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An EDQM Update on Pyrogenicity in European Pharmacopoeia

Dr Mihaela Buda, EDQM, Council of Europe

AFSA-IABS-Humane World for Animals Global Conference, Bangkok, 2-4 December 2025

Ph. Eur. Pyrogenicity Strategy Over the Years

2.6.8 RPT

1971

1987

A. Gel-clot method: limit test
B. Gel-clot method: quantitative test
C. Turbidimetric kinetic method
D. Chromogenic kinetic method
E. Chromogenic end-point method
F. Turbidimetric end-point method

2.6.14 BET

1998

2001

2009

Harmonisation
5.1.10 Guidelines for using the BET

2.6.30 MAT

2010

2.6.40 MAT for vaccines containing inherently pyrogenic components

2024

2020

2.6.32 BET using rFC

Deletion of RPT from 57 Ph. Eur. texts

5.1.13 Pyrogenicity

2025

2026

Suppression of RPT

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"Phasing out the rabbit pyrogen test" project

1987 1998 2001 2009

2.6.14 BET

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Harmonisation 5.1.10 Guidelines for using the BET

2020

2.6.32 BET using rFC

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Suppression of RPT

Suppression of Rabbit Pyrogen Test: Major Milestone Achieved

https://www.edqm.eu/en/-/ph.-eur.-bids-adieu-to-rabbit-pyrogen-test-in-its-monographs

This concerns 57 texts

Non animal pyrogenicity approaches instead (BET, MAT)

- ★ The use of the RPT is **no longer** required in any text of the Ph. Eur.
- ★ Implementation date: **1 July 2025**
- ★ **The chapter itself will be removed from the Ph. Eur. on 1 January 2026**
- ★ A major achievement for animal welfare and the advancement of modern *in vitro* approaches!

Pyrogen testing 2.0: Ethical, Evolving and Eco-friendly

Implementing safe, rapid, state-of-the art and sustainable non-animal approaches worldwide

25-26 February 2026 - European Commission, Albert Borschette Conference Center, Rue Froissart 36, Brussels, and Online

Key highlights:

- Learn more about the implementation of the RPT phase-out strategy across Europe
- Gain insights into the challenges and solutions encountered during the transition to MAT
- **Explore the status of recombinant reagents for use in the bacterial endotoxins test (BET)**
- Understand how other pharmacopoeias and regulatory frameworks outside Europe have adapted in response to the Ph. Eur.'s initiative
- Perspectives from regulators, industry and international stakeholders on the future of animal-free pyrogenicity testing

The European Partnership for Alternative Approaches to Animal Testing

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

Registration OPEN - Participation FREE OF CHARGE

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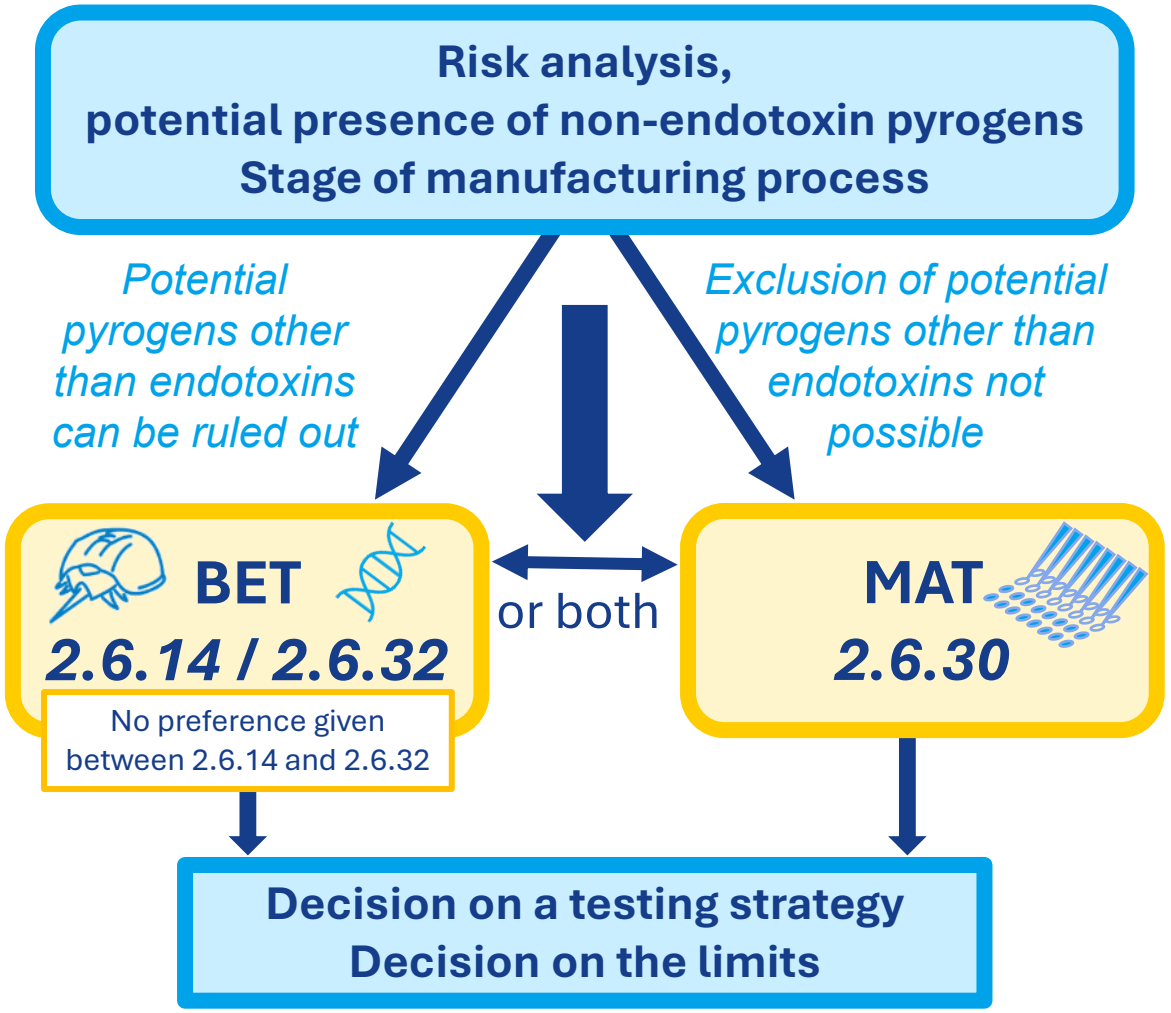
2.6.32 BET using rFC

- ★Currently referenced in 12 Ph. Eur. texts, including:
  - **5.1.13 Pyrogenicity**
  - 5.1.10 Guidelines for using the test for BET
  - 0008 Water, purified
  - 0169 Water for injections
  - 0520 Parenteral preparations
  - 2034 Substance for pharmaceutical use
  - …

What about the 500 other occurrences of chapter 2.6.14?

5.1.13 Pyrogenicity



BET: Inclusion of rFC as New Method G in 2.6.14

- At its March 2025 Session, the Ph. Eur. Commission decided to **revise chapter 2.6.14** *Bacterial Endotoxins* **to include** the test using recombinant factor C (**rFC**) as the 7th BET method (i.e. as new method G)

2.6.14. BACTERIAL ENDOTOXINS(1)

The following 6 methods ▸◊using the amoebocyte lysate and the method using recombinant factor C◊◂ are described in the present chapter:

Method A.	Gel-clot method: limit test
Method B.	Gel-clot method: quantitative test
Method C.	Turbidimetric kinetic method
Method D.	Chromogenic kinetic method
Method E.	Chromogenic end-point method
Method F.	Turbidimetric end-point method
▸◊Method G.◂	▸Fluorimetric end-point method using recombinant factor C◊◂

- This revision will give the possibility to use rFC in all Ph. Eur. texts referring to chapter 2.6.14 (~500 texts) and will give full recognition of the **equivalence of rFC with** all the **LAL** methods

2.6.32. TEST FOR BACTERIAL ENDOTOXINS USING RECOMBINANT FACTOR C

- In addition, chapter 5.1.13 is being revised to reflect changes to chapter 2.6.14 and highlight that considerations regarding **sustainability** should be made when choosing a BET method
- The revised chapters 2.6.14 & 5.1.13 have been released for **public consultation in Pharmeuropa 37.2** (commenting period 1 April - 30 June 2025)

5.1.13. PYROGENICITY

CHOICE OF THE TEST

Bacterial endotoxins from gram-negative bacteria are the most common and most active exogenous pyrogens. The tests for bacterial endotoxins described in general ▸~~chapters~~◂ ▸chapter◂ *2.6.14* ▸~~and 2.6.32~~◂ are thus the analytical methods most widely used to address the pyrogenicity of parenterally administered medicinal products and their components. ▸These methods use amoebocyte lysate from the horseshoe crab (gel-clot, turbidimetric or chromogenic techniques) or recombinant factor C based on the gene sequence of the horseshoe crab (fluorimetric technique). Using the test for bacterial endotoxins◂ ▸~~This approach~~◂ is only appropriate if the presence of non-endotoxin pyrogenic substances can be ruled out.

▸Considerations regarding sustainability should be made when choosing a method (A-G) for the test for bacterial endotoxins in general chapter *2.6.14.* Bacterial endotoxins.◂

Recombinant Cascade Reagent (rCR)

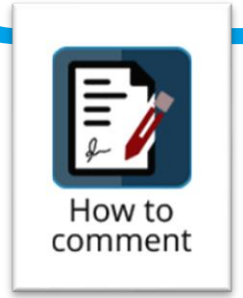
- ★ Kits still recent
- ★ Few peer-reviewed literature
- ★ Few user's data
- ★ No medicinal product approved using rCR in Europe

→ **Alternative method**

On-going revision of general chapter

5.1.10 Guidelines for using the BET

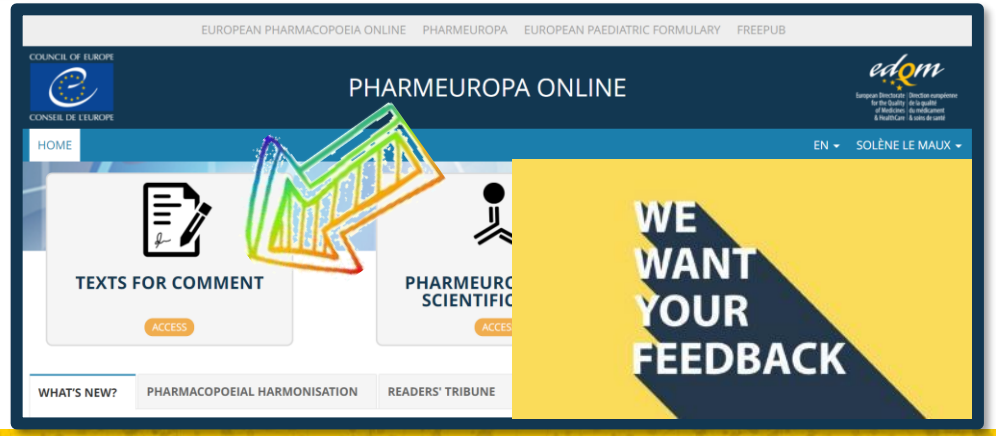
- Better integration of rFC
- Introduction of a reference to rCR to encourage data generation and broader acceptance



Draft will be published in Pharmeuropa for public consultation

proposal

https://pharmeuropa.edqm.eu/home

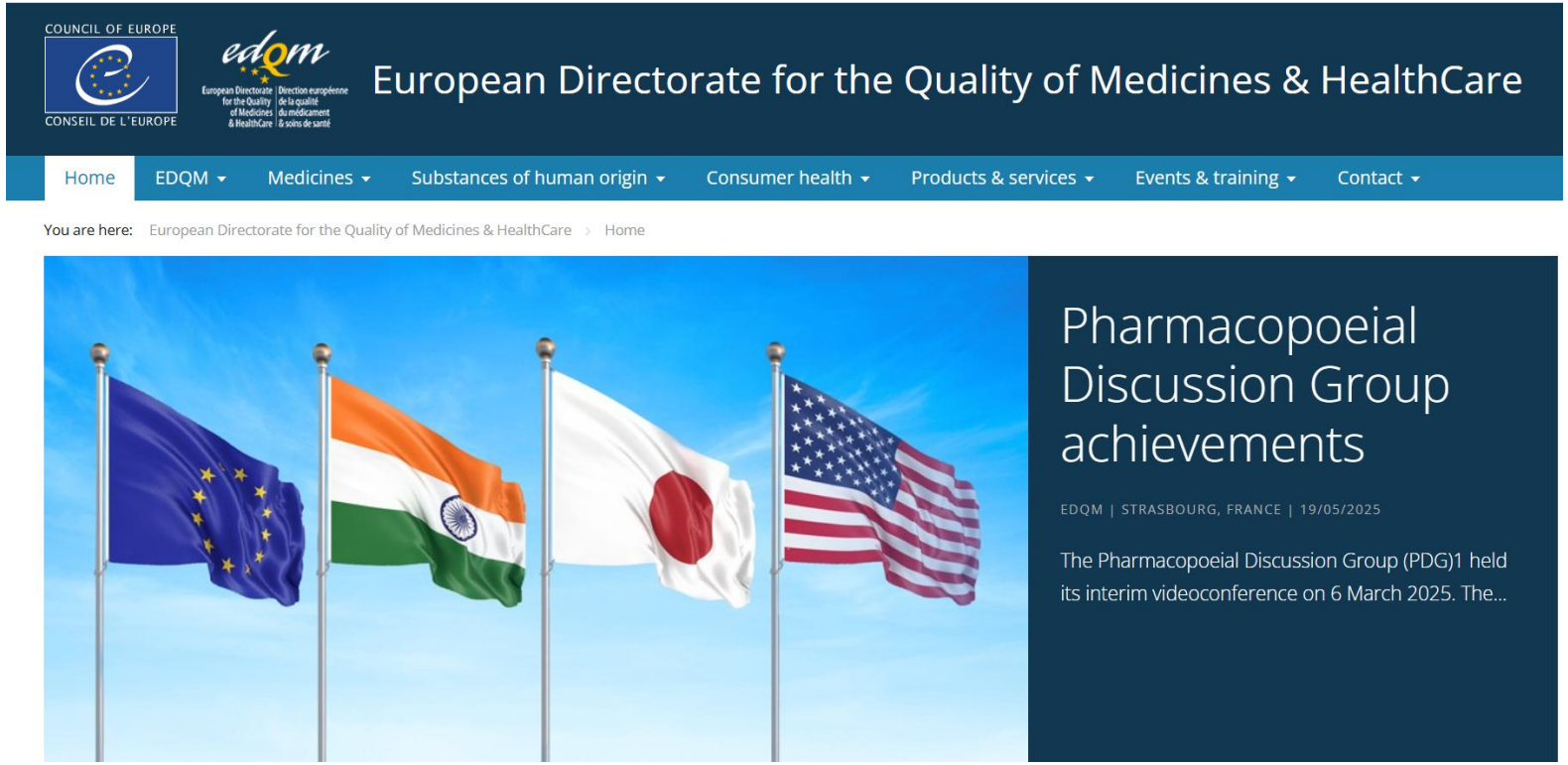


- ➢ When mature…
- ➢ Addition of rCR as **new METHOD H** in *2.6.14* considered

Impact on the Pharmacopoeia Discussion Group (PDG)

- ★ Ph. Eur. informed PDG of this initiative
- ★ PDG committed to follow the same direction at a later stage – Press release from March 2025 meeting, published on the EDQM website on 19 May 2025

https://www.edqm.eu/en/-/pharmacopoeial-discussion-group-achievements-14



The PDG held productive discussions on aligning innovative approaches to the test for Bacterial Endotoxins using recombinant reagents. Through continuous and open dialogue, the PDG reached a major achievement by approving a unified position among the four member pharmacopoeias regarding the goal to include methods using recombinant reagents in the harmonised chapter. Details on this important topic are shown in the Appendix, below.

Impact on the Pharmacopoeia Discussion Group (PDG)

Appendix: PDG position

PDG is committed to making efforts to develop and revise existing test methods, for example, the test for Bacterial Endotoxins (BET), to decrease the use of animals or animal derived reagents.

In PDG's general chapter Bacterial Endotoxins (Q-06), six methods are described that use Limulus or Tachypleus Amoebocyte Lysate (LAL/TAL) as a reagent. This reagent consists of cells (amoebocytes) derived from the horseshoe crab.

PDG recognizes the availability of non-animal derived recombinant reagents as alternatives to replace LAL/TAL in the BET. These alternatives include recombinant factor C (rFC) and synthetic mixtures that mimic the coagulation cascade, referred to as "recombinant cascade reagents" (rCR).

The pharmacopoeias of PDG and the regulatory framework they are embedded into are at different stages of acceptance regarding the performance of recombinant reagents compared to LAL/TAL.

PDG's goal is to include new methods using recombinant reagents in the harmonised chapter.

EDQM's Biological Standardisation Programme: Establishment of Reference Standards for BET/MAT

NEW

Ph. Eur. Endotoxin standard BRP (BSP174) - *replacement batch*

- calibrant for BET
- reference preparations are harmonised between WHO IS, Ph. Eur. BRP and USP RS, ensuring consistent quality and availability across pharmacopoeias
- common value assigned for current batches 10 000 IU/vial, using compendial gelation or photometric assays

Joint WHO/MHRA-EDQM-JP-USP project launched for establishment of harmonised replacement endotoxin standards

Ph. Eur. Non-endotoxin pyrogen (NEP) Reference Reagents for use in MAT (BSP149)

- Ph. Eur. MAT Chapter 2.6.30 - *METHOD VALIDATION FOR NON-ENDOTOXIN MONOCYTE-ACTIVATING CONTAMINANTS*
- need for NEP standards for cell batch qualification, positive control and global acceptance
- controls applicable to the different existing test systems

Joint collaborative study WHO/MHRA, PEI and EDQM

Take away message:

- The EDQM has successfully led a paradigm shift in pyrogen testing by eliminating the RPT from the European Pharmacopoeia
- This milestone reflects a commitment to animal welfare and sustainability in pyrogen testing

More information

www.edqm.eu

https://go.edqm.eu/Newsletter

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