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.

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**)
- Radiopharmaceutical preparations (**0125**)
- Immunoserums for human use, animal (**0084**)

**Dosage form monographs (3)**
- Parenteral preparations (**0520**)
- Preparations for irrigation (**1116**)
- Intravesical preparations (**2811**)

**Individual monographs (50)**
- large-volume parenterals (4)
- blood products (17)
- vaccines for human use (17)
- antibiotics (8)
- other chemical substances (4)
- solutions for organ preservation

**General chapters (3)**
- Plastics
  - Sterile plastic containers for human blood and blood components (**3.3.4**)
  - 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**)

**Plastics**
- Sterile plastic containers for human blood and blood components (**3.3.4**)
- 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**)

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

60 Ph. Eur. texts
Replacement of chapter 2.6.8: proposed strategy

Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022

Potential pyrogens other than endotoxins can be ruled out

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

Decision on a testing strategy
Decision on the limits

New! Chapter 5.1.13. Pyrogenicity

Exclusion of potential pyrogens other than endotoxins not possible

Chapters 2.6.14/2.6.32. BET

or both

Chapter 2.6.30. MAT

2.6.8 in 60 texts of the PhEur

2.6.8 in 60 texts of the PhEur

12

? or both

EUROPEAN
PHARMACOPOEIA

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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

<table>
<thead>
<tr>
<th>Use</th>
<th>Maximum daily dose</th>
<th>Reporting threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary use only</td>
<td>Not applicable</td>
<td>&gt; 0.10 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 0.05 per cent</td>
</tr>
</tbody>
</table>

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.

Pyrogenicity (5.1.13)

Pyrogens are microorganisms that are capable of causing fever or inflammation when introduced into the body. They are often associated with bacterial endotoxins, but can also be caused by other substances such as viruses and fungi. The presence of pyrogens in pharmaceutical products can lead to adverse effects in patients, including fever, chills, and other symptoms of an inflammatory response.

Guidelines for using the test for pyrogenicity (2.6.14). The substance for pharmaceutical use complies with the test for bacterial endotoxins or if it is intended for use in parenteral preparations or if it is a pyrogen-free grade or if it is a pyrogen-free grade, the substance for pharmaceutical use complies with the test for endotoxins. The test procedure is not indicated in the individual monograph, recommendations for using the test for 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 or a pyrogen-free grade, the substance for pharmaceutical use complies with the test for pyrogens. The method are stated in the individual monograph or approved by the competent authority. For pyrogens and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.
The new requirements of general monograph 2034 apply.
Parenteral preparations (0520)

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 are human use, if applicable after reconstitution or dilution, comply with the test for bacterial endotoxins (2.6.14) or, 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 limits 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 mg or more per gram of body mass.

Storage
In a sterile, airtight, tamper-evident container.
Plasma-derived products

HUMAN VON WILLEBRAND FACTOR

DEFINITION
Sterile, freeze-dried preparation of a plasma protein fraction
including factor VIII, von Willebrand factor, factor IX, factor XIII.
This monograph applies to preparations formulated according
to the human von Willebrand factor activity.

Pyrogenicity (5.1.13)

Sterility (2.6.1). It complies with the test.

Pyrogens (5.1.8) or Bacterial endotoxins (2.6.1). It complies
with the test for pyrogens or, preferably and wherever justified
and authorised, with a validated in vitro test such as the test
for bacterial endotoxins.

For the pyrogen test, inject 1 mg (or 0.5 mg if the preparation
contains less than 0.05 IU endotoxin per
International Unit of human von Willebrand factor).

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

Limits for BET maintained,
Endotoxin Equivalents (5.1.13)
# Vaccines for human use

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

*monographs were suppressed from the Ph. Eur. as of July 2023 (Supplement 11.2)
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 Ph. Eur.: 1) new general chapter 5.1.13 Pyrogenicity; 2) monographs on individual vaccines for human use that were revised to delete the reference to the test. The revised individual monographs no longer contain any mention of the rabbit pyrogen test, and, as a result, the requirements of general monograph 0153 for pyrogenicity (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

- **Project started**
  - EPC
  - June 2021

- **Ph. Eur. Groups of Experts**
  - September 2021

- **PharmaLab Conference**
  - (03/03)

- **PDA Endotoxin Workshop**
  - (07/10)

- **NC3R Workshop**
  - (25/11)

- **Strategy finalised**
  - EPC
  - March 2022

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

- **European Microbiology Conference**
  - 04/05

- **PharmaLab Conference**
  - (03/03)

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

- **NC3R Workshop**
  - (19-20/09)

- **EPC**

- **Project started**
  - EPC
  - February 2023 (14-16/02/23)

- **NC3R Workshop**
  - (19-20/09)

Issue 35.1 (Deadline: 2023-03-31)
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

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

Gwenaël Cirefice, Katrin Schütte, Ingo Spreitzer, Emmanuelle Charton, Shahjahan Shaid, Laura Viviani, Michelle Rubbrecht, Irene Manou

European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, Strasbourg, France
European Commission, Brussels, Belgium
Paul-Ehrlich-Institute, Frankfurt am Main, Germany
GSK Vaccines, Wevelgem, Belgium
SIBS/NIC, Basel, Switzerland
PS5 Epidemiology & Pharmacovigilance, Leuven, Belgium
European Partnership for Alternative Approaches to Animal Testing (EPAA), Brussels, Belgium

ARTICLE INFO

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:
Next steps towards the suppression of the rabbit pyrogen test
## Timelines

<table>
<thead>
<tr>
<th>WHAT</th>
<th>WHO</th>
<th>WHEN</th>
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<tr>
<td><strong>Elaboration of new chapter on Pyrogenicity (5.1.13) (and revision of chapter 5.1.10)</strong></td>
<td>BET WP</td>
<td>Jan 2025</td>
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<tr>
<td><strong>Revision</strong></td>
<td>BET WP</td>
<td>Jan 2022</td>
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<tr>
<td>Chapter 2.6.30</td>
<td>BET WP</td>
<td>July 2022</td>
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<td>Jan 2023</td>
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<tr>
<td>Gen. monograph 0520</td>
<td>G12 with BET WP support</td>
<td>Jan 2024</td>
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<tr>
<td>All other Ph. Eur. texts</td>
<td>GoE/WP with BET WP support</td>
<td>Jan 2025</td>
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<tr>
<td>Pyrogens (2.6.8)</td>
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</tbody>
</table>
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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