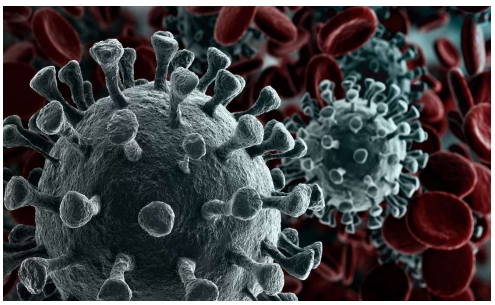
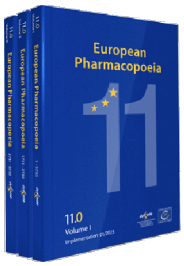
- EDQM/Ph. Eur. achievements in the control of extraneous agents for vaccines & perspectives on HTS





Featured Session on Microbiological and Viral Safety
20 September 2022

Catherine Lang and Gwenael Cirefice, EDQM

Outline

- Extraneous agent testing for vaccines: evolution of the Ph. Eur.
  - Vaccines for human use
    - Drivers for revising Ph. Eur. requirements
    - Evolution of Ph. Eur. 5.2.3 & 2.6.16
    - The concept of Substitution to replace *in vivo* methods as described in Ph. Eur. 5.2.14
  - Vaccines for veterinary use
    - Drivers for revising Ph. Eur. requirements
    - Evolution of Ph. Eur. chapters for veterinary vaccines
    - New Approach brings opportunities & benefits
    - Support to stakeholders in a nutshell
- High Throughput Sequencing for the detection of extraneous agents
- Conclusion

Extraneous agent testing: Evolution of the Ph. Eur.

- Vaccines for human use

Drivers for changing extraneous agent testing (human vaccines)

- 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*" (2010-2013)
  - 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

Drivers for changing extraneous agent testing (human vaccines)

- 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!

Evolution of Ph. Eur. chapters for human vaccines


01/2018:50203

5.2.3. CELL SUBSTRATES FOR THE PRODUCTION OF VACCINES FOR HUMAN USE

This general chapter deals with diploid cell lines and

07/2020:20616

2.6.16. TESTS FOR EXTRANEOUS AGENTS IN VIRAL VACCINES FOR HUMAN USE

INTRODUCTION

A strategy for testing extraneous agents in viral vaccines must be developed based on a risk assessment following the principles of viral contamination risk detailed in general

01/2018:50214

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

PURPOSE

The purpose of this general

er is to provide guidance
in vitro methods as
thods, in cases where a
on is not appropriate for
of one or more *in vitro*
not discuss the details of
e principles are described

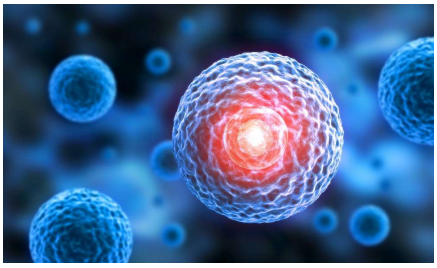
ily to vaccines for human
ciples described may also
ra.

	Ph. Eur. Chapter 5.2.3 *Cell substrates for the production of vaccines for human use*	Ph. Eur. Chapter 2.6.16 *Tests for extraneous agents in viral vaccines for human use*	Ph. Eur. Chapter 5.2.14 *Substitution of in vivo methods for the QC of vaccines*
Scope	**Testing of cell substrates** (including extraneous agent testing)	Extraneous agent **testing of viral seed lots/harvests**	Concept of **Substitution to replace *in vivo* methods**
Year introduced or year of last major update	July 2017 (Ph. Eur. Suppl. 9.3)	July 2017 (Ph. Eur. Suppl. 9.3)	July 2017 (Ph. Eur. Suppl. 9.3)

- Revision of chapters 5.2.3 & 2.6.16
- Elaboration of chapter 5.2.14 (concept of Substitution)

5.2.3 Cell substrates for the production of vaccines for human use

- Scope: diploid cell lines and continuous cell lines used as cell substrates for the production of vaccines

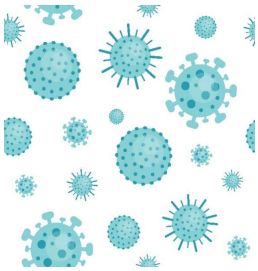


- *Chapter 5.2.3 revised in 2017 (Suppl. 9.3) to introduce the risk assessment, allow the use of broad molecular methods (e.g. HTS), and remove an in vivo test (test in adult mice)*

- Extraneous agents: testing strategy is to be based on a risk assessment considering e.g. choice of permissive cells, nature of cell lines (e.g. insect cells), cell lines shown to express endogenous retroviral particles, *in vivo* tests to be justified if maintained
- A strategy is given in chapter 5.2.3. Alternative strategies could focus on more extensive testing of the MCB or WCB

2.6.16 Tests for extraneous agents in viral vaccines for human use

- Applies to starting materials and substrates used for production and control of viral vaccines (virus seed lots, virus harvests, control cells/eggs)



- *Chapter 2.6.16 revised in 2017 (Suppl. 9.3) to introduce the risk assessment, allow the use of broad molecular methods (e.g. HTS), and remove two in vivo tests (tests in adult mice, guinea pigs)*
- Panel of *in vivo* and *in vitro* methods
  - Cell culture methods
  - *In vivo* tests (suckling mice, fertilised eggs): to be justified if maintained
  - Molecular methods (for specific extraneous agent or broad virus detection)

- Testing strategy (package of suitable tests) is to be built based on a risk assessment

5.2.14 Substitution of *in vivo* methods for the QC of vaccines

- Chapter elaborated to facilitate the transition to *in vitro* methods (e.g. HTS), applies to human and vet vaccines
- Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible
- Envisages the possibility that the relevance and performance of the *in vitro* method be demonstrated without such head-to-head comparison: concept of "substitution" as an alternative approach for replacement
- Focus on the scientific rationale behind the *in vitro* methods and the validation package

Extraneous agent testing: Evolution of the Ph. Eur.

- Veterinary vaccines

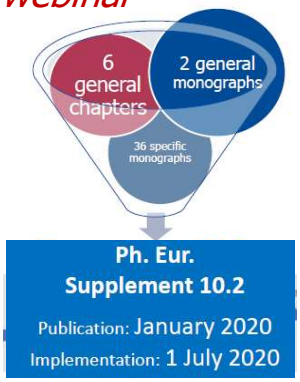
Drivers for changing extraneous agent testing (vet. vaccines)

- move from prescriptive methods to any suitable culture or other **fit-for-purpose** method representing current practice and capable of detecting specified extraneous agents (based on a list) identified thanks to a **risk assessment approach**, with a focus on modern ***in-vitro*** **methods** (for ex. based on nucleic acid amplification technology + allow the use of broad molecular methods (e.g. HTS) – chapter *2.6.37*).
- Strong Commitment of the Ph. Eur. to the **3Rs**
- **GMP and consistency approach** allowing the omission of unnecessary testing (risk assessment)
- Covering **starting materials** of animal and human origin and **all materials** (master seed, substrates – eggs, cells etc)
- Covering the **entire production process**, from the sourcing of raw materials to the final product stage
- There was also a general reflection on EA testing at EU and international level (VICH)

of Ph. Eur. requirements!

Evolution of Ph. Eur. chapters for veterinary vaccines

Work with all stakeholders: hearings, collaboration with international partners e.g. EMA, April 2020 webinar

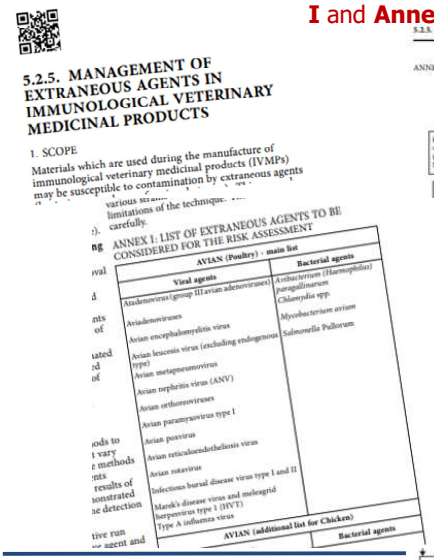


- Revision of chapter 5.2.5 (*Management of extraneous agents in IVMPs*) Risk management & compilation of all requirements, also includes **2 Annexes** (**I List** of EA; **II** Testing strategy: example of a **decision tree**)
- Elaboration of chapter 2.6.37 (*Principles for the detection of extraneous viruses in IVMPs using culture methods*) - General principles and examples of parameters to be taken into account to use **fit-for-purpose culture methods** (manufacturers have to check that the method is able to detect what they are looking for)
- Deletion of chapters 2.6.24 & 2.6.25

These chapters, which contained detailed protocols for testing extraneous agents, have been suppressed from the Ph. Eur. **on 1st of July 2020**, but are still accessible in Ph. Eur. archives https://pheur.edqm.eu/app/arch/search/

Evolution of 44 Ph. Eur. texts for veterinary vaccines

07/2020:50205 Revision of chapter **5.2.5** (Add **Annex I** and **Annex II**)



5.2.5. MANAGEMENT OF EXTRANEOUS AGENTS IN IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

1. SCOPE

Materials which are used during the manufacture of immunological veterinary medicinal products (IVMPs) may be susceptible to contamination by extraneous agents

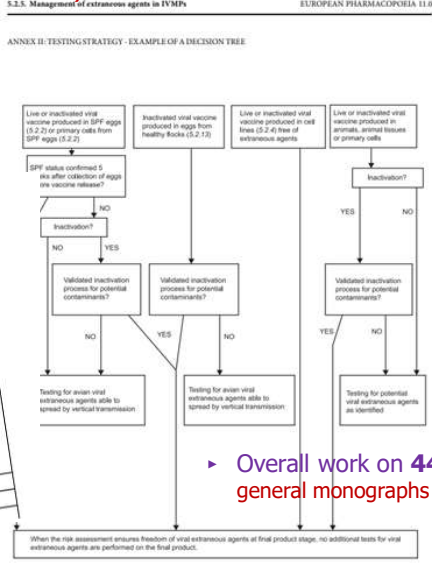
various [illegible]

limitations of the technique[illegible]

carefully.

ANNEX I: LIST OF EXTRANEOUS AGENTS TO BE CONSIDERED FOR THE RISK ASSESSMENT

AVIAN (Poultry) - main list	
Viral agents	Bacterial agents
Aviadenovirus (group III avian adenoviruses)	Arthrobacterium (Haemophilus) paragallinarum
Avian encephalomyelitis virus	Chlamydia spp.
Avian leucosis virus (excluding endogenous type)	Mycobacterium avium
Avian metapneumovirus	Salmonella Pullorum
Avian nephritis virus (ANV)	
Avian orthoreovirus	
Avian paramyxovirus type 1	
Avian poxvirus	
Avian reticuloendotheliosis virus	
Avian rotavirus	
Infectious bursal disease virus type 1 and II	
Marek's disease virus and meleagrid herpesvirus type 1 (HVT)	
Type A influenza virus	
AVIAN (additional list for Chicken)	Bacterial agents



5.2.5. Management of extraneous agents in IVMPs EUROPEAN PHARMACOPOEIA 11.0

ANNEX II: TESTING STRATEGY - EXAMPLE OF A DECISION TREE

Live or inactivated viral vaccine produced in SPF eggs (5.2.2) or primary cells from SPF eggs (5.2.2)

Inactivated viral vaccine produced in eggs from healthy flocks (5.2.13)

Live or inactivated viral vaccine produced in cell lines (5.2.4) free of extraneous agents

Live or inactivated viral vaccine produced in animals, animal tissues or primary cells

SPF status confirmed 5 [illegible] after collection of eggs [illegible] vaccine release?

Inactivation?

Validated inactivation process for potential contaminants?

Testing for avian viral extraneous agents able to spread by vertical transmission

Testing for potential viral extraneous agents as identified

When the risk assessment ensures freedom of viral extraneous agents at final product stage, no additional tests for viral extraneous agents are performed on the final product.

EUROPEAN PHARMACOPOEIA 11.0

07/2020:20637 corrected 10.3

2.6.37. PRINCIPLES FOR THE DETECTION OF EXTRANEOUS VIRUSES IN IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS USING CULTURE METHODS

This general chapter describes general principles that apply to culture methods for the isolation and detection of extraneous viruses in all materials used during the [illegible] immunological veterinary

- Elaboration of chapter **2.6.37** (Principles for the detection of extraneous viruses in IVMPs using culture methods)
- Deletion of chapters **2.6.24 & 2.6.25**
- Overall work on **44** Ph. Eur. texts (**6** general chapters, **2** general monographs and **36** vaccine specific monographs)

New Approach brings opportunities & benefits (vet. vaccines)

- New approach based on **risk assessment** allows **reduction in testing** during manufacture and **deletion of unnecessary tests** for EAs on final product
- Comprehensive requirements for EA testing are **centralized in chapter 5.2.5**, Ph. Eur. now cover **all species**, this brings more clarity (no duplication, no discrepancies)
- Flexibility to choose any suitable (validated) method to detect possible specific EA identified as a risk – **fit-for-purpose** sensitive techniques reflecting progress in science (state-of-the-art)
- Methods no longer described in detail, building in flexibility of approach and allowing tailoring to individual product needs
- Use of **up-to-date** and currently used modern methods result in reduction of *in vivo* testing (3Rs)
- Absence of EA checked during **the entire production process**
- Coordinated Ph. Eur./EU approach, important also in the context of VICH
- Better control of the risk of extraneous agents contamination ➔ **improve the safety**

Support to stakeholders in a nutshell (veterinary vaccines)

- Training of assessors via the EU National Training Centre database in October 2019
- ➡ Elaboration of a Q&A document by EMA/CVMP/IWP (1st Published in June 2020)

Questions and answers on management of extraneous agents in immunological veterinary medicinal products (IVMPs) (PDF/187.75 KB)

First published: 26/06/2020
EMA/CVMP/IWP/669993/2019

https://www.ema.europa.eu/en/questions-answers-management-extraneous-agents-immunological-veterinary-medicinal-products-ivmps#current-effective-version-section

- EDQM event on 1st April 2020, enabling a continued dialogue between Ph. Eur. experts, EU regulators and industry (at the start of the confinement) **Free e-Learning**

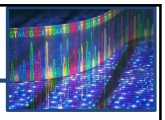
https://www.edqm.eu/en/-/training-live-broadcast-on-the-management-of-extraneous-agents-in-ivmps

- ➡ 15V publications available for free
  - "What has changed and why" published on Pharmeuropa online https://www.edqm.eu/documents/52006/77330/Veterinary-vaccines-E.pdf
  - Revision of the technical guide https://www.edqm.eu/documents/52006/66555/Technical-guide-elaboration-use-monographs-vaccines-immunological-veterinary-medical-products.pdf
  - Summary of the webinar (including concrete examples) https://www.edqm.eu/documents/52006/245619/document-summarising-training-european-pharmacopoeias-new-approach-management-extraneous-agents-ivmps.pdf

+ Search Database online | Knowledge Database

High Throughput sequencing (HTS) for the detection of extraneous agents

Perspectives on HTS



- Ph. Eur. chapters 5.2.3, 2.6.16 & 2.6.37 mention HTS and foresee its use as part of the testing strategy for extraneous agents for human & vet vaccines
- However, HTS methods are currently not described in details in any regulatory document and no guidance for their validation is available
- The availability of regulatory standards including validation guidelines in the Ph. Eur. will serve as a reference for regulators and manufacturers, while:
  - HTS is planned to be introduced in the revised ICH Q5A guideline (*Viral safety evaluation of biotechnology products*)
  - FDA has recently developed panels of viruses as reference preparations for HTS (adopted by WHO ECBS)

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
- Proposed content: description of the technology, **guidelines for method validation**

- Under elaboration by the HTS Drafting Group of Ph. Eur. Group of Expert 15 (international group of regulators, OMCLs, industry)

Conclusion

Conclusion

- The Ph. Eur. provides a flexible approach for extraneous agent testing of vaccines, that is based on a risk assessment for the specific product and manufacturing process & the use of a panel of analytical tools

  → Improvement & modernisation of control strategy for improved vaccine safety, progress for 3Rs, target for convergence of requirements

- The concept of substitution described in Ph. Eur. 5.2.14 can be applied to the replacement of *in vivo* tests for the detection of extraneous agents

- In the Ph. Eur., HTS has been introduced within the testing strategy for human and vet vaccines. The future chapter 2.6.41 on HTS will provide a detailed description of the technology together with validation guidelines to support industry and regulators

Thank you for your attention

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High Throughput Sequencing: How could the Ph. Eur. help in the exercise to validate HTS methods?

Siemon Ng

Collaboration, Innovation and Scientific Excellence: the European Pharmacopoeia 11th Edition
Date: 19-21 September 2022

Outline

- High Throughput Sequencing Technology
- Adventitious Virus Detection in Biologics
- Recent Advancements and Progress in HTS adventitious agent detection
- A New Ph. Eur General Chapter For HTS
- Development in Other Areas
- Summary and Conclusions

High Throughput Sequencing Technology

- High-throughput sequencing (HTS) are technologies that can rapidly sequence millions of DNA (or RNA) from a single biological samples. HTS has quickly become an important tool in scientific research, drug development and medical diagnostics.

- Multiple technologies and platforms
  - Read length from a hundreds of nucleotides to 50+ Kb
    - Short-reads (e.g. Illumina, Ion Torrent)
    - Long-reads technology (e.g. PacBio, Oxford Nanopore)
  - Up to 10 billion reads simultaneously
  - Direct sequencing of DNA and RNA molecules
  - Epigenetics and modified bases

Application of HTS as an Analytical Platform

- Adventitious agent detection: Detect and identify both known and unknown adventitious agents with a high level of sensitivity.

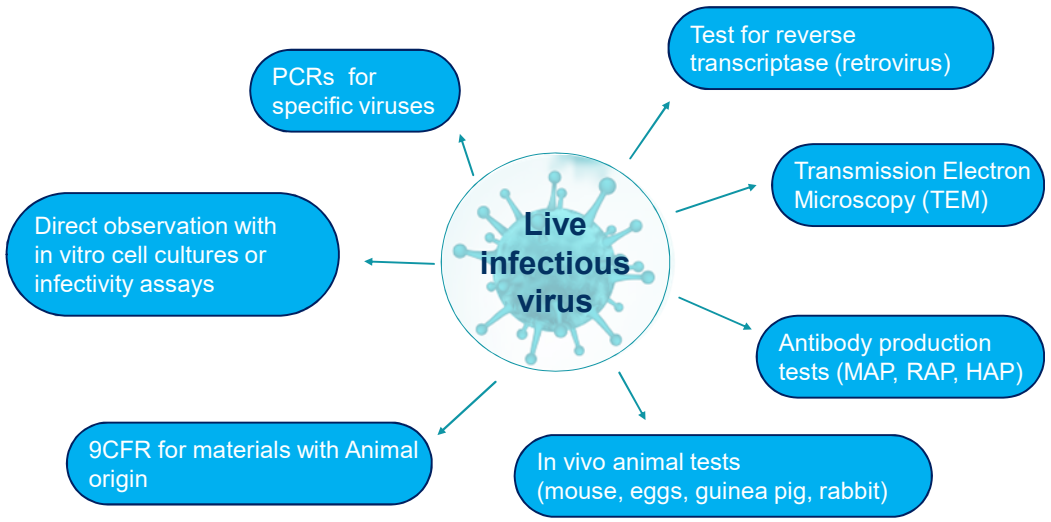
- Assessment of genomic stability or variants: Detect low frequency variants or genomic rearrangements

- Identity testing: Verification of engineered construct(s) or mutations at multiple loci simultaneously.

- Genome characterization: Whole genome sequencing to obtain baseline genomic information

Adventitious Virus Detection In Biologics

Viral Safety Testing Package and Adventitious Virus Testing



Notch THERAPEUTICS

HTS as an Emerging Technology

JOURNAL OF VIROLOGY, June 2010, p. 6033–6040 Vol. 84, No. 12
0022-538X/10/$12.00 doi:10.1128/JVI.02690-09

Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus▽†

Joseph G. Victoria,[1,2] Chunlin Wang,[3] Morris S. Jones,[4] Crystal Jaing,[5] Kevin McLoughlin,[5] Shea Gardner,[5] and Eric L. Delwart[1,2]*

Blood Systems Research Institute, San Francisco, California 94118[1]; Dept. of Laboratory Medicine, University of California, San Francisco, California 94118[2]; Stanford Genome Technology Center, Stanford, California 94304[3]; Clinical Investigation Facility, David Grant USAF Medical Center, Travis AFB, California 94535[4]; and Lawrence Livermore National Laboratory, Livermore, California 94551[5]

Received 22 December 2009/Accepted 25 March 2010

- March 2010: Use of HTS detected an unexpected adventitious virus in a commercial vaccine.
- May 2010: VRBPAC Meeting
- Aug 2010: FDA letter to vaccine manufacturers

Please describe any plans you may have to implement additional adventitious agent testing methods as part of your manufacturing process as these methods become available including, but not limited to, screening for PCV and PCV DNA as well as any additional in-process testing for adventitious agents that you may have recently added, but not reported to the agency. In this regard, please consider any animal derived materials (e.g., culture medium, albumin, enzymes, lipids, etc) and the point at which they are used in your product manufacture, any adventitious agent related quality control testing performed by the material vendor or done in-house, and any applicable viral clearance or inactivation steps provided by your manufacturing process.

- Highlighted that there are gaps in the traditional adventitious virus testing package
- Demonstrated that HTS has the potential to detect and identify both known and unknown adventitious viruses.

Updates to European Phamacopoeia to include HTS

- Evolution of European Pharmacopoeia
  - Ph. Eur. Chapter 5.2.14: "Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines", version 9.3 published in July 2017, creation
  - Ph. Eur. Chapter 5.2.3: "Cell Substrates for the production of vaccines for human use", version 9:0 and updated version 9.3 in July 2017, revision
  - Ph. Eur. Chapter 2.6.16: "Tests for extraneous agents in viral vaccines for human use", version 9.3 published in July 2017, revision
    - ↳in vivo tests for adventitious agents only if it provides a risk mitigation evidenced by the Viral Risk Assessment
- Also updates to other regulatory wording

Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines World Health Organization

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: degenerate NAT for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and 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 approval by the NRA (*51*).

01/2018:50203

5.2.3. CELL SUBSTRATES FOR THE PRODUCTION OF VACCINES FOR HUMAN USE

01/2018:20616 corrected 9.4

2.6.16. TESTS FOR EXTRANEOUS AGENTS IN VIRAL VACCINES FOR HUMAN USE

New, sensitive molecular methods with broad detection capabilities are available. These new approaches include high-throughput sequencing (HTS) methods, nucleic acid amplification techniques (NAT) (e.g. polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), product-enhanced reverse transcriptase (PERT) assays) for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays, and mass spectrometry with broad-spectrum PCR. These methods may be used either as an alternative to *in vivo* tests and specific NAT or as a supplement/alternative to *in vitro* culture tests based on the risk assessment and with the agreement of the competent authority.

Other Rationale for the revisions

- 3Rs Directive in Europe:

20.10.2010 EN Official Journal of the European Union L 276/33

DIRECTIVES

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 22 September 2010
on the protection of animals used for scientific purposes

- NIH publication highlighting the poor sensitivity of *in vivo* methods

Contents lists available at ScienceDirect

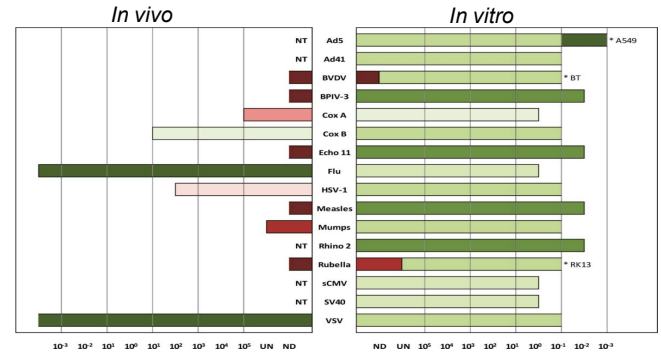
Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products☆

James Gombold[a], Stephen Karakasidis[a], Paula Niksa[b], John Podczasy[a], Kitti Neumann[a], James Richardson[a], Nandini Sane[a], Renita Johnson-Leva[a], Valerie Randolph[a], Jerald Sadoff[a], Phillip Minor[c], Alexander Schmidt[a], Paul Duncan[b], Rebecca L. Sheets[c,*]

- Compared between in vivo and in vitro assays using a panel of 16 viruses across 9 viral families
- Only 6 of the 11 that were tested using in vivo assays were detected



Notch THERAPEUTICS

Recent Advancements with Adventitious Agent Detection by HTS

Scientific Advancements

Biologicals 67 (2020) 94–111

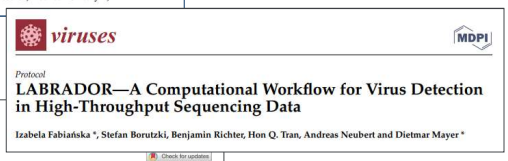
Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Report of the second international conference on next generation sequencing for adventitious virus detection in biologics for humans and animals☆

Arifa S. Khan[a,*], Johannes Blümel[b], Dieter Deforce[c], Marion F. Gruber[d], Carmen Jungbäck[d], Ivana Knezevic[e], Laurent Mallet[f,1], David Mackay[g], Jelle Matthijnssens[h], Maureen O'Leary[i,2], Sebastiaan Theuns[j], Joseph Victoria[i], Pieter Neels[k]



viruses

MDPI

Protocol

LABRADOR—A Computational Workflow for Virus Detection in High-Throughput Sequencing Data

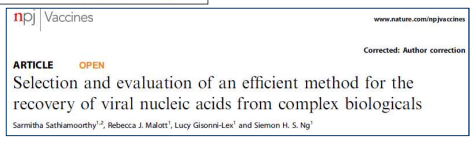
Izabela Fabiańska *, Stefan Borutzki, Benjamin Richter, Hon Q. Tran, Andreas Neubert and Dietmar Mayer *

npj | Vaccines

ARTICLE OPEN

Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection

Robert L. Charlebois[1], Sarmitha Sathiamoorthy[2], Carine Logvinoff[3], Lucy Gisonni-Lex[1], Laurent Mallet[1] and Siemon H. S. Ng[1]✉



npj | Vaccines

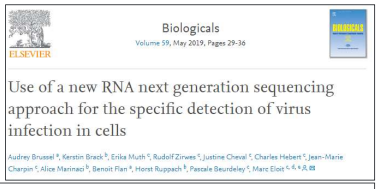
www.nature.com/npjvaccines

Corrected: Author correction

ARTICLE OPEN

Selection and evaluation of an efficient method for the recovery of viral nucleic acids from complex biologicals

Sarmitha Sathiamoorthy[1,2], Rebecca J. Malott[1], Lucy Gisonni-Lex[1] and Siemon H. S. Ng[1]

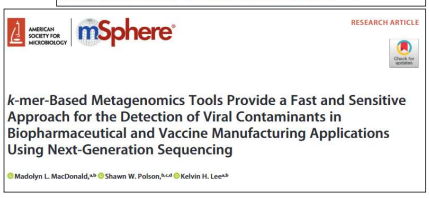


Biologicals

Volume 59, May 2019, Pages 29-36

Use of a new RNA next generation sequencing approach for the specific detection of virus infection in cells

Audrey Brussel[a], Kerstin Brack[b], Erika Muth[c], Rudolf Zirwes[c], Justine Cheval[c], Charles Hebert[c], Jean-Marie Charpin[c], Alice Marinacci[b], Benoit Flan[a], Horst Ruppach[b], Pascale Beurdeley[c], Marc Eloit[c,d,e,f]✉

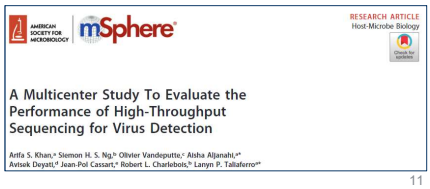


mSphere

RESEARCH ARTICLE

k-mer-Based Metagenomics Tools Provide a Fast and Sensitive Approach for the Detection of Viral Contaminants in Biopharmaceutical and Vaccine Manufacturing Applications Using Next-Generation Sequencing

Madolyn L. MacDonald,[a,b] Shawn W. Polson,[b,c,d] Kelvin H. Lee[a,b]



mSphere

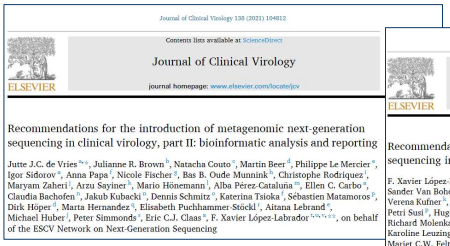
RESEARCH ARTICLE Host-Microbe Biology

A Multicenter Study To Evaluate the Performance of High-Throughput Sequencing for Virus Detection

Arifa S. Khan,[a] Siemon H. S. Ng,[b] Olivier Vandeputte,[c] Aisha Aljanahi,[a,e] Avisek Deyati,[d] Jean-Pol Cassart,[e] Robert L. Charlebois,[b] Lanyn P. Tallaferro[a,e]

Notch THERAPEUTICS

Alignment in perspectives



Journal of Clinical Virology 138 (2021) 104812

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Recommendations for the introduction of metagenomic next-generation sequencing in clinical virology, part II: bioinformatic analysis and reporting

Jutte J.C. de Vries[a,c], Julianne R. Brown[b], Natacha Couto[c], Martin Beer[d], Philippe Le Mercier[e], Igor Sidorov[a], Anna Papa[f], Nicole Fischer[g], Bas B. Oude Munnink[a], Christophe Rodriguez[f], Maryam Zaheri[g], Arzu Sayiner[h], Mario Hönemann[i], Alba Pérez-Cataluña[j,k], Ellen C. Carbo[a], Claudia Bachofen[l], Jakub Kubacki[m], Dennis Schmitz[n], Katerina Tsioka[f], Sébastien Matamoros[o], Dirk Höper[d], Marta Hernandez[q], Elisabeth Puchhammer-Stöckl[r], Aitana Lebrand[q], Michael Huber[s], Peter Simmonds[t], Eric C.J. Claas[a], F. Xavier López-Labrador[q,u,v,zz], on behalf of the ESCV Network on Next-Generation Sequencing

Journal of Clinical Virology 134 (2021) 104691

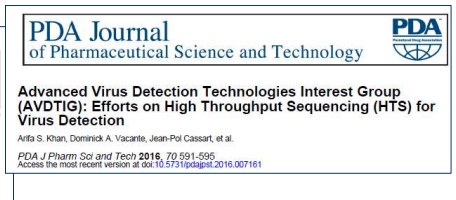
Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Recommendations for the introduction of metagenomic high-throughput sequencing in clinical virology, part I: Wet lab procedure

F. Xavier López-Labrador[a,b], Julianne R. Brown[c], Nicole Fischer[d], Heli Harvala[e], Sander Van Boheemen[f], Ondrej Cinek[g], Arzu Sayiner[h], Tina Vasehus Madsen[i], Eeva Auvinen[j], Verena Kufner[k], Michael Huber[k], Christophe Rodriguez[l], Marcel Jonges[m,n], Mario Hönemann[o], Petri Susi[p], Hugo Sousa[q,r,s], Paul E. Klapper[t], Alba Pérez-Cataluña[u], Marta Hernandez[w], Richard Molenkamp[x], Lia van der Hoek[m,n], Rob Schuurman[y], Natacha Couto[z,aa], Karoline Leuzinger[b,ab], Peter Simmonds[c], Martin Beer[d], Dirk Höper[d], Sergio Kamminga[ac], Mariet C.W. Feltkamp[ac], Jesús Rodríguez-Díaz[w], Els Keyaerts[ad], Xiaohui Chen Nielsen[i], Elisabeth Puchhammer-Stöckl[ae], Aloys C.M. Kroes[af], Javier Buesa[ag], Judy Breuer[ah], Eric C. J. Claas[g], Jutte J.C. de Vries[g,ai], on behalf of the ESCV Network on Next-Generation Sequencing



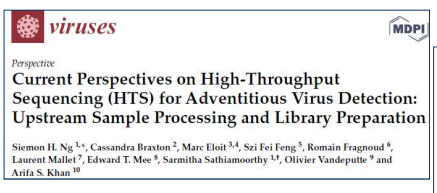
PDA Journal of Pharmaceutical Science and Technology

Advanced Virus Detection Technologies Interest Group (AVDTIG): Efforts on High Throughput Sequencing (HTS) for Virus Detection

Arifa S. Khan, Dominick A. Vacante, Jean-Pol Cassart, et al.

PDA J Pharm Sci and Tech **2016**, *70* 591-595

Access the most recent version at doi:10.5731/pdajpst.2016.007161



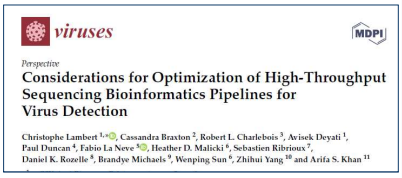
viruses

MDPI

Perspective

Current Perspectives on High-Throughput Sequencing (HTS) for Adventitious Virus Detection: Upstream Sample Processing and Library Preparation

Siemon H. Ng [3,*], Cassandra Braxton [2], Marc Eloit [3,4], Szi Fei Feng [5], Romain Fragnoud [6], Laurent Mallet [7], Edward T. Mee [8], Sarmitha Sathiamoorthy [1,†], Olivier Vandeputte [9] and Arifa S. Khan [10]



viruses

MDPI

Perspective

Considerations for Optimization of High-Throughput Sequencing Bioinformatics Pipelines for Virus Detection

Christophe Lambert [1,*], Cassandra Braxton [2], Robert L. Charlebois [3], Avisek Deyati [1], Paul Duncan [4], Fabio La Neve [5], Heather D. Malicki [6], Sebastien Ribrioux [7], Daniel K. Rozelle [8], Brandye Michaels [9], Wenping Sun [6], Zhihui Yang [10] and Arifa S. Khan [11]

nature biotechnology

PERSPECTIVE

https://doi.org/10.1038/s41587-020-0507-2

Viral contamination in biologic manufacture and implications for emerging therapies

Paul W. Barone[1], Michael E. Wiebe[1], James C. Leung[1], Islam T. M. Hussein[1], Flora J. Keumurian[1], James Bouressa[2,25], Audrey Brussel[3,26], Dayue Chen[4,27], Ming Chong[5], Houman Dehghani[6,28], Lionel Gerentes[7], James Gilbert[8,29], Dan Gold[9], Robert Kiss[10,30], Thomas R. Kreil[11], René Labatut[3], Yuling Li[12,31], Jürgen Müllberg[13], Laurent Mallet[14,31], Christian Menzel[15], Mark Moody[16,31], Serge Monpoeho[17], Marie Murphy[4], Mark Plavsic[18,34], Nathan J. Roth[19], David Roush[19], Michael Ruffing[20], Richard Schicho[21,35], Richard Snyder[22], Daniel Stark[23], Chun Zhang[24,36], Jacqueline Wolfrum[1], Anthony J. Sinskey[1] and Stacy L. Springs[1]✉

Notch THERAPEUTICS

Reference Standards and Database

Vaccine 34 (2016) 2035–2043

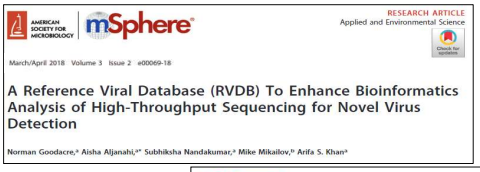
Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Development of a candidate reference material for adventitious virus detection in vaccine and biologicals manufacturing by deep sequencing

Edward T. Mee[a,*], Mark D. Preston[b], CS533 Study Participants[1], Philip D. Minor[a], Silke Schepelmann[a]

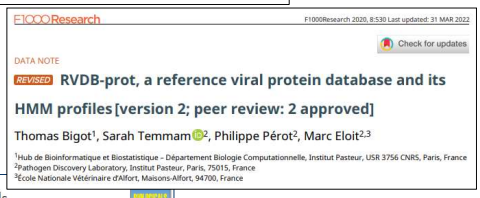


RESEARCH ARTICLE
Applied and Environmental Science

March/April 2018 Volume 3 Issue 2 e00069-18

A Reference Viral Database (RVDB) To Enhance Bioinformatics Analysis of High-Throughput Sequencing for Novel Virus Detection

Norman Goodacre,[a] Aisha Aljanahi,[a*] Subhiksha Nandakumar,[a] Mike Mikailov,[b] Arifa S. Khan[a]



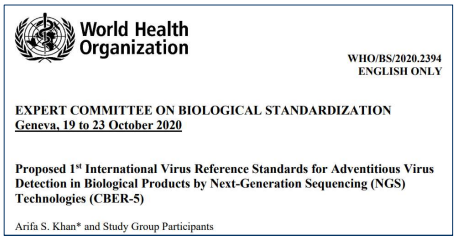
F1000Research 2020, 8:530 Last updated: 31 MAR 2022

DATA NOTE

REVISED RVDB-prot, a reference viral protein database and its HMM profiles [version 2; peer review: 2 approved]

Thomas Bigot[1], Sarah Temmam[1,2], Philippe Pérot[2], Marc Eloit[2,3]

[1]Hub de Bioinformatique et Biostatistique – Département Biologie Computationnelle, Institut Pasteur, USR 3756 CNRS, Paris, France
[2]Pathogen Discovery Laboratory, Institut Pasteur, Paris, 75015, France
[3]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, 94700, France



World Health Organization

WHO/BS/2020.2394
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 19 to 23 October 2020

Proposed 1st International Virus Reference Standards for Adventitious Virus Detection in Biological Products by Next-Generation Sequencing (NGS) Technologies (CBER-5)

Arifa S. Khan* and Study Group Participants

Biologicals

Volume 64, March 2020, Pages 76-82

Meeting report

Report of the 2019 NIST-FDA workshop on standards for next generation sequencing detection of viral adventitious agents in biologics and biomanufacturing ☆

Megan H. Cleveland, Bharathi Anekella, Michael Brewer, Pei-Ju Chin, Heather Couch, Eric Delwart, Jim Huggett, Scott Jackson, Javier Martin, Serge Monpoeho, Tom Morrison, Siemon H.S. Ng, David Ussery, Arifa S. Khan

Notch THERAPEUTICS

How could the Ph. Eur. help in the exercise to validate HTS methods?

Creation a Ph. Eur General Chapter

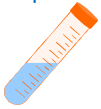
- Expert group 15: ”Human Vaccines and Sera” is working towards a new Ph. Eur. general chapter on “High Throughput Sequencing for the detection of extraneous agents (2.6.41)”
- This chapter will provide details on important considerations when implementing a HTS for adventitious virus detection assay and provide guidance on the validation of such a method.
- Support the implementation the HTS technology in a GMP environment

Describe different HTS technology and the multiple possible approaches

- Provide a standardization of critical components that are part of the assay

Topics to be covered:

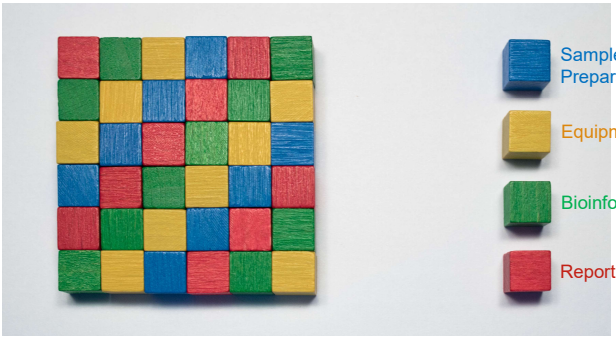
- Different analysis (Genomics, Transcriptomics, Targeted) and sample types (Cell banks, Viral seeds, Harvest)

Sample Preparation	Sequencing	Bioinformatics	Reporting
			
• Pre-treatment / Enrichments • Nucleic acid extraction • DNA or RNA Library preparation • Recommend indexing	• Short reads and long reads • RNA Sequencing • Appropriate read depth, coverage • Considerations for multiplexing	• Bioinformatics pipeline • Removal of expected sequences • Assembly, mapping, alignment • Database requirements (i.e. suitable breadth of detection)	• Interpretation of results • Tool-box for Follow-up investigations • Report summary • Acceptance Criteria

Validation Approach

- Recommends a modular approach where individual components can be qualified / validated separately before validating the entire method
  - Individual elements or parameters can be changed without major impact on overall method validation

- Key elements for method validation



Opportunity to streamline the method validation

- Validation of a complex method and platform that doesn't necessarily follow ICH Q2
  - Breadth of detection (specificity) demonstrated using a few model viruses representing different types of viruses
    - Minimum characterization requirements for model viruses
  - Propose to minimized the number of replicates to two or three runs
    - Achieve a balance between cost and demonstrating consistency
    - How to demonstrate the equivalency of the results between replicates?
  - Validation parameters
    - Demonstrating reproducibility for qualitative vs quantitative assays
    - Sensitivity and LOD

- Clarification that a head-to-head comparison is not needed to replace in vivo tests

Promote Standardization for the use of HTS

- Provide the first strategic frame-work for the HTS validation approach
- Clarify regulatory expectation
- Promote the use of the HTS technology in a GMP environment
- Potentially serve as a foundation for evaluating HTS dossier submission

HTS in Other Areas

Expansion of HTS applications

Transfusion Medicine and Hemotherapy

Review Article

Transfus Med Hemother 2019;46:87–93
DOI: 10.1159/000499088

Received: November 14, 2018
Accepted: February 21, 2019
Published online: March 15, 2019

Viral Metagenomics of Blood Donors and Blood-Derived Products Using Next-Generation Sequencing

Sophie Waldvogel-Abramowski[a, b] Sofiane Taleb[c] Marco Alessandrini[d] Olivier Preynat-Seauve[e, f]

ELSEVIER

Food Microbiology
Volume 79, June 2019, Pages 96-115

The use of next generation sequencing for improving food safety: Translation into practice

Balamurugan Jagadeesan [a], Peter Gerner-Smidt [b], Marc W. Allard [c], Sébastien Leuillet [d], Anett Winkler [e], Yinghua Xiao [f], Samuel Chaffron [g], Jos Van Der Vossen [h], Silin Tang [i], Mitsuru Katase [j], Peter McClure [k], Bon Kimura [l], Lay Ching Chai [m], John Chapman [n], Kathie Grant [o]

Expansion of HTS applications

- Gene and Cell Therapy

Published online 8 December 2020 *Nucleic Acids Research, 2021, Vol. 49, No. 3 e16*
doi: 10.1093/nar/gkaa1152

Genome-wide integration site detection using Cas9 enriched amplification-free long-range sequencing

Joost van Haasteren[†], Altar M. Munis[†], Deborah R. Gill and Stephen C. Hyde*

Gene Medicine Group, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Biotechnology Journal
Systems & Synthetic Biology · Nanobiotech · Medicine

Research Article

Enhanced CHO Clone Screening: Application of Targeted Locus Amplification and Next-Generation Sequencing Technologies for Cell Line Development

Samuel H. Aeschlimann, Christian Graf, Dmytro Mayilo, Hélène Lindecker, Lorena Urda, Nora Kappes, Alicia Leone Burr, Marieke Simonis, Erik Splinter, Max van Min, Holger Laux

First published: 22 February 2019 | https://doi.org/10.1002/biot.201800371 | Citations: 4

BIOTECHNOLOGY PROGRESS

Society for Biological Engineering

AIChE

RESEARCH ARTICLE

Multiplexed clonality verification of cell lines for protein biologic production

Sofie A. O'Brien, Juhi Ojha, Paul Wu, Wei-Shou Hu

First published: 07 February 2020 | https:

Nat Biotechnol. 2014 Oct;32(10):1019-25. doi: 10.1038/nbt.2959. Epub 2014 Aug 17.

Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping

Paula J P de Vree [1], Elzo de Wit [2], Mehmet Yilmaz [3], Monique van de Heijning [3], Petra Klous [3], Marjon J A M Verstegen [4], Yi Wan [4], Hans Teunissen [4], Peter H L Krijger [4], Geert Geeven [4], Paul P Eijk [5], Daoud Sie [5], Bauke Ylstra [5], Lorette O M Hulsman [6], Marieke F van Dooren [6], Laura J C M van Zutve, Marion Cornelissen [8], Pieter van der Vlies [9], Mohamed Lamkanfi [1], John A Foekens [14], Jo, Max van Min [3], Erik S

nature biotechnology

Article | Published: 15 June 2020

CHANGE-seq reveals genetic and epigenetic effects on CRISPR–Cas9 genome-wide activity

Cicera R. Lazzarotto, Nikolay L. Malinin, Yichao Li, Ruochi Zhang, Yang Yang, GaHyun Lee, Eleanor Cowley, Yanghua He, Xin Lan, Kasey Jividen, Varun Katta, Natalia G. Kolmakova, Christopher T. Petersen, Qian Qi, Evgheni Strelcov, Samantha Maragh, Giedre Krenciute, Jian Ma, Yong Cheng & Shengdar Q. Tsai

Nature Biotechnology 38, 1317–1327 (2020) | Cite this article

Nat Biotechnol. 2020 November ; 38(11): 1317–1327. doi:10.1038/s41587-020-0555-7.

CHANGE-seq reveals genetic and epigenetic effects on CRISPR-Cas9 genome-wide activity

Cicera R. Lazzarotto[1], Nikolay L. Malinin[1], Yichao Li[1], Ruochi Zhang[2], Yang Yang[2], GaHyun Lee[1], Eleanor Cowley[3], Yanghua He[1,4], Xin Lan[1], Kasey Jividen[1], Varun Katta[1], Natalia G. Kolmakova[5], Christopher T. Petersen[6], Qian Qi[1], Evgheni Strelcov[7,8], Samantha Maragh[5], Giedre Krenciute[6], Jian Ma[2], Yong Cheng[1], Shengdar Q. Tsai[1,*]

Conclusion

Summary and Conclusion

- HTS is a technology capable of known and unknown viral adventitious agent in a biological samples.
- Recent achievements and alignment within the community towards the implementation of a viral adventitious agent detection by HTS as a GMP specification test.
- Drafting of a new Ph. Eur. general chapter will provide a suitable approach towards validation of the HTS assay and promote wider adaption of the HTS technology

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

edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

New pyrogenicity strategy

of the European Pharmacopoeia

Dr. Emmanuelle Charton
Head of DivB
European Pharmacopoeia Department

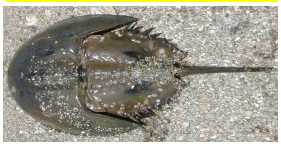
Assays for pyrogens / endotoxins in the Ph. Eur.

1971

Pyrogens (2.6.8)
(*"Rabbit Pyrogen Test"*)

Pyrogen detection

1987



LAL is a lyophilised amoebocyte lysate obtained from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*)

BET (2.6.14) & Guidelines for using the BET (5.1.10)

- *BET using recombinant Factor C (2.6.32) [NEW]*

2020

Endotoxin detection
(e.g. LPS from Gram- bacteria)

2010

Monocyte-activation test (2.6.30)

- *Monocyte activation test for vaccines containing inherently pyrogenic components (2.6.40) [Draft Phpa 33.3 NEW]*

2023?

Pyrogen detection

Replacement of the Rabbit Pyrogen Test

The RPT continues to be widely performed although:

- Several EDQM conferences (1988, 2011) have been held on the issue
- Chapter *2.6.8. Pyrogens* encourages the replacement

> In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. Wherever possible and after product-specific validation, the pyrogen test is replaced by the monocyte-activation test (*2.6.30*).

- Ph. Eur. has introduced amendments to relevant texts to encourage users to perform *in vitro* tests for the control of pyrogens

In view of the situation, the complete removal of the RPT from the Ph. Eur. is necessary if the aim is to move towards the exclusive use of *in vitro* tests for the control of pyrogens

2.6.8. Pyrogens in the Ph. Eur.

General monographs (3)

- Substances for pharmaceutical use (*2034*) BET WP
- Radiopharmaceutical preparations (*0125*) G14
- Immunosera for human use, animal (*0084*) G15

Dosage form monographs (3)

- Parenteral preparations (*0520*)
- Preparations for irrigation (*1116*) G12
- Intravesical preparations (*2811*)

Pyrogens (*2.6.8*)
(Rabbit pyrogen test)
60 texts

General chapters (3)

Plastics

- Sterile plastic containers for human blood and blood components (*3.3.4*) G16
- Sets for the transfusion of blood and blood components (*3.3.7*)

Vaccines for human use

- Carrier proteins for the production of conjugated polysaccharide vaccines for human use (*5.2.11*) G15

Individual monographs (50)

- solutions (4) G12 DIA WP
- blood products (17) G6B
- vaccines for human use (17) G15
- antibiotics (8) G7
- other chemical substances (4) G10D G9 CRB WP

Replacement of chapter *2.6.8: proposed strategy*

Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022

***2.6.8** in 60 texts of the PhEur*

***2.6.8** in 60 texts of the PhEur*
5.1.13
Pyrogenicity

New! Chapter *5.1.13.* Pyrogenicity

Potential pyrogens other than endotoxins can be ruled out

Risk analysis, potential presence of non-endotoxin pyrogens Stage of manufacturing process

Exclusion of potential pyrogens other than endotoxins not possible

USER

Chapters *2.6.14/ 2.6.32.* BET or both **Chapter *2.6.30.* MAT**

Decision on a testing strategy
Decision on the limits

https://go.edqm.eu/NewPyrogenicityStrategy

Activities and actions at the level of GoEs and WPs

GoE 9
(Inorganic chemistry)

GoE 10D
(Organic chemistry – (semi)synthetic products)

GoE 7
(Antibiotics)

GoE 14
(Radiopharmaceutical preparations)

GoE 12
(Dosage forms and PT procedures)

GoE 16
(Plastic materials, plastic containers and closures)

CRB WP
(Carbohydrates)

DIA WP
(Dialysis)

GoE 6B
(Human plasma and plasma products)

GoE 15
(Vaccines for human use)

BET WP
+ ad hoc experts
Chair of the WP

- ***5.1.13*** Pyrogenicity
- ***2034*** *Substances for pharmaceutical use*
- ***5.1.10*** *Guidelines for using the test for bacterial endotoxins*

Texts are being processed for Pharmeuropa!

Activities and actions at the level of GoEs and WPs

GoE 9 (Inorganic chemistry)

GoE 10D (Organic chemistry – (semi)synthetic products)

GoE 7(Antibiotics)

GoE 14
(Radiopharmaceutical preparations)

GoE 12
(Dosage forms and PT procedures)

GoE 16
(Plastic materials, plastic containers and closures)

CRB WP (Carbohydrates)

More than 50 hours of live discusssions in plenary meetings of Groups of Experts!

DIA WP (Dialysis)

GoE 6B
(Human plasma and plasma products)

GoE 15
(Vaccines for human use)

Substances for pharmaceutical use (2034)

Related substances. Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1 or in Table 2034.-2 for peptides obtained by chemical synthesis.

Table 2034.-1. – *Reporting, identification and qualification of organic impurities in active substances*

Use	Maximum daily dose	Report-ing threshold	Identification threshold	Qualification threshold
				> 0.15 per cent or a daily intake of > 1.0 mg (whichever is
				per cent
Veterinary use only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

Table 2034.-2. – *Reporting, identification and qualification of*

Sterility (*2.6.1*). If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, or if offered as sterile grade, the substance for pharmaceutical use complies with the test for sterility.

Bacterial endotoxins (*2.6.14*). The substance for pharmaceutical use complies with the test for bacterial endotoxins if ... as a bacterial endotoxin-free grade or if it is intended ... of parenteral preparations or prepared ... without a further appropriate procedure for removal of bacterial endotoxins. The limit, where ... monograph, is determined ... with the recommendations of general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*.

Pyrogens (*2.6.8*). ... test for pyrogens is ... rather than the test for bacterial endotoxins ... pyrogen-free grade is offered, ... pharmaceutical use complies with the test ... limit and test method are stated in the individual monograph and approved by the competent authority ... validation for bacterial endotoxins ... and pyrogens ... for bacterial endotoxins may ... the test for pyrogens.

Pyrogenicity (*5.1.13*)

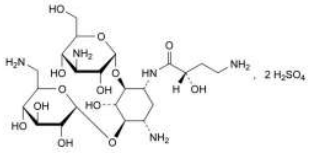
microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Individual monographs on substances for pharmaceutical use

01/2019:1290
corrected 10.0

AMIKACIN SULFATE

Amikacini sulfas



$C_{22}H_{43}N_5O_{13}S_2$ [39831-55-5] M_r 782

DEFINITION

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulfate.

Antimicrobial substance obtained from kanamycin A.

Semi-synthetic product derived from a fermentation product.

Content: 96.5 per cent to 102.0 per cent (dried substance).

Loss on drying (*2.2.32*): maximum 13.0 per cent, determined on 0.500 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 3 h.

Pyrogens (*2.6.8*). If intended for use in the manufacture of parenteral ... appropriate procedure for the ..., it complies with the test for pyrogens. ... of the rabbit's mass 5 mL of a solution ... the substance to be examined in ... *Injections*.

ASSAY

The new requirements of general monograph *2034* apply

mobile phase and dilute to 10.0 mL with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Parenteral preparations (0520)

Parenteral preparations

07/2021:0520

PARENTERAL PREPARATIONS

Pyrogenicity (*5.1.13*)

necessarily apply
veterinary use
preparation is

DEFINITION

Parenteral preparations are sterile preparations intended for administration into the human or animal body. They may be administered by injection, infusion or implantation.

They are liquid, semi-solid or solid preparations containing one or more active substances in a suitable vehicle. Liquid preparations for injection or infusion are solutions, colloidal dispersions, emulsions or suspensions.

Sterility (*2.6.1*). Parenteral preparations comply with the test.

Bacterial endotoxins - pyrogens. Parenteral preparations for human use, if applicable after reconstitution or dilution, comply with the te bacterial endotoxins (*2.6.14*) where justified and auth ith the test for pyroge Recommendati its for bacterial given in gener e lim preparations is e

Where the label states om bacterial endotoxins or th preparation complies with the test *4*) or with the test for pyroge

Parenteral pre ary u st for bacterial en *4*) or with the ns (*2.6.8*) when the be injected in a sing 15 mL or more and equivalent to a dose of 0.2 mL more per kilogram of body mass.

STORAGE

In a sterile, airtight, tamper-evident container.

Plasma-derived products

HUMAN VON WILLEBRAND FACTOR

Factor humanus von Willebrandi

Pyrogenicity (*5.1.13*)

DEFINITION

Sterile, freeze-dried preparation of a plasma protein fractio Willebrand factor tion factor VIII, . It prepared fro ine on *Humi* paration may conta

This monograph applies to preparations formulated accordi to the human von Willebrand factor activity.

The potency of the preparation, reconstituted as stated on t label, is not less than 20 IU of human von Willebrand factor per millilitre.

Sterility (*2.6.1*). It complies with the test.

Pyrogens (*2.6 Bacterial endotoxin 6.14*). It complies with the test ns or, preferal here justified and author dated as the test for bacterial t

For the pyrogen te of the rabbit's mass a volume equivalen IU of human von Willebrand fac

Where the endo e preparation to be examin is less than 0.0 dotoxin per International U of human von Willebr factor.

Limits for BET maintained, Endotoxin Equivalents(5.1.13)

Vaccines for human use

Monograph/chapter		Requirement for RPT
Hepatitis B-containing vaccines	- Hep B (1056) - DT-Hep B* (2062) - DTaP-Hep B* (1933)	- RPT on the final lot
3-O-Desacyl-4'-monophosphoryl lipid A (MPL) (2537)		- RPT on an intermediate
Haemophilus influenza type b-containing vaccines	- Hib (1219) - DTaP-Hib (1932) - DTaP-IPV-Hib (2065) - DTwP-IPV-Hib* (2066)	- RPT as a process validation requirement - RPT on the final lot if any vaccine component prevents the determination of endotoxin
	- DTaP-IPV-Hep B-Hib (2067) - Hib-Men C (2622)	- RPT as a requirement during product development
Meningococcal vaccines	- Men PS vaccine (0250)	- RPT on an intermediate and on the final lot
	- Men C conjugate vaccine (2112) - Men A, C, W135, Y conjugate vaccine (3066)	- RPT as a process validation requirement
Pneumococcal vaccines	- Pneumococcal polysaccharide vaccine (0966)	- RPT on final lot
	- Pneumococcal conjugate vaccine (2150)	- RPT as a requirement during product development
Rabies vaccine (0216)		- RPT on the final lot in case non-endotoxin pyrogens are present
Tick-borne encephalitis vaccine (1375)		- RPT on the final lot
Carrier proteins for the production of conjugated vaccines (5.2.11)		- RPT for *N. meningitidis* outer membrane protein complex (OMP)

+ Revise general monograph *Vaccines for human use* (*0153*)

*monographs will be suppressed from the Ph. Eur. as of July 2023 (Supplement 11.2)

General monograph *Vaccines for human use* (*0153*)

NOTE ON THE GENERAL MONOGRAPH

***Pyrogenicity**. The section on Bacterial endotoxins in the Tests part of the monograph has been replaced with a new section on Pyrogenicity, referring to new general chapter 5.1.13 Pyrogenicity which provides guidance for selection and implementation of a suitable test for pyrogenicity (test for bacterial endotoxins or monocyte-activation test).*

In addition, a statement has been introduced under General provisions in the Production part of the monograph to stress the need to characterise pyrogenicity during development studies and whenever revalidation is necessary.

This revision of general monograph 0153 is part of a broader exercise affecting multiple Ph. Eur. texts and aiming at the complete suppression of the rabbit pyrogen test from the Ph. Eur.

As part of this exercise, the following texts have been published in the same issue of Pharmeuropa: 1) new general chapter 5.1.13 Pyrogenicity; 2) monographs on individual vaccines for human use that were revised to delete the reference to th en test. The revised individual monographs no longer contain any mentio sting and, as a result, the requirements of general monograph 0153 for under General provisions and Tests) will apply.

Importantly, the revision of the monograph does not call into question established manufacturers' strategies to control the pyrogenicity of their products using the test for bacterial endotoxins that were authorised by the competent authority, and is not intended to prompt a retrospective assessment on pyrogenicity.

PRODUCTION

General provisions. The production method for a given product must have been shown to yield consistently batches comparable with the batch of proven clinical efficacy, immunogenicity and safety in man.

Product specifications including in-process testing should be set. Specific requirements for production including in-process testing are included in individual monographs. Where justified and authorised, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test.

Unless otherwise justified and authorised, vaccines are produced using a seed-lot system. The methods of preparation are designed to maintain adequate immunogenic properties, to render the preparation harmless and to prevent contamination with extraneous agents.

Pyrogenicity is characterised during development studies and controlled whenever revalidation is necessary. Guidance for selection of a suitable pyrogenicity test is given in general chapter *5.1.13*.

TESTS

Vaccines comply with the tests prescribed in individual monographs including, where applicable, the following: [...]

~~**Bacterial endotoxins**. Unless otherwise justified and authorised, a test for bacterial endotoxins is carried out on the final product. Where no limit is specified in the individual monograph, the content of bacterial endotoxins determined by a suitable method (*2.6.14*) is less than the limit approved for the particular product.~~

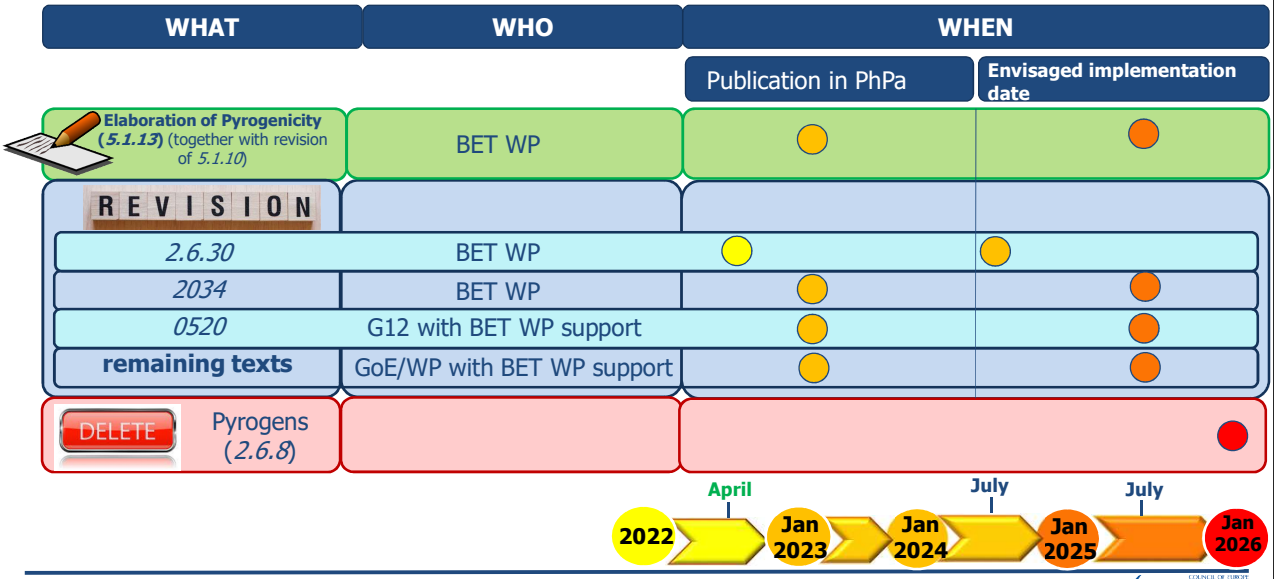
Pyrogenicity. The vaccine complies with a suitable test for pyrogenicity. Guidance for selection of a test is given in general chapter *5.1.13*. Where no limit is specified in the individual monograph, it complies with the limit approved for the particular product.

NOTES ON THE TEXTS

- *"It should be noted that the exercise will ultimately lead to the suppression of general chapter 2.6.8 from the Ph. Eur. Manufacturers still using the rabbit pyrogen test are strongly encouraged to take the necessary steps to proceed with its replacement by a suitable in vitro alternative (e.g. the monocyte-activation test), in line with the new requirements of this general monograph."*

- *"Importantly, the revision of this text does not call into question strategies involving the test for bacterial endotoxins that are already used by manufacturers to control the pyrogenicity of their products and have been authorised by the competent authority, nor is it intended to prompt a retrospective assessment of pyrogenicity."*

Timelines



Communication to stakeholders

EPC
Project started

Ph. Eur. Groups of Experts

PharmaLab Conference (25/11)

European Microbiology conference 04/05

EPC
Strategy finalised

2021 | June | September | October | November | 2022 | March | May | June | September

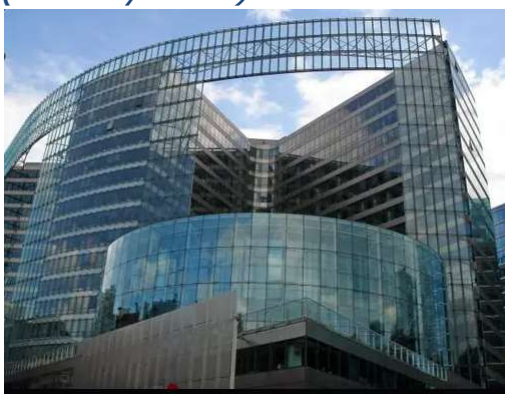
PDA Endotoxin Workshop (07/10)

NC3R Workshop (03/03)

EDQM 11th edition Conference (19-21/09)

EPAA/EDQM International Public Conference

To mark the first official milestone of the strategy, i.e. the publication of revised Ph. Eur. texts omitting the RPT in Pharmeuropa 35.1 (January 2023)



Date: **14-16 February 2023**

Venue: European Commission premises, Brussels

Acknowedgements

- The BET Working Party and its Chair, Dr. Ingo Spreitzer
- All the experts in Groups of Experts and Working Parties (6, 6B, 7, 9, 10D, 12, 14, 15, 16, CRB, DIA) and their respective Chairs
- All EDQM European Pharmacopoeia Department staff members who worked on the revised and new texts, with particular thanks to Dr Gwenaël Ciréfice who co-ordinated the exercise

Thank you for your attention

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3R & Pyrogen detection in the vaccine industry

Shahjahan Shaid - Global Quality

Disclosure

Shahjahan Shaid is an employee of the GSK group of companies.
This work was sponsored by GlaxoSmithKline Biologicals SA.

Agenda

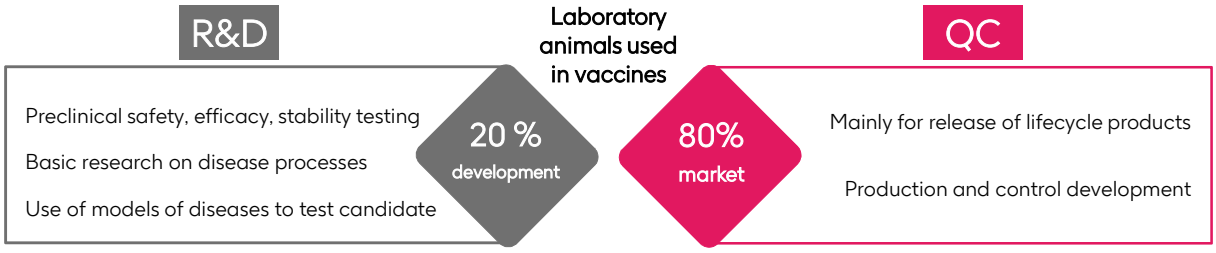
1. Why is GSK using animal tests
2. GSK´s commitment to shift towards non-animal tests
3. 3R Achievements, future perspectives and challenges of pyrogen testing

Why is GSK using animal tests

A small but crucial part of the business

Why is GSK Vaccines using animal tests

Animal tests are required by compendia for lifecycle vaccines



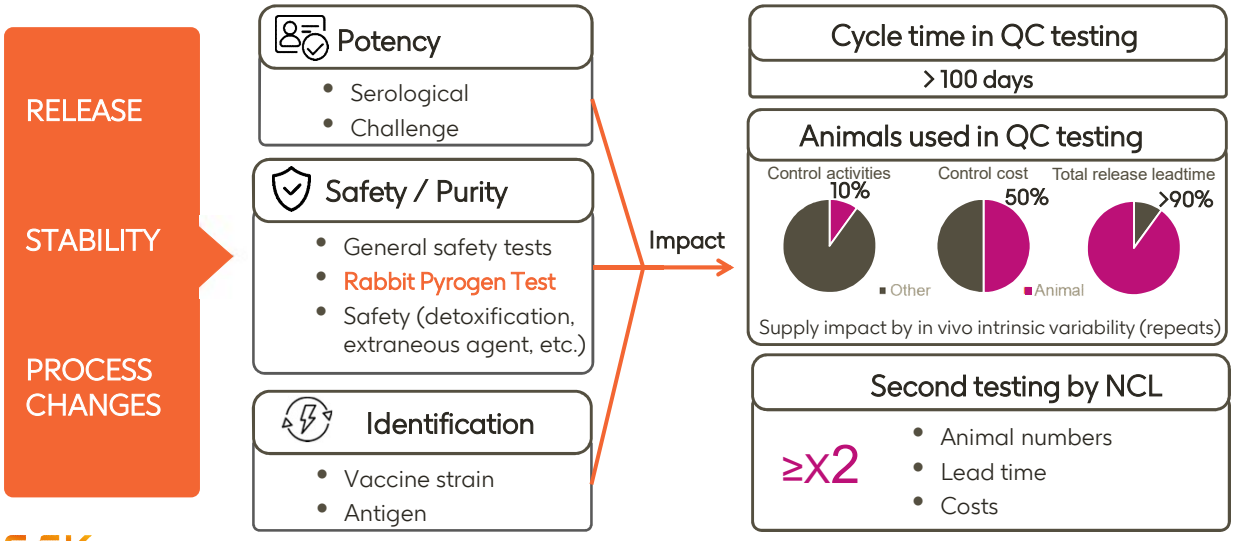
Animal assays are required by WHO and regional Pharmacopeias which are often not aligned

e.g. EDQM has prioritized a substitution where scientifically possible.

The substitution in a biologics license application might therefore be rejected by certain countries and can take years due to number of countries involved for each product.

Impact of *in vivo* assays in lifecycle vaccines

Animal tests have a disproportionate high impact on release compared to other test



GSK's Vaccine commitment to shift towards non-animal tests

3R strategy: created to respond to the changing environment

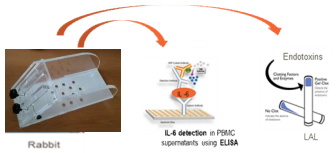
GSK

3R improves the quality of science by addressing animal welfare

Replacement is the first priority

REPLACE Information gathered with a different approach

Development and use of models and tools to address scientific questions without the use of animals.



RPT → MAT & BET

REDUCE Animals and information

Appropriately designed & analyzed animal experiments that are robust & reproducible & truly added knowledge base

REFINE Increased welfare

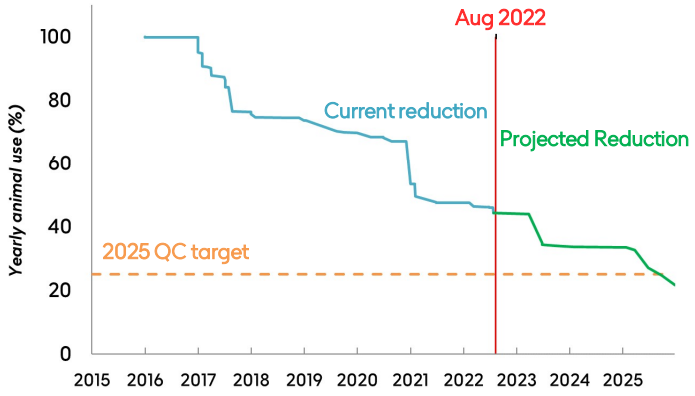
Advancing animal welfare research by exploiting the latest technologies and improving the understanding of the impact of welfare on scientific outcomes

Handling, containment, stress, temperature rise

3R & GSK: Prioritize replacement to further reduce animal use by 75% from 2016-2025

Animal use in lifecycle has been already reduced by 75% from 2007 to 2015

More information @ GSK web site **The 3R at GSK**



Conducting animal studies with high standards of humane care and treatment is GSK's moral responsibility and priority.

Historically refinement had the highest priority. Having those high animal welfare standards and the availability of new non-animal-technologies allows a focus on replacement

3R Portfolio in QC

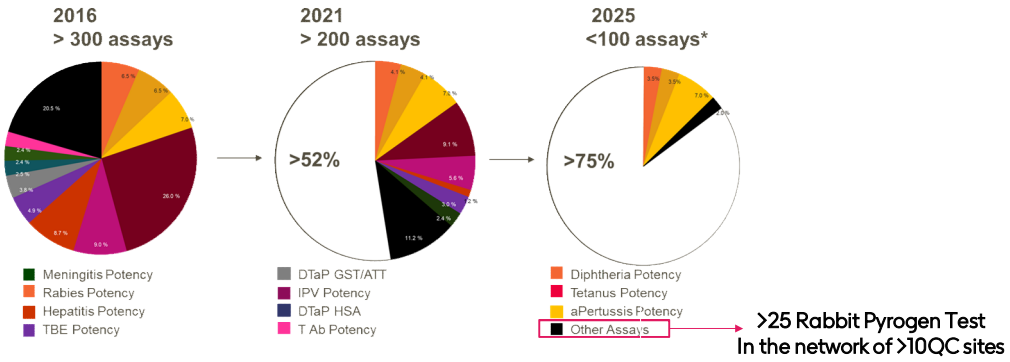
Replacement 80%

Reduction 15%

Refinement 5%

3R & GSK: Animal assays are conducted in Release stability and for process changes

A substitution at all three stages simultaneously is time consuming.



The RPT is a small but crucial part of GSK 3R strategy to ensure patient safety without animal testing.

3R Achievements, future perspectives and challenges of pyrogen testing

GSK

Pyrogen detection

Several techniques are available allowing non-animal detection of pyrogens

Exogenous pyrogens

Non-endotoxin pyrogens

- Components from Gram-positive bacteria
- Yeast and mould
- Virus
- Particles of the environment

Endotoxins

Components from Gram-negative bacteria (LPS)

Pyrogens detection

MAT: EP 2.6.30

- Recognizes pyrogens with a high sensitivity.
- Established in the PEP
- Mentioned in the Ch.P and USP

RPT: EP 2.6.8

- Measure of the **rabbit** body temperature after injection of the product.
- Former gold standard.

Endotoxins detection

BET Guideline : EP 5.1.10

Guidelines for using the test for bacterial endotoxins (and MAT)

rFC: EP 2.6.32

- Non-animal-derived reagent that can detect endotoxins.
- Accepted as equivalent in the E.P.

LAL: EP 2.6.14

- 1st commercial available endotoxin method
- Best established and harmonized alternative to the RPT
- Broad acceptance from authorities around the world

Rabbit Pyrogen Test will be removed in the EP by 2025

Substitution with non-animal technologies is mandatory in EU (directive 2010/63)

RPT will be an alternative method not described in the EP leaving the European manufacturers to ensure patients safety and animal ethics with MAT, rFC, TAL, LAL.

1. **Remove and rely on controls at other steps**
2. **Substitute by the BET test as a release**
3. **Replace by the MAT test as a release**

Substitute RPT with a risk-based approach

Avoid test duplication and rely on the most effectual test

1 **Remove and rely on controls at other steps**

A B +C

Lifecycle based on the consistency approach:

- Based upon the principle that the **quality of a biologic** is the result of the **strict application of a quality system and consistent production.**
- Subsequent **batches are determined to be similar to clinically evaluated batches** and therefore acceptable for release

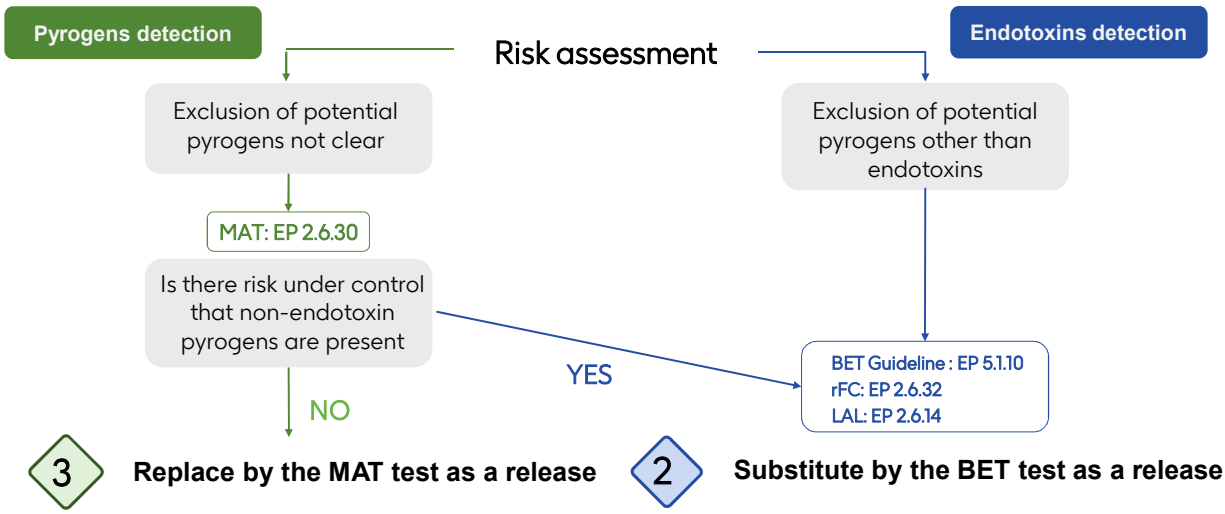
Pyrogen testing in Incoming Material (e.g. Antibiotics)

Pyrogenicity is

- Reduced in the manufacturing process
- Tested at intermediate drug product level
- Analyzed by the Supplier and part of the CoA
- Evaluated again at a later manufacturing steps

Pyrogen detection method should depend on a risk-based approach

Without the use of RPT



Substitute RPT with a risk-based approach

The manufacturing level and overall test schedule of the product needs to be considered as well

2 **Substitute by the BET test as a release**

- The **choice** of pyrogen detection strategy should **depend on a risk-based approach**.
- This should consider lifecycle, manufacture process, technology novelties, QC testing and intrinsic pyrogenic characteristics of the product.
- Modern vaccine manufacturers have **thoroughly validated controls, specifications and limits** embedded in a **GMP manufacturing process. Contaminants are** therefore appropriately **controlled.**
- The **absence of LPS** in materials combined with ruling out the presence of non-endotoxin pyrogens is a **strong indicator to justify the absence of exogenous pyrogens**.

Final container (e.g. Meningococcal A,C,W vaccine)	• There is no intrinsic pyrogenicity in the product, or matrix interference with rFC or BET • Pyrogenicity is evaluated as a combination of rFC/BET and further test e.g bioburden sterility and environmental monitoring

Substitute RPT with a risk-based approach

The manufacturing level and overall test schedule of the product needs to be considered as well

3 **Replace by the MAT test as a release**

- MAT recognizes all human pyrogens with high sensitivity **using human monocytes** to mimic fever responses to pyrogens in vivo
- Depending on the interleukin-6 **(IL-6)** response variability, two assays are described:
  - Semi quantitative for not inherently pyrogenic substances
  - **Full quantitative** for both inherently pyrogenic and non-pyrogenic substances
- MAT allows **validation according to** current **ICH guidelines**
- **MAT is established in QC and R&D to ensure a sustainable retirement of RPT in GSK vaccines**

Final container (e.g. Meningococcal B vaccine)	Endotoxin test is not applied. Instead, MAT is the method of choice due to • The intrinsic pyrogenicity of the product • Matrix interference with the rFC/BET tests.

Will GSK abolish the RPT?

RPT use has been drastically reduced. An abolition in lifecycle is a realistic target

R&D = NO

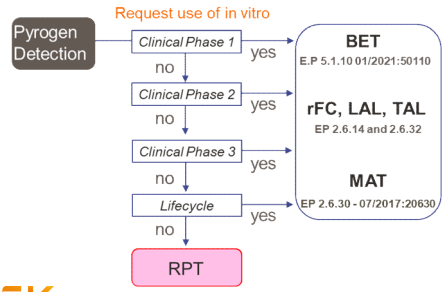
- GSK always proposes *in vitro* test if applicable
  This is not always accepted by all authorities
- If RPT is requested, GKS aims to have RPT as interim release test while proving equivalence or superiority of alternatives

RPT use in vaccines

~5%	2016	~95%
~50%	2022	~50%
100%	2025	0%

QC = YES

- The most suitable alternatives have been identified, validated, or even submitted for approval
- Target: all approvals before 2025
- If specific authorities request RPT, it will be reintroduced for the region



2016	> 25 RPT
2022	< 5 RPT
2025	0 RPT

Exemplary benefits for a replacement

<0.1% animal, Ethics, Compliance
>10d reduced test time, 25-75k€ ingross gains

To avoid the use of RPT beyond lifecycle, harmonization is required

RPT is still considered as gold standard in certain regions beyond Europe, impacting abolition at R&D level

While the BET is preferred by the user and widely accepted as a substitution method by Health authorities it is not always applicable.

The regulatory landscape beyond Europe regarding acceptance of MAT

WHO	**Only states RPT (proposal has been drafted)**
USA	USP 151 – Validated and equivalent in vitro test may be used in place of RPT **where appropriate**
China	**MAT** is **included to be conducted in addition to RPT** for guideline 9301: "Application of Safety Tests for Injection"
Rep. of Korea	**No mention of MAT** making it an **alternative method**.
Japan	**No mention of MAT** making it an **alternative method**. Requires proving superiority in terms of accuracy and precision
India	Considers **MAT a suitable alternative test requires to show equivalence**
Brazil	Pyrogen test required. Expected to add **MAT in their Pharmacopoeia**

To avoid the use of RPT beyond lifecycle, harmonization is required

RPT remains a gold standard in certain regions hindering its abolition in industry

- The **Ph.Eur.** is progressively **allowing the use of non animal tests as an equal** assay to RPT.
- **Beyond Europe *in vivo*** is usually the **gold standard** and *in vitro* assays are usually considered as alternative methods, **requiring** the user **demonstrate** for each product or process that **the *in vitro* test is equal or superior.**
- As manufacturer, both *in vivo* and *in vitro* assays must be validated and performed in parallel.
- An **acceptance of MAT as equivalent to RPT beyond Europe would allow to abolish the RPT** completely **in development** and limit the decision to whether BET or MAT is the most suitable for lifecycle testing.

The announced deletion of the RPT in the Ph.Eur. hopefully will start a deeper reflection in other compendia's.

Thanks to all the partners in GSK and outside

Substituting animal testing is providing business benefits in terms of ethics, quality, speed and cost