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

The Eur. Ph. strategy for the replacement of the Rabbit Pyrogenicity Test

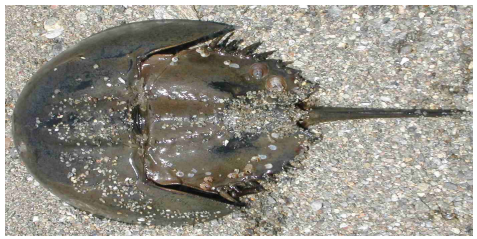
- HSI Webinar, 27 March 2024
- Transition to non-animal based vaccine batch release testing
- Policy and regulations theoretical aspects and case studies
- *Emmanuelle Charton, EDQM*

New Ph. Eur. Pyrogenicity strategy

1971

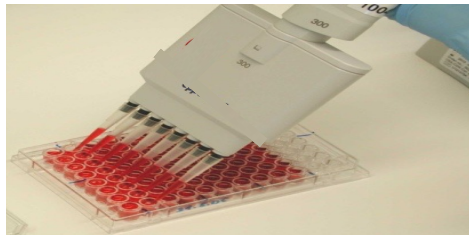
Pyrogens (2.6.8)

1987



BET (2.6.14)

2010



MAT (2.6.30)

2020

BET using rFC (2.6.32)

The RPT continues to be widely performed

Experts of the Ph. Eur.

The European Partnership for Alternative Approaches to Animal Testing

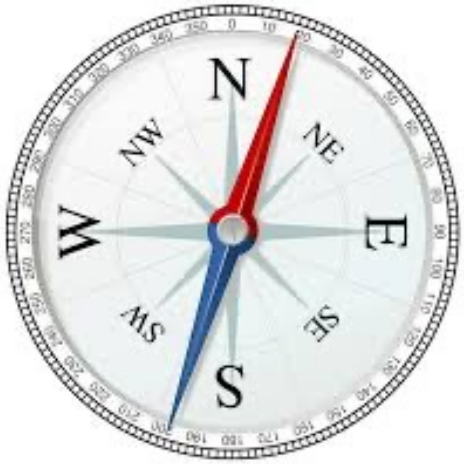
Proposal

- **New chapter *5.1.13* Pyrogenicity**
- **Deletion of the rabbit pyrogen test from 60 Ph. Eur. texts by 2025 and suppression of chapter *2.6.8* from the Ph. Eur. by 2026**

Public consultation in Pharmeuropa 35.1

New Ph. Eur. Pyrogenicity strategy

→ Published on Pharmeuropa webpage: https://go.edqm.eu/NewPyrogenicityStrategy



Strategy for removing or replacing the rabbit pyrogen test:
New pyrogenicity strategy of the European Pharmacopoeia Commission
September 2022

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 Ph. Eur. 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)

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

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

USER

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

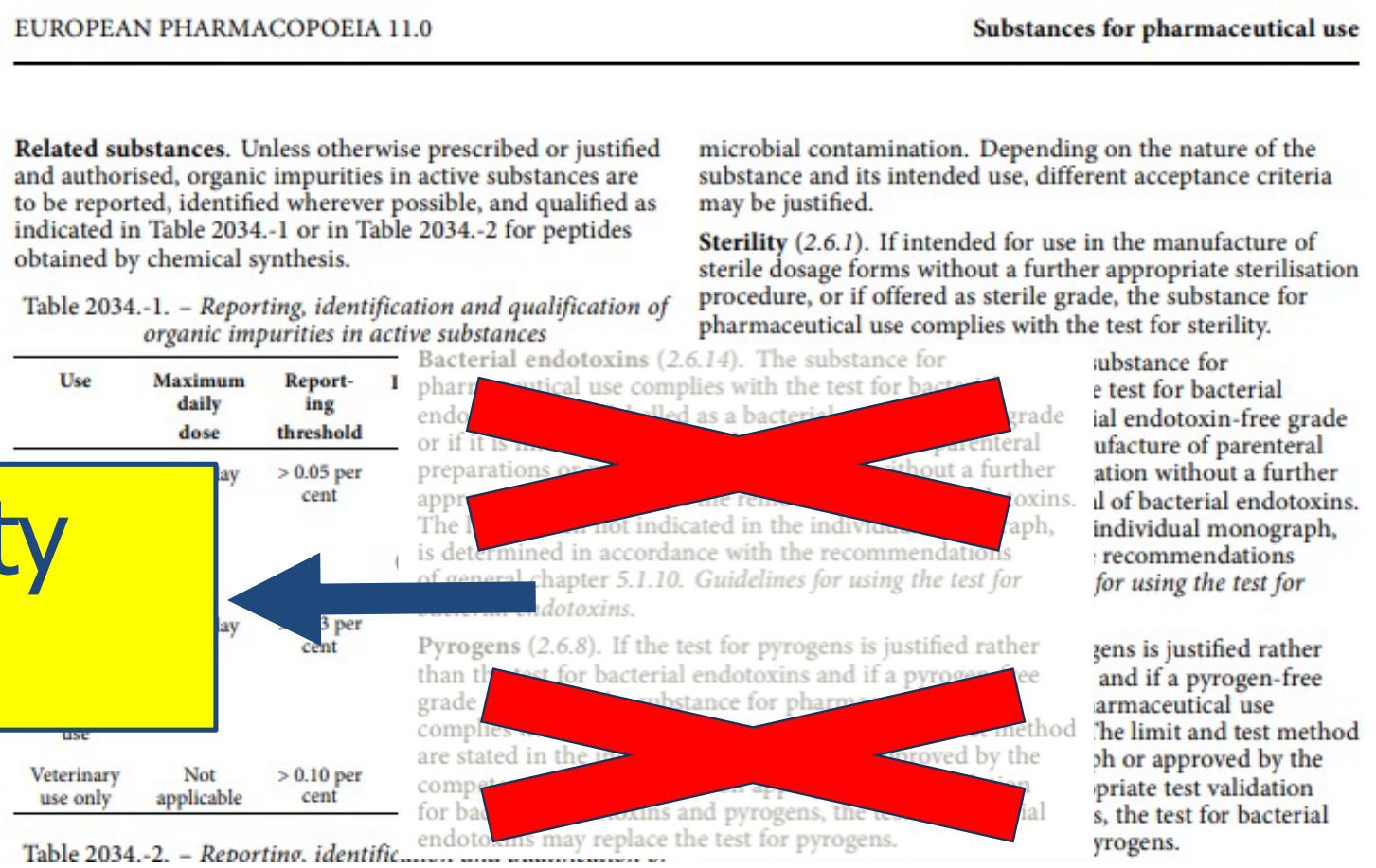
Chapters *2.6.14/ 2.6.32.* BET

or both

Chapter *2.6.30.* MAT

Decision on a testing strategy
Decision on the limits

Substances for pharmaceutical use (2034)



EUROPEAN PHARMACOPOEIA 11.0 **Substances for pharmaceutical use**

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	I
		> 0.05 per cent	
		3 per cent	
Veterinary use only	Not applicable	> 0.10 per cent	

Table 2034.-2. – *Reporting, identific*

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

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, labelled as a bacterial endotoxin-free grade or if it is intended for the manufacture of parenteral preparations without a further appropriate test for bacterial endotoxins. The limit is not indicated in the individual monograph, is determined in accordance with the recommendations of general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*.

Pyrogens (*2.6.8*). If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade of the substance for pharmaceutical use complies with the limit and test method are stated in the individual monograph or approved by the competent authority, the appropriate test validation for bacterial endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

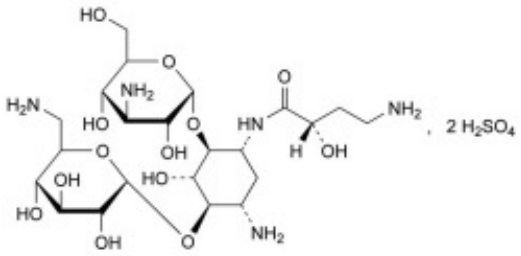
Pyrogenicity (*5.1.13*)

Individual monographs on substances for pharmaceutical use

01/2019:1290
corrected 10.0

AMIKACIN SULFATE

Amikacini sulfas



$C_{22}H_{47}N_5O_{21}S_2$ M_r 782
[39831-55-5]

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 preparations without a further purification procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of rabbit's mass 5 mL of a solution containing 25 mg of the substance to be examined in *water for injections R*.

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*)

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 test for bacterial endotoxins (*2.6.14*). Where justified and authorised, with the test for pyrogens (*2.6.8*). Recommendations on the limits for bacterial endotoxins are given in general chapter *5.1.10*. The limit for intravitreal preparations is expressed per eye.

Where the label states that the preparation is free from bacterial endotoxins or that it is pyrogenic, the preparation complies with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*), respectively.

Parenteral preparations for veterinary use comply with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*) where the volume to be injected in a single dose is 15 mL or more and is equivalent to a dose of 0.2 mL or 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

DEFINITION

Sterile, freeze-dried preparation of a plasma protein fractio
Willebrand factor
tion factor VIII,
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.

Pyrogenicity (*5.1.13*)

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

Pyrogens (2.6.8) **or Bacterial endotoxins** (2.6.14). It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated *in vitro* test such as the test for bacterial endotoxins.

For the pyrogen test, inject per kilogram of the rabbit's mass a volume equivalent to not less than 100 IU of human von Willebrand factor.

Where the test for bacterial endotoxins is used, the preparation to be examined contains less than 0.05 IU of endotoxin per International Unit of human von Willebrand 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 were 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 esting 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.~~

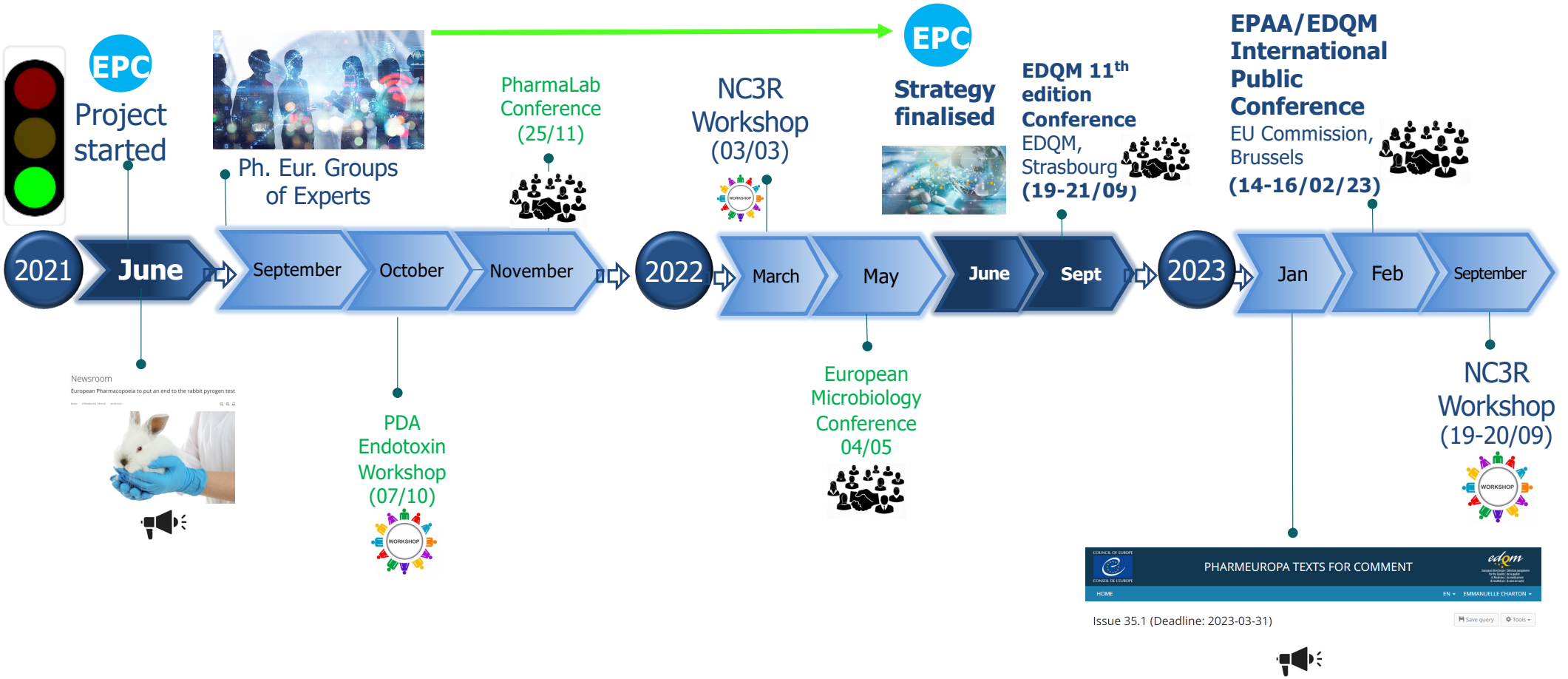
Pyrogenicity. The vaccine complies with a suitable test for pyrogenicity. Guidance for selection of a test is given in general chapter *5.1.13*. Where no limit is specified in the individual monograph, it complies with the limit approved for the particular product.

Explanatory notes in the revised Ph. Eur. texts (selected extracts)

- *"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."*

Communication to stakeholders

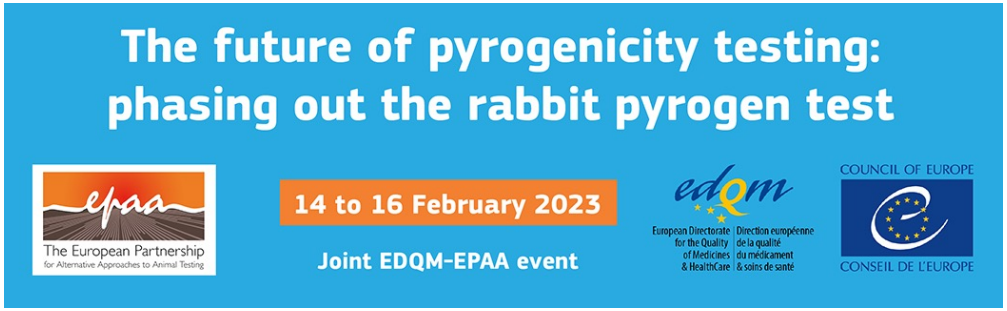


EPAA/EDQM International Public Conference

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



EPAA/EDQM International Public Conference

Hosted by the European Commission in Brussels

250 participants from Industry, Academia, Regulatory Authorities (worldwide), WHO, Pharmacopoeias (European, United States, Japan, Brazil, China, India), National Control Laboratories, MAT kit manufacturers and developers, service providers

Take home messages:

- In Europe, stakeholders are showing great enthusiasm towards the Ph. Eur. strategy aimed at phasing out the Rabbit Pyrogen Test (RPT)
- Outside Europe, the strategy is generally seen positively, however, alternative methods such as MAT are not described in detail nor even mentioned in most Pharmacopoeias, where the RPT is still required in monographs. The journey towards complete removal might therefore take longer
- International convergence toward the same goal is important
- Implementing the MAT has been facilitated greatly in the last years by the standardisation of reagents and the increase in available kits
- **The time has come to switch from *in vivo* RPT to *in vitro***

Slides, video and training resources: https://www.edqm.eu/en/-/joint-edqm-epaa-event-the-future-of-pyrogenicity-testing-phasing-out-the-rabbit-pyrogen-test

Biologicals 84 (2023) 101702

Contents lists available at ScienceDirect

Biologicals

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

The future of pyrogenicity testing: Phasing out the rabbit pyrogen test. A meeting report

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ARTICLE INFO

Keywords:
Rabbit pyrogen test
Alternative methods
Monocyte-activation test
Non-animal testing

ABSTRACT

The rabbit pyrogen test (RPT) was the benchmark for pyrogenicity testing, but scientific advancements have provided innovative and humane methods, such as the *in vitro* monocyte-activation test (MAT). However, transitioning from the RPT to the MAT has been challenging. The European Directorate for the Quality of Medicines & HealthCare, the Council of Europe, and the European Partnership for Alternative Approaches to Animal Testing jointly hosted an international conference entitled "The future of pyrogenicity testing: phasing out the rabbit pyrogen test". The conference aimed to show how the European Pharmacopoeia intends to remove the RPT from its texts by 2026, facilitate the use of MAT, and identify gaps in the suppression of RPT. The events contributed to a better understanding of the barriers to RPT replacement and acceptance of *in vitro* alternatives.

→ Link to article:
https://www.sciencedirect.com/science/article/pii/S1045105623000404

Next steps towards the suppression of the rabbit pyrogen test

Timelines

WHAT	WHO	WHEN: Publication in PhPa	WHEN: Envisaged implementation date
Elaboration of new chapter on *Pyrogenicity* (*5.1.13*) (and revision of chapter *5.1.10*)	BET WP	●	●
REVISION			
Chapter 2.6.30	BET WP	●	●
Gen. monograph 2034	BET WP	●	●
Gen. monograph 0520	G12 with BET WP support	●	●
All other Ph. Eur. texts	GoE/WP with BET WP support	●	●
DELETE	Pyrogens (*2.6.8*)		●

The project is on track for the deletion of the rabbit pyrogen test from the Ph. Eur. in 2025!

Acknowledgements

- The BET Working Party and its Chair, Dr. Ingo Spreitzer
- All the experts in Ph. Eur. Groups of Experts and Working Parties (6, 6B, 7, 9, 10D, 12, 14, 15, 16, CRB, DIA) and their respective Chairs
- Dr Gwenaël Ciréfice

Thank you for your attention

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