CMC Strategy Forum Europe - From LAL to Recombinant Methods - Regulatory Expectations and Challenges in the EU

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Introduction

- Bacterial endotoxin testing (BET) is a **mandatory** safety requirement across Ph.Eur., USP, JP and ICH regions to ensure parenteral product quality
- Historically, testing relied on Limulus Amebocyte Lysate (LAL) **derived from horseshoe crabs, raising sustainability, variability and supply-chain concerns**
- Recombinant technologies (rFC, rCR) provide animal-free, highly specific and reproducible alternatives aligned with global **3Rs objectives**
- rBET maturity, platform knowledge, and regulatory challenges

Evolution of Regulatory Standards (Ph. Eur., USP, JP, ICH)

Ph. Eur.

- rFC was first introduced via general chapter 2.6.32, which became legally effective on 1 January 2021
- Since 1 April 2024, revised monographs Water for Injection (0169) and Purified Water (0008) allow the direct use of rFC for endotoxin testing without comparison to 2.6.14.

USP

- The revised USP <1085> (published July 2025, official February 2026) formally recognizes recombinant reagents and clarifies their compendial status
- USP <86>, official May 2025, provides detailed approaches for endotoxin testing using rFC and rCR, as alternatives to USP <85>

JP

- General Information G4-4-180 (2021) aligns Japan with Ph. Eur. and USP, enabling use of both rFC and rCR and describing their generations

ICH Q4B Annex 14

- Confirms EP General Chapter 2.6.14 Bacterial endotoxins, JP 4.01, and USP <85> BET chapters as interchangeable across ICH regions
- This harmonization applies only to traditional LAL-based BET, not recombinant methods

Evolution of Pyrogen & Endotoxin Testing in Europe

1980s - 2000s: Establishment of LAL as the Standard

- LAL becomes the primary method for bacterial endotoxin testing
- Broad regulatory acceptance across global pharmacopeias

2016 - 2020: Progressive Phase-Out of RPT

- EMA/EDQM 3Rs initiative accelerates transition away from animal-based methods
- Risk-based approaches increasingly used to justify MAT or no testing for NEPs

2016 - 2027: Entry of Recombinant Technologies - rFC

- 2016: rFC included in Ph. Eur. via General Chapter 5.1.10. Guidelines for using the BET as an alternative method
- 2020: rFC published as Ph. Eur. 2.6.32. (suppl. 10.3)
- rFC recognised as a validated, animal-free BET alternative
- rFC January 2027 will become part of Ph. Eur. 2.6.14 as Method G

2024 - 2026: Transition Toward Full Animal-Free Testing - rCR

- rCR identified as a promising next-generation recombinant method; Pharmeuropa 2026 introduction of a reference to rCR
- Ph. Eur. encourages data generation to support future inclusion of rCR, large volumes of peer-reviewed data and user experience are essential and need to be supported by the approval of medicinal products for which rCRs were used
- Currently, bacterial endotoxin tests involving rCR or any other new reagents are considered to be alternative methods replacing a pharmacopoeial test, as described in the General Notices. Equivalence with one of the methods described in general chapter 2.6.14 is to be demonstrated in accordance with the General Notices and to the satisfaction of the competent authority.

EMA 3Rs Initiative

Two major focus areas:

- Withdrawal of Rabbit Pyrogen Test (RPT) → replaced by MAT and/or risk assessment
- Phasing out LAL BET → recombinant reagents: rFC and/or alternative rCR

  rBET as a key enabler of sustainability (reducing animal-derived materials)


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 December 2016
EMA/CHMP/CVMP/JEG-3Rs/450091/2012
Committee for Medicinal Products for Human Use (CHMP)
Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches

Industry Perspective

- LAL → rFC / rCR

  - Need for harmonization between USP and Ph. Eur.

  - Request for a more flexible approach to submitting the changes (downgrade the variation category)

- rFC and rCR target the same biological mechanism as traditional LAL — activation of Factor C by endotoxin — but in a fully synthetic, recombinant format.

- Advantages of recombinant methods:
  - Higher specificity, because rFC bypasses the Factor G pathway and therefore avoids false-positive activation by β-glucans
  - Improved reproducibility and lower lot-to-lot variability, as recombinant assays are produced under controlled biotechnological conditions and do not rely on harvested biological material.
  - Sustainability benefits, as no horseshoe crabs are used, addressing supply chain vulnerability and 3Rs/ethical requirements.

- Multiple peer-reviewed validation studies have consistently demonstrated that rFC performs equivalently to LAL with respect to linearity, specificity, accuracy, precision, and robustness.
  Examples include comparative studies in vaccine matrices, water systems, and biopharmaceutical sample

Advantages & Sustainability Impact – rBET methods

- Elimination of the use of endangered horseshoe crab species (Limulus polyphemus, Tachypleus tridentatus), since recombinant methods do not require animal-derived lysate.
- Traditional LAL relies on blood harvested from horseshoe crabs, including Limulus polyphemus and Tachypleus tridentatus, both of which are recognized as vulnerable or endangered in parts of the world. rBET avoids this entirely because it is produced biotechnologically.
- More stable and resilient supply chains thanks to controlled recombinant production rather than dependence on wild animal populations.
- rBET is manufactured through cell-based expression systems, improving standardization, reducing geographic supply limitations, and protecting against shortages linked to crab population stress.
- Comparable or superior analytical performance to LAL across a wide range of product types.
- Peer-reviewed studies show that rFC provides equivalent sensitivity, accuracy, linearity, and precision compared to LAL—including in challenging vaccine and biopharmaceutical matrices.
- The transition to recombinant reagents is widely recognized as aligned with global 3Rs objectives aimed at reducing animal use in testing and improving sustainability.

EU regulators view / BWP Position: BET Replacement (LAL → rFC / rCR)

- BWP Interested Parties Meeting October 2025 with EFPIA and VE (Enabling Global Implementation of Changes to Pyrogenicity and Endotoxin Testing)
- Participation in the EDQM symposium: Pyrogen testing 2.0: Ethical, Evolving and Eco-Friendly, implementing safe, rapid, state-of-the art and sustainable non-animal approaches worldwide, February 2026
- The Quality Innovation Group (QIG) organised a LLFG meeting to advance sustainability goals in pharmaceutical manufacturing - Implementing Recombinant Endotoxin Testing, March 2026
- The aligned nature of the cascade performed in the rCR and the traditional LAL method offer potential advantages → promising alternative
- USP and Ph. Eur. lack harmonization → impacts adoption
- rCR expected to follow rFC Ph.Eur. pathway, inclusion in Ph. Eur. 5.1.10 as alternative test
- Full validation and equivalence to LAL still required for rCR
- Regulatory confidence must be built through the data, not variation category debate

EU regulators view / BWP Position: Variation Categories

Current classification:

- LAL → rFC / rCR = Type II variation

According to the Interpretation of the Union format for Manufacturer Importer Authorisation (europa.eu) endotoxin testing (LAL, rFC or rCR) is classified as a 'biological testing' so it would fall under a type II category (under the current variations guideline (C/2025/5045) if it is introduced for testing AS (or FP or excipient), or a IB for testing *starting material/reagent/intermediate used in the manufacturing process of the active substance. (*Q.I.b.2, Q.II.c.2 and Q.II.d.2)

Regulatory intentions:

- BWP considers Type II var for rFC too strict
- BWP has prepared Q/A introducing a more flexible approach, will be publish on the EMA website in summer 2026
- Q/A: when replacing LAL with a pharmacopoeial **rFC test**, Applicants may instead submit a **Type IB variations** (Q.I.b.2. (z), Q.II.c.2. (z), Q.II.d.2 (z))
- Q/A: **rCR test** remains **Type II** until compendial status changes, Applicants currently must provide full analytical method validation data
- Additional details on the rFC and rCR assays can be found on the EDQM website: https://faq.edqm.eu/display/FAQS/PYROGENICITY

International Regulatory Dynamics

- **ICMRA** (International Coalition of Medicines Regulatory Authorities) accepted collaborative review of **PACMP for LAL → rCR**

https://www.ema.europa.eu/en/partners-networks/international-activities/multilateral-coalitions-initiatives/international-coalition-medicines-regulatory-authorities-icmra

- PACMP encouraged for portfolio-wide implementation, including a recommendation to use the **scientific advice procedure** to ensure a smoother regulatory process at the time of implementation
- Global harmonization efforts ongoing
- Regional regulatory frameworks differ, but none preclude rFC or rCR use

ICMRA acts as a forum to support **international cooperation** among **medicines regulatory authorities**.

Scientific & Practical Considerations

Advantages of rFC / rCR:

- Non-animal origin, lower variability, sustainable supply chain
- rCR (Factor C + Factor B + proclotting enzyme) theoretically more closely mimics the full LAL cascade

Challenges of rFC / rCR:

- Demonstrating equivalence to LAL across a range of product types and complex matrices. This is critical for us to understand the risks associated with the change
- Limited number of products that have successfully implemented rFC and rCR
- Global harmonization and possible divergent expectations across regions
- **Insights into managing technical challenges (e.g. LER)**
- Interference in complex matrices (e.g., proteins, surfactants) can be more pronounced with rFC, but is typically mitigated through appropriate dilution or matrix-adapted preparation. This is documented in studies comparing rFC and LAL in biopharmaceutical and vaccine matrices, where rFC performance improved with dilution

Technical challenge: Low Endotoxin Recovery (LER) effect

- LER is described as the failure to detect spiked endotoxin when tested using the bacterial endotoxins test (BET)
- LER occurs when endotoxin recovery remains at <50% of the spiked endotoxin concentration for two consecutive time points at any point over the duration of the study
- Based on current knowledge, two main factors contribute to LER:
    1. Endotoxin masking caused by endotoxin-binding proteins present in a sample
    2. Endotoxin masking caused by certain buffer components (drug matrix formulation)
- Endotoxin Recovery Studies (ERS) are not part of compendial endotoxin method suitability testing, but are defined as supplemental studies
- The current hypothesis is that LER mainly occur as a consequence of micelle formation when the drug substance is formulated with Polysorbate 20 or Polysorbate 80 together with the buffer salts sodium phosphate and/or sodium citrate
- BWP Interested Parties Meeting, October 2024
- Publishing of the BWP Q&A on the EMA website https://www.ema.europa.eu/en/human-regulatory-overview/research-and-development/scientific-guidelines/biological-guidelines/questions-answers-biological-medicinal-products

Updated Q/A - December 2025: When should LER be investigated?

- Definition of LER - reduced ability to detect spiked endotoxin using LAL or rFC assays1
- Cause of LER - linked to endotoxin masking; mechanism not fully understood
- Risk formulations - products containing surfactant (e.g., polysorbate) + chelator (e.g., EDTA, citrate, phosphate, histidine) require LER assessment
- When to test - perform LER studies for all products with identified risk (new MAA and already approved)
- Study design - spike undiluted samples with known endotoxin and monitor recovery over time
- Process relevance - include relevant temperatures and manufacturing hold times
- Regulatory requirement for new MAAs - include LER data in Module 3

[1] *Schwarz, H., Gornicec, J., Neuper, T. et al. Biological Activity of Masked Endotoxin. Sci Rep 7, 44750 (2017). https://doi.org/10.1038/srep44750*

Updated Q/A - December 2025: When should LER be investigated?

- Minimum time-points - evaluate at least 4 time points to ensure valid and accurate results
- LER definition - two consecutive data points falling <50% recovery = LER
- Mitigation strategy - optimize compendial method or develop an alternative method
- Specification setting - for LER-positive products, set endotoxin limits as low as reasonably achievable
- CTD placement - LER studies in 3.2.P.5.3 or 3.2.P.2.3 with cross-reference
- QC vs LER - QC sample hold times differ from hold time applied during LER studies; QC samples may be diluted/frozen, LER samples not
- Focus on FP - LER studies should focus on finished product; AS only if matrix differs
- Reference standard - PDA Technical Report No. 82 recommended for study design (primarily focuses on protein-based products, also applicable to vaccines and gene therapy)

Future Outlook

- rCR expected to enter Ph. Eur. as an alternative method
- Downgrade of variation category for rFC
- Future regulatory evolution must be data-driven, agency confidence should be built through shared analytical data, not assumptions
- Real-world examples of successful rBET implementation will be available, with sufficient experience, reduced reliance on formal equivalence testing may be considered in appropriate regulatory forums
- Increasing regulatory alignment through ICMRA and 3Rs groups
- Gradual phase-out of LAL tests
- Faster adoption supported by PACMP frameworks
- Opportunities for regulators to support sustainability through guidance, Q&A, and international alignment

Conclusion – EU perspective

- EU is transitioning from animal-based to recombinant testing
- rFC and rCR have a defined regulatory pathway
- Transitioning to rBET reduces environmental impact and improves analytical consistency
- rBET methods are scientifically beneficial compared to conventional BET, and their implementation is supported by the EU regulators
- Global harmonization remains the key challenge
- Strong collaboration between industry and regulators is essential; industry is encouraged to engage early with QIG (e.g., 1:1 meeting) to discuss platform scope and supporting data packages
- Platform knowledge needs to be substantiated by data submissions to regulatory authorities; establishing platform analytical procedures (ICH Q2) can streamline lifecycle management and support efficient implementation across portfolios
- Further regulatory evolution should be based on shared data, not variation category debate

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THANK YOU FOR YOUR ATTENTION

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