Joint EDQM-EPAA Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

14-15 February 2023

Joint EDQM-EPAA Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

Opening Session

Animals in science

Phasing out the rabbit pyrogenicity test – challenges and opportunities

EDQM-EPAA Pyrogenicity Event
14-16 February 2023
Susanna Louhimies

Controversial views on animal use in research and testing

- *Animals recognised as sentient beings in the EU*
- *Ethical concerns*
- *Animal welfare concerns*
- *Sensitive and often an emotional topic*

Unique legislation laying out a Union objective

*"…this Directive represents an important step towards achieving the **final goal of full replacement** of procedures on live animals for scientific and educational purposes **as soon as it is scientifically possible to do so**"*

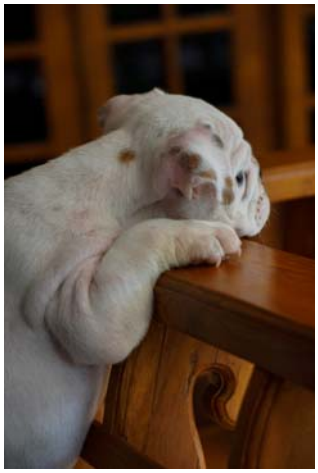


Photo by Maneesha Mohan - Unsplash

Impatience is growing

2021 EP Resolution:

- *Draw up an EU-wide action plan to drive active phase-out of animal use in research and testing*

EU Citizens' Initiative "Save cruelty free cosmetics" (2022)

- *Commit to a legislative proposal plotting a roadmap to phase-out all animal testing in the EU*

Legal framework in the EU – Directive 2010/63/EU

- *Legislation to protect animals used in science since 1986*
- *Fully revised in 2010 – amended in 2019 to further improve transparency*
  - ***Level playing field for industry and academia***
  - ***Improved transparency and enforcement***
  - ***Implementation of the Three Rs is a legal obligation***

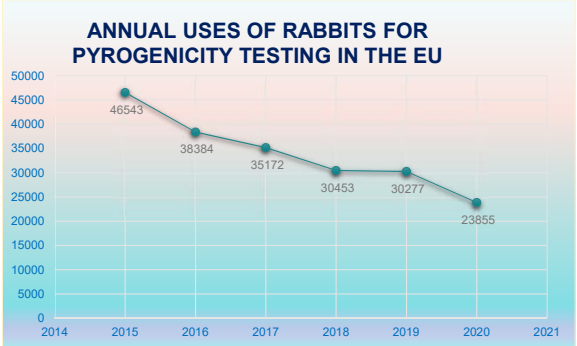
The Three Rs

➢ ***REPLACE***

➢ ***REDUCE***

➢ ***REFINE***

Total RPT use in the EU



- *MAT test adopted in the European Pharmacopeia in 2009*
- *Directive 2010/63/EU took effect in 2013*

➢ *In 2015, almost 50K uses of rabbits in RPT persist*

Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm

Replacement a legal requirement

Photo by Vincent Pelletier - Pexels

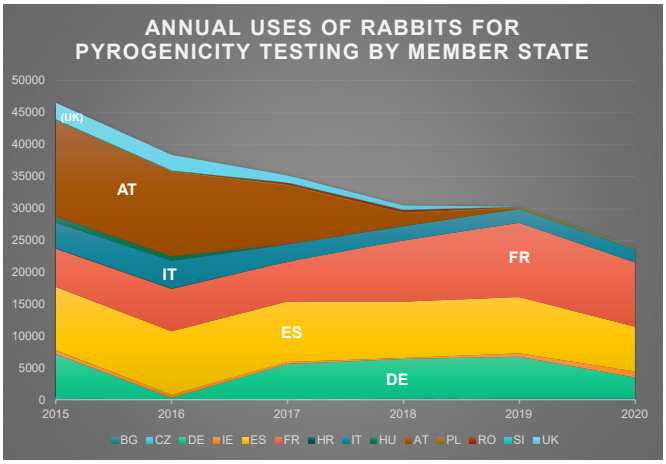
➢ *Article 13 of the Directive:*

Animal use can no longer be authorised) if another method, not entailing the use of animals, is recognized under the legislation of the Union.*

*) *unless product specific validation fails*

Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm

RPT use in the EU by MS



- *2015: 14 MS reported RPT*
- *2020: down to 10 MS of which 93,5% uses by 4 MS*
- ➢ *2021 indications: total RPT use significantly higher than in 2020*
- ➢ ***Success stories**: AT from the highest RPT use in 2015 to no further use since 2019*

Including estimated 2020 data (publication in February 2023)
https://ec.europa.eu/environment/chemicals/lab_animals/alures_en.htm

European Commission

Learning from others: an example of an approach by a MS 1/2

1. *New project applications and monitoring of on-going projects*
   - **New project application to include a detailed justification of why RPT is required**
   - **CROs required to have robust system for the implementation of Replacement and appropriate scrutiny to assess requests for RPT (product property information and efforts made to validate an alternative)**
   - **Only when alternative is confirmed unfeasible, can a CRP progress to RPT**
   - **Limited duration of project authorisations with periodic updates on progress to developing and validating alternatives; timelines for regulatory submission**

European Commission

Learning from others: an example of an approach by a MS 2/2

2. *Inspection process*
   - **Compliance reviewed during inspections**
   - **CROs internal processes for compliance**
   - **Review of RPT records to verify that only those products that do not have regulatory accepted non-animal alternative available are tested**
3. *Refinement*
   - **Group housing**
   - **Use of enriched floor pens**

Current efforts at EU level

Photo by FeeLoona - Pixabay

- *RPT use discussed regularly with MS*
  - **meetings of MS authorities twice a year**
  - **bilaterally**
- *Annual reporting changed to require explanation of justifications for animal use where alternatives are available (MS narratives from 2021 data onward)*
- *EPAA-EDQM project on RPT*

Conclusions and next steps

*Between 2015 - 2020, RPT use **decreased by 49%** - downward trend **not yet confirmed** – a general decrease in animal use in 2020*

- *What issues slow down the transition to alternatives?*
- *What needs to happen to replace all RPT in EU?*
- *What can be learnt from the success stories?*

European Commission

Thank you for your attention!

I wish you a productive and successful event!

More information at:

https://ec.europa.eu/animals-in-science

The views expressed in this presentation are solely those of the presenter and do not reflect the official view of the European Commission.

Logo photograph © Novo Nordisk

European Commission

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

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

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Joint EDQM-EPAA Event

The future of pyrogenicity testing: phasing out the rabbit pyrogen test

Petra Doerr
Director, EDQM, Council of Europe

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1. The future of pyrogenicity testing

14 FEBRUARY 2023

09:00-10:30 – **Opening session**

11:10-12:00 – **In-depth exploration of the monocyte-activation test (MAT)**

13:30-15:00 – **MAT (cont.)**

15:30-17:00 – **Pulling the rabbit out of the hat: Industry perspectives**

15 FEBRUARY 2023

09:00-10:30 – **Pulling the rabbit out of the hat: Industry perspectives (cont.)**

11:00-12:00 – **Regulatory Session: So what will rabbit-free pyrogen testing look like in Europe? How about the rest of the world?**

13:30-17:30 – **Regulatory Session (cont.)**

16 FEBRUARY 2023 (morning only)

EDQM-EPAA MAT Training Session

Hands on experience, case studies, troubleshooting with technicians from different laboratories

09:00-09:10 – **Welcome and Opening**

09:10-09:50 – **Qualification of Peripheral Blood Mononuclear Cells (PBMCs)**

09:50-10:20 – **Freezing and thawing of PBMCs**

10:40-12:00 – **Cell handling in the MAT assay**

12:15-12:30 – **Readout options**

12:30-13:00 – **Round table on the technical topic: Regulatory Acceptance**

13:00-13:10 – **Closure and Goodbye**

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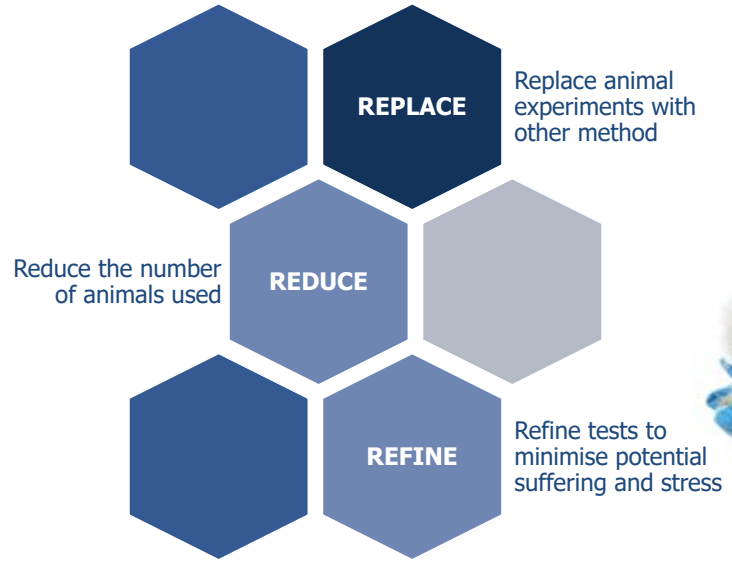
2. The 3R principles



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3. Roots of the initiative

- 1986 – Council of Europe's European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes
- 1998 – Ratification by the EU

COUNCIL OF EUROPE
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European Treaty Series - No. 123

European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes

Strasbourg, 18.III.1986

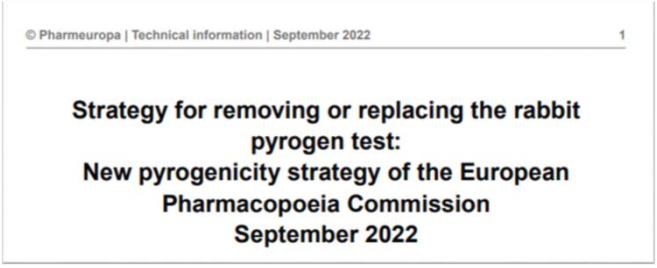
Preamble

The member States of the Council of Europe, signatory hereto,

Recalling that the aim of the Council of Europe is to achieve a greater unity between its members and that it wishes to co-operate with other States in the protection of live animals used for experimental and other scientific purposes;

Recognising that man has a moral obligation to respect all animals and to have due consideration for their capacity for suffering and memory;

Accepting nevertheless that man in his quest for knowledge, health and safety has a need to use animals where there is a reasonable expectation that the result will be to extend knowledge or be to the overall benefit of man or animal, just as he uses them for food, clothing and as beasts of burden;



© Pharmeuropa | Technical information | September 2022 1

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

60 texts in *Pharmeuropa* 35.1 for consultation
as of 1 January 2023

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4. Objectives of this joint event

- **INFORM** users of this change and offer support in relation to the public consultation
- **FACILITATE** the use of the MAT, the *in vitro* alternative to the RPT
- **IDENTIFY** any gaps in the suppression of the RPT
- **ENCOURAGE PARTICIPATION** in ongoing discussions

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HARMONISATION OF THE THREE RS IN BIOLOGICALS: STRIKING THE RIGHT NOTE

Dr. Katrin Schutte, European Commission
Dr. Shahjahan Shaid, GSK
co-chairs of the EPAA Biologicals project team

CONTENTS

1. The EPAA Partnership
2. The EPAA Biologicals Team and Project background
3. 1st focus area Safety testing of vaccines
4. 2nd focus area Pyrogenicity testing
5. Pyrogen detection methods
6. Lack of international harmonisation
7. Benefits of the MAT-assay

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European Partnership for Alternative Approaches to Animal Testing (EPAA) in 2022

The European Partnership for Alternative Approaches to Animal Testing

Collaboration between the European Commission and Industry stakeholders from 8 sectors (est. 2005)

Vision: The replacement, reduction and refinement (3Rs) of animal use for meeting regulatory requirements through better and more predictive science.

3Rs Mission:

- Promote development & acceptance
- Foster cross-sector knowledge-sharing
- Increase international collaboration
- Facilitate stakeholder dialogue

38 Companies (including 1 SME)

5 DG's of the EC

DG GROW
DG ENV
DG SANTE
DG JRC
DG RTD

Including Partner Agencies

Mirror Group (Advisory body)
Emily McIvor (Chair), Dr Tuula Heinonen, Dr Christiane Hohensee, Dr Helena Kandarova; Sirpa Pietikaïnen (MEP), Prof. Vera Rogiers
(Thank you to Ana Barros, Belén Pintado, Prof. Horst Spielmann & Marina Pereira)

8 Sectoral Associations

EPAA website: https://ec.europa.eu/growth/sectors/chemicals/epaa_en
E-mail: GROW-EPAA@ec.europa.eu

EPAA BIOLOGICALS PROJECT TEAM MEMBERS

- GSK — Co-chairs
- European Commission (DG Environment) — Co-chairs
- Sanofi Vaccines
- ZOETIS
- Novo Nordisk
- EFPIA (Pharmaceutical Industry Association)
- Animal Health Europe (Industry Association)
- EDQM (EU Directorate for the Quality of Medicines, Council of Europe)
- VACCINES EUROPE (EU vaccines producers)
- Humane Society International

BIOLOGICALS PROJECT BACKGROUND

- **Biologicals** (vaccines, hormones, immunoglobulins, blood products) are manufactured by biological processes of inherent variability and require a strict quality control strategy to secure consistent quality from batch to batch.
- Required **safety and efficacy (potency control) tests** (in vivo/in vitro) stated in monographs of relevant pharmacopoeias.
- Differences in test requirements and protocols between countries still give rise to unnecessary repetition of testing.
- Article 13 of **EU Directive 2010/63** asks that a procedure using animals is not carried out if a non-animal method for obtaining the same result is recognized under EU legislation.

Global harmonization of 3Rs in biologicals is an EPAA priority within its focus on international convergence of testing requirements.

FIRST FOCUS AREA: VACCINES TESTING

Deletion/waiving of **general safety tests** (abnormal toxicity test (ATT) or GST, TABST)) for **human vaccines** at WHO level / **veterinary vaccines** at VICH level and from national regulatory requirements.

Rationale: Since introduction of Good Manufacturing Practice and the use of adequate/stringent Quality Control measures, the relevance of the ATT has become highly questionable.

EPAA successes:

- International workshop on deletion of these tests in **2015** and publication: http://www.sciencedirect.com/science/article/pii/S1045105617300647
- ATT deleted from 49 monographs of the **European Pharmacopoeia** since 2019
- **OIE** allowed waiving of TABST based on EPAA request as part of the 2018 OIE *Terrestrial Manual*
- **WHO** recommendation in 2018 for immediate discontinuation of inclusion of the ATT/GST test in all WHO documents on vaccines and biologicals published in the Technical Report Series (including WHO Recommendations, Guidelines and manuals).

5. New focus area: pyrogenicity testing

- The **in-vivo Rabbit Pyrogen Test** is still conducted for Biological products despite alternative assays being available since long
- **Lack of international harmonisation** of testing requirements suspected here as well
- *In vitro* alternatives **BET** (Bacterial Endotoxin Test) and **MAT** (Monocyte Activation Test) assays are in Ph.Eur.
- **Can EPAA facilitate** the implementation of *in vitro* alternatives to the Rabbit Pyrogen Test?
- **Biologicals team conducted a survey mid 2018** on users' experience with in vivo /in vitro tests for pyrogens

6. Pyrogenicity survey 2018 : top-line results

Responses from 28 companies & testing institutes:

- 17 use the RPT in vivo assay
- 12 say MAT is applicable to their products in principle
- 22 say BET is applicable to their products in principle

Responses from 5 Member States:

- 3 MS say in-vivo assay is not conducted
- 1 MS: accepts in-vivo assay when pyrogenicity of substances other than bacterial endotoxins have to be tested
- 1 MS: use of the in-vivo assay has gone down, follow-up with each user to help apply alternative method

Products that the in-vivo assay is still used for:

- Blood products - Vaccines - Antibiotics - Excipients of pharma products - Medical devices

6. Pyrogenicity survey: top-line results 2

Main reasons given for still conducting the in-vivo assay:

- In-vivo test used for detection of non-microbial pyrogens
- BET not a full replacement assay, does not detect all pyrogens (only certain bacterial endotoxins)
- Technical difficulties with the in-vitro assays (endotoxin masking and with product specific validation
- Legal requirement in other jurisdictions (China, Japan, US)
- Long time for variation approval of changing to in-vitro test
- Cost (especially MAT)*

* not a legally valid reason not to use alternative!

In-vivo test used for detection of non-microbial pyrogens

Rabbit Pyrogen Test will be removed in the EP by 2025. Substitution with non-animal technologies is mandatory in EU (Directive 2010/63)

Pyrogens detection

MAT: EP 2.6.30
- Recognizes pyrogens with a high sensitivity.
- Established in the PEP
- Mentioned in the Ch.P

RPT: EP 2.6.8
- Measure of the rabbit body temperature after injection of the product.
- Former gold standard.

RPT will be a method not described in the EP. European manufacturers will have to ensure patients safety regarding Non-endotoxin pyrogens by relying on the MAT.

BET not a full replacement assay, does not detect all pyrogens (only certain bacterial endotoxins)

Exogenous pyrogens

Non-endotoxin pyrogens (NEP)

- 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

Pyrogen detection method with a risk-based approach

NEP

Risk assessment

Endotoxins detection

Exclusion of potential NEP is not clear

MAT: EP 2.6.30

Is the risk under control that non-endotoxin pyrogens are present

NO

YES

Exclusion of potential pyrogens other than endotoxins (NEP)

BET Guideline : EP 5.1.10
rFC: EP 2.6.32
LAL: EP 2.6.14

2 Replace by the MAT test as a release

1 Substitute by the BET test as a release

Technical difficulties with the in-vitro assays (endotoxin masking) and with product specific validation

- MAT, BET, RPT differ due to their biochemical reaction in sensitivity, specificity and readout. Is a full comparison useful or even needed?
- E.g. they are differently affected by matrix effects from e.g. adjuvants. Effects can occur in MAT, BET, rFC that are not detectable in the RPT. This can impact method performance.
- Those matrix interference and endotoxin masking as well as cytotoxic effect require attention in the method validation.
- Expertise and product adaptation of the in vitro methods are required to overcome those constraints.

Legal requirement in other jurisdictions (China, Japan, US)

Today the pyrogen methods are not aligned between the Compendia's. If this trend continues, there will be no "common ground" ?

	Non-endotoxin pyrogens			Endotoxin pyrogens		
	RPT	MAT	Other methods	LAL	rFC	rLAL
E.P	Not foreseen	Sole method	Not mentioned	Foreseen	Equivalent method	To be determined
WHO	Foreseen	Proposed*	Not mentioned	Foreseen	Proposed*	To be determined
USP	Foreseen	Not mentioned	Not mentioned	Foreseen	To be determined	To be determined
China	Foreseen	Supplemental**	In discussion	Foreseen	Available	To be determined
Korea	Foreseen	Not mentioned	In discussion	Foreseen	Expected	To be determined
Japan	Foreseen	Guideline**	Not mentioned	Foreseen	Alternative method	To be determined
India	Foreseen	Available	Not mentioned	Foreseen	Alternative method	To be determined
Brazil	Foreseen	Foreseen	Not mentioned	Foreseen	Expected	To be determined

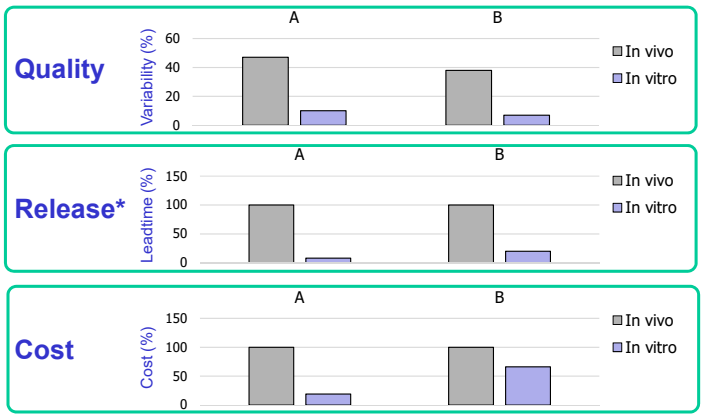
*NC3r led project ** mentioned in guidelines

Long time for variation approval of changing to in-vitro test

- The non-alignment likely triggers prolonged approval times due to Questions when replacement methods are submitted.
- Extensive parallel testing of RPT, BET and MAT are requested. Shifting timelines to fade out RPT.
- A comparison between the three methods is challenging due to their analytical target profiles and read outs
- **How can we move away from considering the RPT as the gold standard outside of Europe?**

Cost of Replacing the RPT by MAT

In vitro replacement does not jeopardize quality, it improves it with more reliable analytics.



<u>Activity Based Costing</u> to consider overhead and indirect cost.

Improve availability of Vaccines :
- Faster release (repeats, OOS, etc.)
- Reduced stock planning
- Avoidance of write offs
- Shortened test time
- Robust assay: less deviations and repeats

Reduce Touch time:
- In vitro is less labor intense
- Allows automation
- Streamlined due to robust assay

Reduced costs of goods e.g. assay reagents
Capacity of Scale
Reduced costs for National Control
Lower infrastructure maintenance

Scope of the workshop

- The different experts will address the mentioned concerns and share strategies to overcome them.
- Several Health authorities will present their perception on the future of pyrogen testing
- A half day training will allow deep dive on technical procedures and questions with the in vitro MAT method.

Thanks for your attention!

- EPAA website:
  http://ec.europa.eu/growth/sectors/chemicals/epaa_en

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

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Pulling the rabbit out of the hat: how the European Pharmacopoeia is tackling the rabbit pyrogen test

of the European Pharmacopoeia

Dr. Emmanuelle Charton
Head of DivB
European Pharmacopoeia Department

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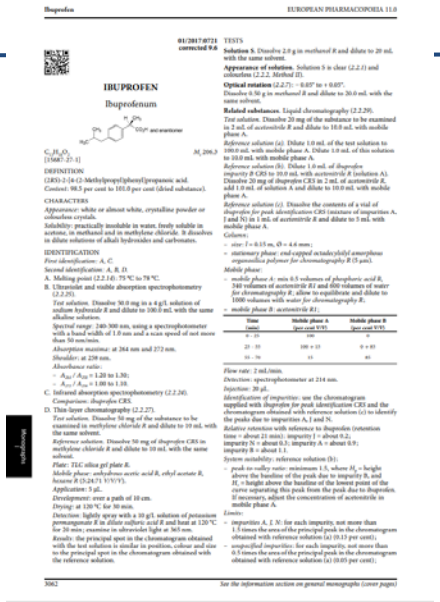
The European Pharmacopoeia



Ibuprofen EUROPEAN PHARMACOPOEIA 11.0

01/2017:0721 corrected 9.6

IBUPROFEN

Ibuprofenum

DEFINITION

(3RS)-2-[4-(2-Methylpropyl)phenyl]propanoic acid.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: practically insoluble in water, freely soluble in acetone, in methanol and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D.

A. Melting point (2.2.14): 75 °C to 78 °C.

B. Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. Dissolve 50.0 mg in a 4 g/l solution of sodium hydroxide R and dilute to 100.0 ml with the same alkaline solution.

Spectral range: 240-300 nm, using a spectrophotometer with a band width of 1.0 nm and a scan speed of not more than 50 nm/min.

Absorption maxima: at 264 nm and 272 nm.

Shoulder: at 259 nm.

Absorbance ratio:

- A_{264} / A_{272} = 1.20 to 1.30;
- A_{259} / A_{272} = 1.00 to 1.10.

C. Infrared absorption spectrophotometry (2.2.24).

Comparison: ibuprofen CRS.

D. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 50 mg of the substance to be examined in methylene chloride R and dilute to 10 ml, with the same solvent.

Reference solution. Dissolve 50 mg of ibuprofen CRS in methylene chloride R and dilute to 10 ml, with the same solvent.

Plate: TLC silica gel plate R.

Mobile phase: anhydrous acetic acid R, ethyl acetate R, hexane R (5:24:71 V/V/V).

Application: 5 µl.

Development: over a path of 10 cm.

Drying: at 120 °C for 30 min.

Detection: lightly spray with a 10 g/l solution of potassium permanganate R in dilute sulfuric acid R and heat at 120 °C for 20 min; examine in ultraviolet light at 365 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S. Dissolve 2.0 g in methanol R and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (2.2.1) and colourless (2.2.2, Method II).

Optical rotation (2.2.7): −0.05° to +0.05°.

Dissolve 0.50 g in methanol R and dilute to 50.0 ml with the same solvent.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 20 mg of the substance to be examined in 2 ml of acetonitrile R and dilute to 10.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 10.0 ml with mobile phase A.

Reference solution (b). Dilute 1.0 ml of ibuprofen impurity B CRS to 10.0 ml with acetonitrile R (solution A). Dissolve 20 mg of ibuprofen CRS in 2 ml of acetonitrile R, add 1.0 ml of solution A and dilute to 10.0 ml with mobile phase A.

Reference solution (c). Dissolve the contents of a vial of ibuprofen for peak identification CRS (mixture of impurities A, I and N) in 1 ml of acetonitrile R and dilute to 5 ml with mobile phase A.

Column:

- size: l = 0.15 m, Ø = 4.6 mm;
- stationary phase: end-capped octadecylsilyl amorphous organosilica polymer for chromatography R (5 µm).

Mobile phase:

- mobile phase A: mix 0.5 volumes of phosphoric acid R, 340 volumes of acetonitrile R1 and 600 volumes of water for chromatography R; allow to equilibrate and dilute to 1000 volumes with water for chromatography R;
- mobile phase B: acetonitrile R1;

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 25	100	0
25 - 35	100 → 15	0 → 85
35 - 70	15	85

Flow rate: 2 ml/min.

Detection: spectrophotometer at 214 nm.

Injection: 20 µl.

Identification of impurities: use the chromatogram supplied with ibuprofen for peak identification CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, I and N.

Relative retention with reference to ibuprofen (retention time = about 21 min): impurity I = about 0.2; impurity N = about 0.3; impurity A = about 0.9; impurity B = about 1.1.

System suitability: reference solution (b):

- peak-to-valley ratio: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ibuprofen. If necessary, adjust the concentration of acetonitrile in mobile phase A.

Limits:

- impurities A, I, N: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- unspecified impurities: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);

3062 See the information section on general monographs (cover pages)

Example of quality standard on Ibuprofen

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What is the European Pharmacopoeia?

- A compilation of > **2800** documentary standards for the quality control of medicines
- Binding in the **39** signatory member states **and in the EU**
- Used as a reference world-wide, including in 31 observer countries, from all continents
- Plays a major role in protection of public health
- Facilitates the free movement of medicinal products in Europe and beyond

Covering

- All stages of the life-cycle of a medicine from development through to production and market surveillance
- All components used during the production process … from raw materials, intermediates of synthesis to medicinal products

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Members & Observers

European Pharmacopoeia:

39 member states and the EU
(Albania in February 2020);

31 observers
(Ethiopia in Nov 2022)

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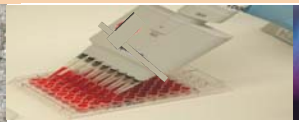
New Pyrogenicity strategy

1971	1987	2010	2020
			
Pyrogens (2.6.8)	BET (2.6.14)	MAT (2.6.30)	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:

Pharmeuropa 35.1

New chapter *5.13* 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

New Pyrogenicity strategy

- https://go.edqm.eu/NewPyrogenicityStrategy

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

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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

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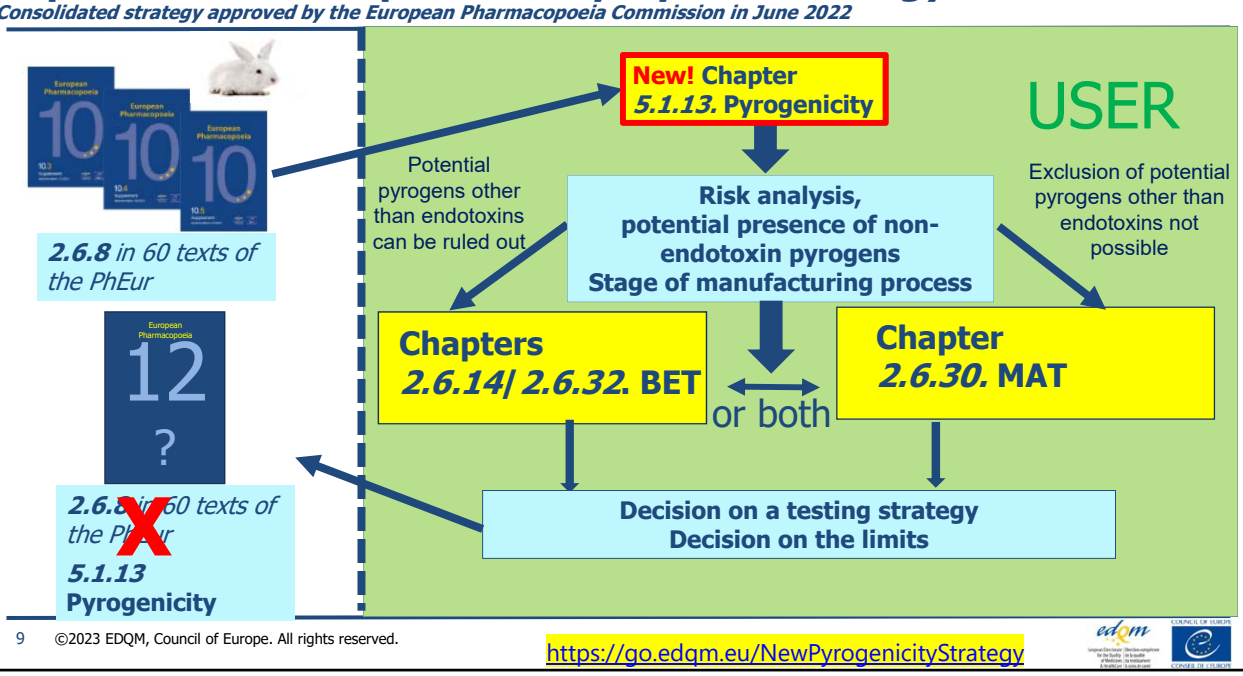
Replacement of chapter *2.6.8: proposed strategy*

Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022



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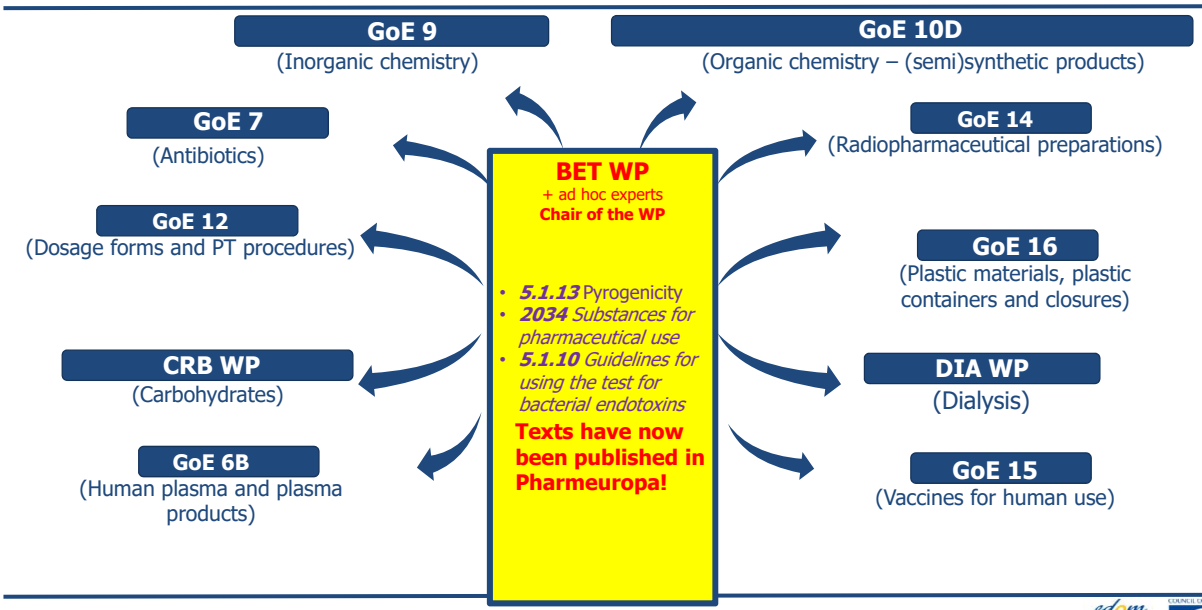
https://go.edqm.eu/NewPyrogenicityStrategy

Activities and actions at the level of GoEs and WPs



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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)

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Content and structure of the Ph. Eur.

- ✓ Apply to ALL texts
- ✓ Provide basic information, rules and conventional expressions
- ✓ Address general issues
- ✓ Essential reading

General notices

General chapters

- ✓ General methods & texts
- ✓ Editorial convenience
- ✓ Not mandatory "per se"
- ✓ Part of the standard when referred to in a monograph
- ✓ Can be used when there is no monograph → may need validation

General monographs

Individual monographs

- ✓ Classes of substances and dosage forms
- ✓ Mandatory to all substances/preparations within the definition scope
- ✓ Not cross-referenced in individual monographs

- ✓ Substance based
- ✓ Specific
- ✓ Not stand-alone
- ✓ Take account of approved products

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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*

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
use				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 complies with the test for bacterial endotoxins if as a bacterin-free grade or if it is intended of parenteral preparations or prep without a further appropriate proced bacterial endotoxins. The limit, wh monograph, is determined ice with ndations *10. Guidelines for the test for bacterial endotoxins.*

Pyrogens (*2.6.* test for pyrogen ified rather than the test f dotoxi ogen-free grade is offered, ical use complies with the tes mit and test method are stated in the ind proved by the competent aut alidation for bacterial en nd pyrogen or bacterial endotoxins may ace the test for pyrog

Pyrogenicity (*5.1.13*)

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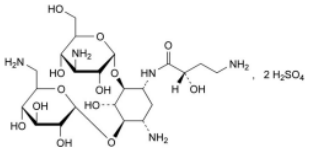
Individual monographs on substances for pharmaceutical use

01/2019:1290
corrected 10.0

AMIKACIN SULFATE

Amikacini sulfas



$C_{22}H_{45}N_5O_{21}S_2$ 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* intended for u he manufacture of parenteral r appropriate procedure for th , it complies with the test for pyrogens. Is m of the rabbit's mass 5 mL of a soluti he substance to be examined in *njections*

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.

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Parenteral preparations (0520)

Parenteral preparations

07/2021:0520

PARENTERAL PREPARATIONS

Pyrogenicity (*5.1.13*)

necessarily apply
veterinary use
he 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 gene e limi 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 is (*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.

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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 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 e

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

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

Limits for BET maintained, Endotoxin Equivalents(5.1.13)

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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.

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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."*

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Timelines

WHAT	WHO	WHEN: Publication in PhPa	WHEN: Envisaged implementation date
Elaboration of Pyrogenicity (*5.1.13*) (together with revision of *5.1.10*)	BET WP	●	●
REVISION			
2.6.30	BET WP	●	●
2034	BET WP	●	●
0520	G12 with BET WP support	●	●
remaining texts	GoE/WP with BET WP support	●	●
DELETE Pyrogens (*2.6.8*)			●

2022 → April → Jan 2023 → Jan 2024 → July → Jan 2025 → July → Jan 2026

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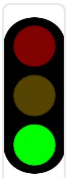
Communication to stakeholders



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)

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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

Today!

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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

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Thank you for your attention

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