Industry perspective on the regulatory impact of recombinant Endotoxin testing

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Global Regulatory Affairs
EDQM, Feb, 26th, 2026

Disclaimer

Abdelmoughit Kaoukab is an employee of the GSK group of companies.
This work was sponsored by GlaxoSmithKline Biologicals SA.

Limulus Amoebocyte Lysate (LAL) and recombinant Factor C (rFC)

- LAL-based methods are used for endotoxin testing
- LAL is derived from the blood of various horseshoe crab species
- Endotoxin testing is a mandatory release test to ensure patient safety
- LAL testing is described in the European Pharmacopoeia (Eur. Ph 2.6.14) and USP <85>
- Testing is performed at different manufacturing steps: incoming material, water, drug substance, intermediates, adjuvant, drug product
- rFC is an alternative to LAL and is described in Eur. Ph 2.6.32 and USP <86>

Why recombinant Factor C (rFC) ?

- Reduces ecological impact: wild stock derived from an endemic threatened species
- rFC is produced by recombinant expression and does not require horseshoe crab blood
- Recombinant manufacturing provides more reliable supply compared with reliance on wild-harvested raw material
- Recombinant production yields a defined reagent with reduced biological variability, supporting more consistent assay performance
- Regulatory recognition: rFC methods are described in Eur. Ph. 2.6.32 and USP <86>
- EMA feedback on recent submissions for transitioning to rFC

Vaccine strategy for products in development

- New vaccines will be developed using recombinant Factor C (rFC)
- LAL-based comparability assessments will not be applicable if implemented during clinical studies
- Verification studies will be performed in accordance with product development plans and regulatory expectations

Changes in EU

- The change from LAL to rFC is not impacting the acceptance criteria

- Testing performed as per Eur. Ph does not require reporting validation in module 3. Reference to the analytical procedure is considered sufficient

- Validation/verification can be shared during inspection or upon request during the review of the submissions:

    - **Method validation and comparability**: comparability to be assessed in Water for Injection (WFI) for both LAL and rFC-based methods
    - **Method performance verification and comparability** : comparability to be assessed in drug product samples for both LAL and rFC-based methods
    - **Method verification**: each sample, where endotoxin test is conducted, will be tested with rFC for interfering factors

- No submission is planned when M3 is not impacted. Changes are managed in the Pharmaceutical Quality System (PQS)

Regulatory classification of approved vaccines

- EU: Reporting category agreed with EMA: Type IB
  - Q.1.b.2.d Other change to an analytical procedure (including replacement or addition) for a starting material/reagent/intermediate
  - Q.II.d.2.d Other change to an analytical procedure for a finished product (including replacement or addition

- United States of America:

  - Post Approval Supplement
  - Agreed approach agreed with FDA during a type C meeting

- Japan:
  - Partial Change Application
  - Consultation is planned prior submission

- Canada: Annual Notification

- China: major change

Changes for other countries including US, China and Japan

- The EU rationale, verification and justification remains valid
- Impact on Module 3 may vary depending on regional requirements or otherwise agreements
- Endotoxin might not be applicable at level of the drug product; therefore, method performance verification and comparability will be conducted at level of drug substance

Transition plan

- Transition to rFC will be phased, not implemented in a single step, due to the large number of assays across the manufacturing processes and sites
- Rollout will proceed in waves aligned with validation and verification progress
- During the transition period, both LAL and rFC based methods may coexist at different manufacturing step
- When the same test at the same process step is performed at two different sites, transition may only proceed once both sites completed their respective validation and verification steps

Questions ?

Transition from LAL test for endotoxin to rFC methods

Veterinary Industry Position

Dr Cat Stirling

Director Global Regulatory Affairs

26th Feb 2026

Context

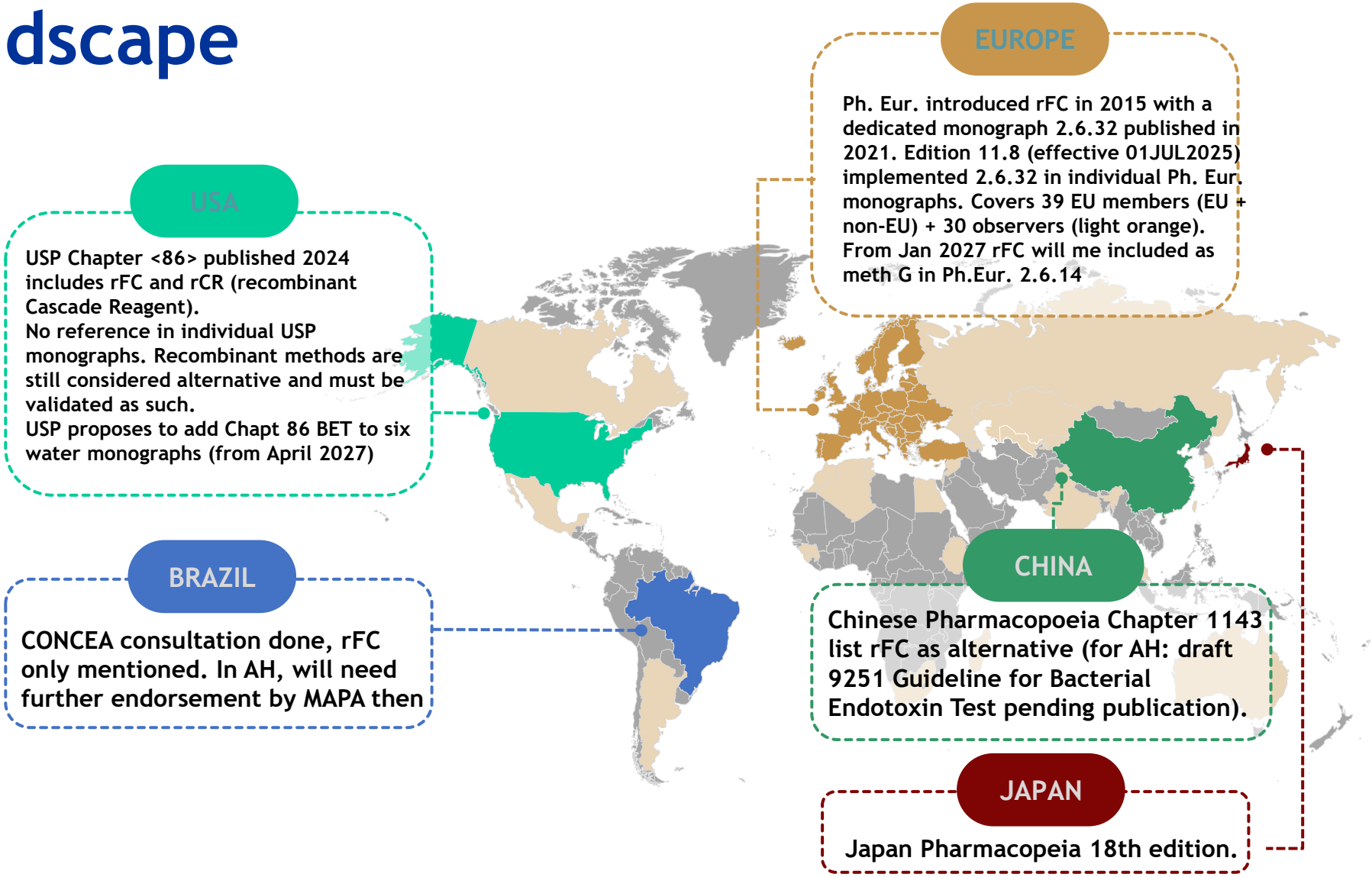
- Endotoxins are heat-stable lipopolysaccharides (LPS) from Gram-negative bacteria.
- They may contaminate e.g. raw materials, injectables, sterile solvents, vaccines, mAbs, and medical devices.
- They pose serious risks to humans and animals: causing fever, inflammation, septic shock.
- Endotoxin-free products are critical for safety across human and animal health sectors.

History & Concerns

- Historically, amoebocyte lysate from the Horseshoe crab (LAL or TAL) are used for bacterial endotoxin testing (BET).
- Testing Principle: In presence of endotoxins, Factor C binds with LPS molecules and triggers a cascade of reactions (coagulation), allowing detection and measurement.
- The use in the pharmaceutical industry may have contributed to Horseshoe Crab population decline.
- Supply risk due to reliance on one endangered species only.
- Animal variability affects test reliability.
- Work on alternatives started more than two decades ago. Among others, recombinant Factor C (rFC) reagent was developed as non-animal derived alternative.

Regulatory landscape

- EU: Compendial test
- Outside EU: Alternative to Compendial test
- Current regulatory context is rather favorable to implement non-animal alternatives and has been addressed by key markets
- Concern: The absence of harmonization across regions is presenting significant challenges for developing a coherent transition strategy



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global animal health association

Industry Transition

- **Large product scope** impacted and **at various stages**:
  - Drug development, raw materials, in-process control upstream/downstream in a GMP environment, and release tests.

- **Multiple instruments and multiple kits per each supplier available for rFC/rCR detection:**
  - Uncertainty, for acceptance of recombinant reagents other than rFC/cCR by regulatory bodies globally, complicates choice of equipment and method.
  - rFC vs rCR decision – rCR has higher validation requirement (need to comply with alternative method validation requirements)

- **Contract Manufacturing Organizations (CMO) constraints:**
  - Challenge for equipment standardization and planning.

Industry transition - Validation Challenges

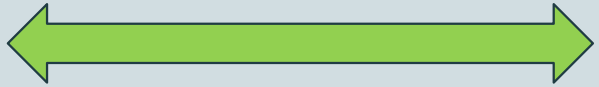
Significant validation effort required across regions

Equipment, validation methods & data may differ according to the regions. Example of Challenges:

- Validate against endotoxins standard solution (EU/US)
- Equivalence or superiority to be demonstrated *versus* former tests (Japan)
- Complex validation matrix for globally commercialized products
- 3 batches per product to confirm comparative test results
- Raw materials: as many validations as suppliers

- **Proposal: Validate per product range with shared features (rather than per product).**

Validation Matrix

Drug product manufactured and tested in EU EEA	Product for EU EEA	For export to NON-EU EEA	Drug Product manufactured and tested in NON-EU EEA
BET with LAL/TAL official and compendial*			BET with LAL/TAL official and compendial*
rFC official and compendial*	Compendial method verification	Full Method validation	rFC alternative method
rCR alternative	Full method validation	Full method validation	rCR alternative method

*if referenced in individual monograph

Regulatory Approval

- **EU:**
  - Variation and approval required to switch to rFC. Type: Variation Requiring Assessment (VRA). In EU rFC 2.6.32 is compendial.

- **International:**
  - rFC alternative method requires full method validation. Requirements and acceptability for rFC need to be checked with regulators internationally.

Challenge:

Need to maintain duplicate testing over several years until all the approvals are received.

Vet industry has started to see CVMP request commitments to transition in new product submissions where LAL is currently used.

Why is this important?

Is a switch mandatory – EDQM Q&D indicates that Ph.Eur.5.1.13 does not recommend one method over the other but also that sustainability should be considered when choosing method. rFC is not considered

Is a switch to rFC/rCR mandatory?

Vet industry has started to see CVMP request commitments to transition in new product submissions where LAL is currently used.

BUT

Is a switch mandatory?

- EDQM Q&D indicates that Ph.Eur.5.1.13 does not recommend one method over the other but also that sustainability should be considered when choosing method. rFC is not considered superior to LAL but the applicant would need to decide based on a risk-based assessment

Industry needs clear guidance globally to advance the switch

Expected pragmatic approaches

- **Cross-site validation**: One site validates and transfer the technological method to others.
- **Product range matrix approach**: Range-based validation for products sharing common features should be acceptable.
- The expectation is that **no specification** changes are needed.
- **Active Ingredient via Finished Product validation**: Worst-case approach may delay approvals if validation is need for both (may be the case with non-biologicals)
  (API End of Shelf Life in FP method validation)?

Worldwide regulators engagement for harmonization of recombinant BET shift is key.

Conclusion

- Industry progressing toward recombinant reagent adoption for BET.
  - Voluntary unless pushed by regulatory questions
- Large scope, multi-year effort.
- Burden due to dual testing.
- Requires investment, training, SOP updates.
- Validation burden high; pragmatic range-based approach suggested.
- Regulatory clarity needed for rCR acceptance and Compendia harmonization.

↳ CVMP/CHMP exchange as well as collaboration with other regions for harmonized regulatory approach is critical to phase out LAL and transition to rFC/rCR

THANK YOU

Source: File:Limules.jpg - Wikimedia Commons

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RECOMBINANT BACTERIAL ENDOTOXIN TESTING: A GENERATIONAL CHANGE

JAY BOLDEN, ELI LILLY AND COMPANY

FEBRUARY 2026

Lilly

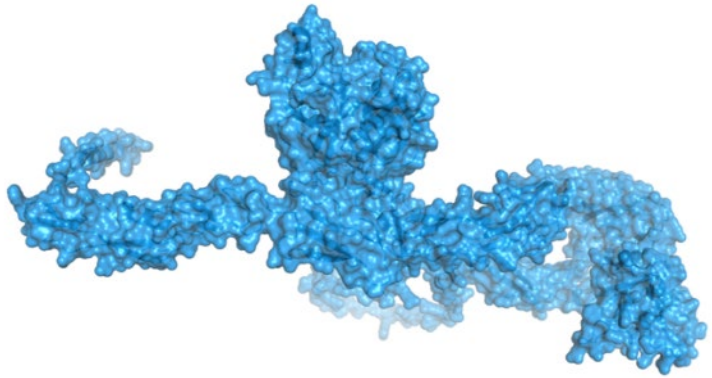
Jay Bolden is a member of the USP Microbiology Expert Committee. The views expressed are the author's alone and not those of USP, including the USP Microbiology Expert Committee.

Evolution of Bacterial Endotoxins Testing

Rabbit Pyrogen Test

Bacterial Endotoxins Test: LAL & TAL

Image courtesy of Jack Levin

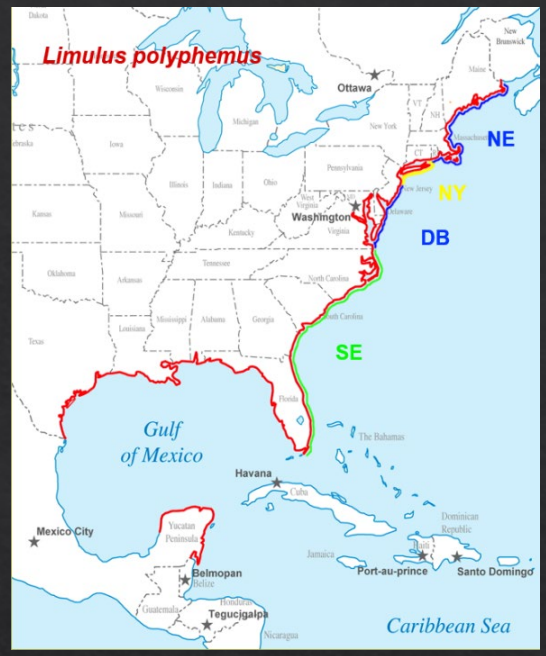


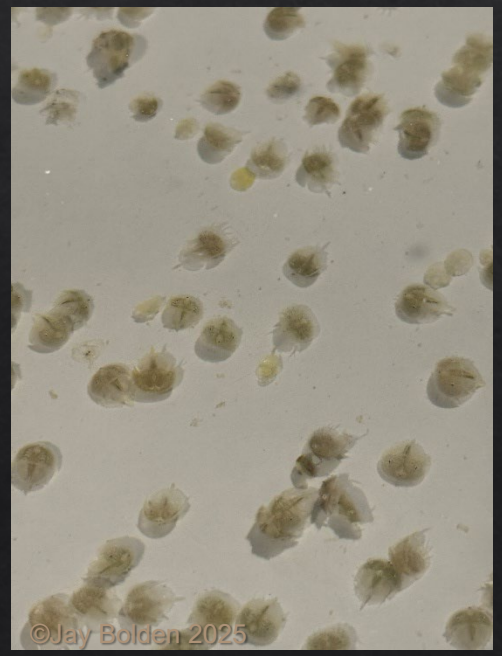
Recombinant Bacterial Endotoxins Test

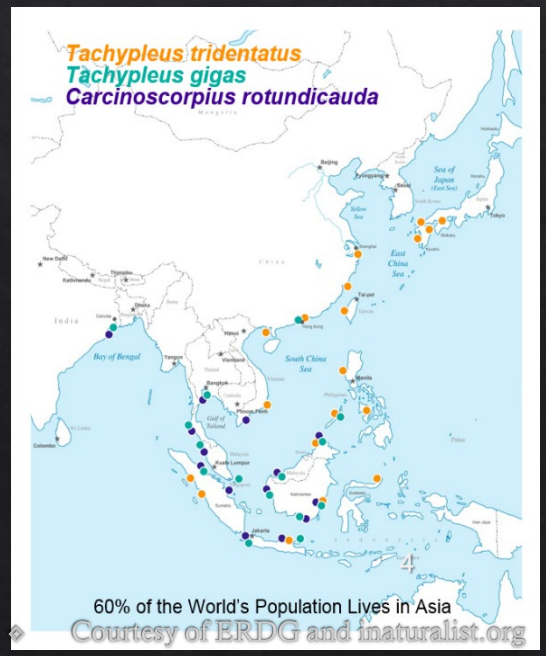
Image courtesy of Lynne Ding; Tim Cernak/Tesko Chaganti using Alphafold3 from sequence in J. Genet. Eng. Biotechnol., 2023, 21, 44.

Horseshoe Crab Distribution

Genus / Species	Common Name	IUCN Status
Limulus polyphemus	American horseshoe crab	Vulnerable
Carcinoscorpius rotundicauda	Mangrove horseshoe crab	Data Deficient*
Tachypleus gigas	Indo-Pacific horseshoe crab	Data Deficient*
Tachypleus tridentatus	Tri-spine horseshoe crab	Endangered

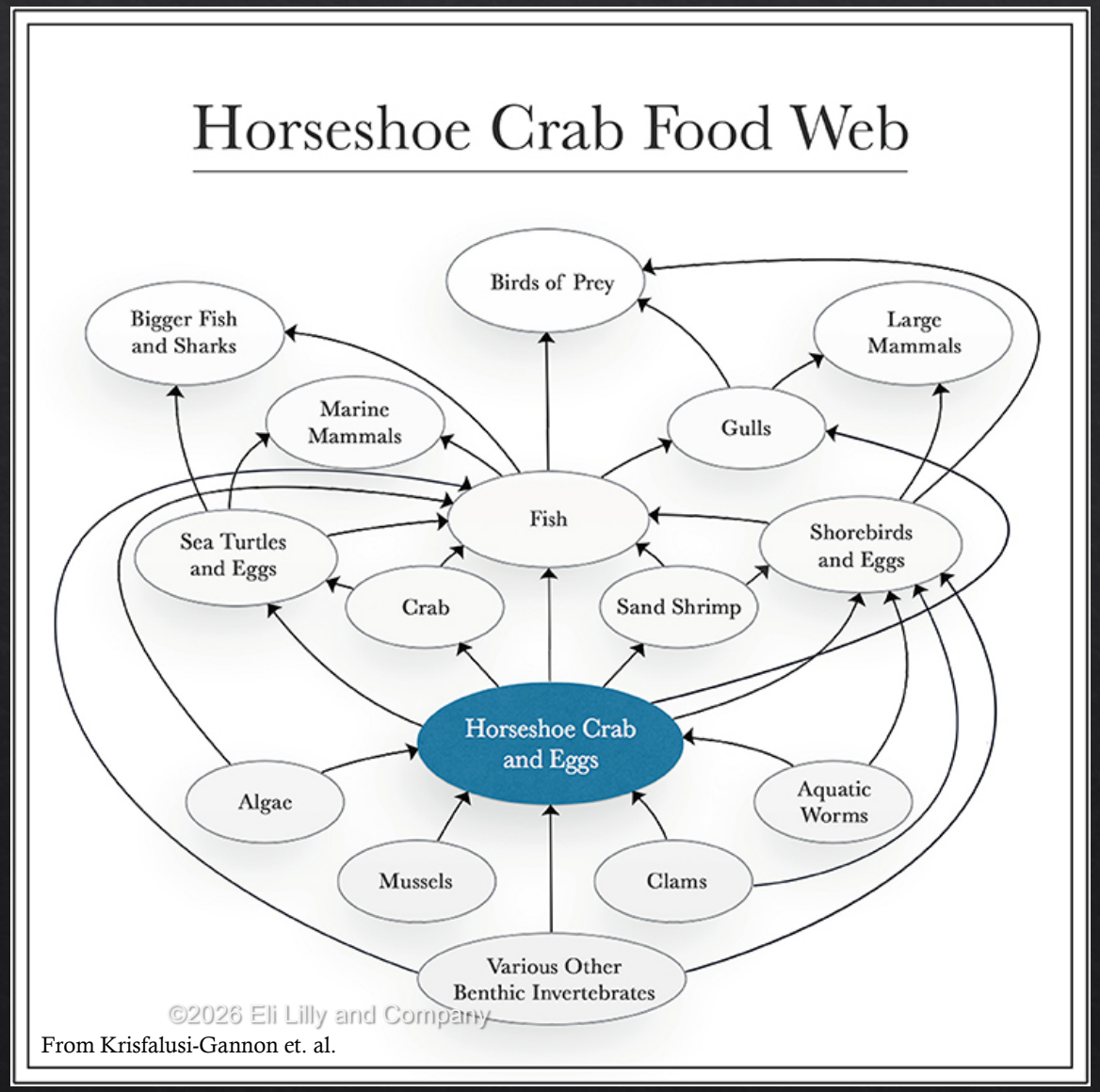






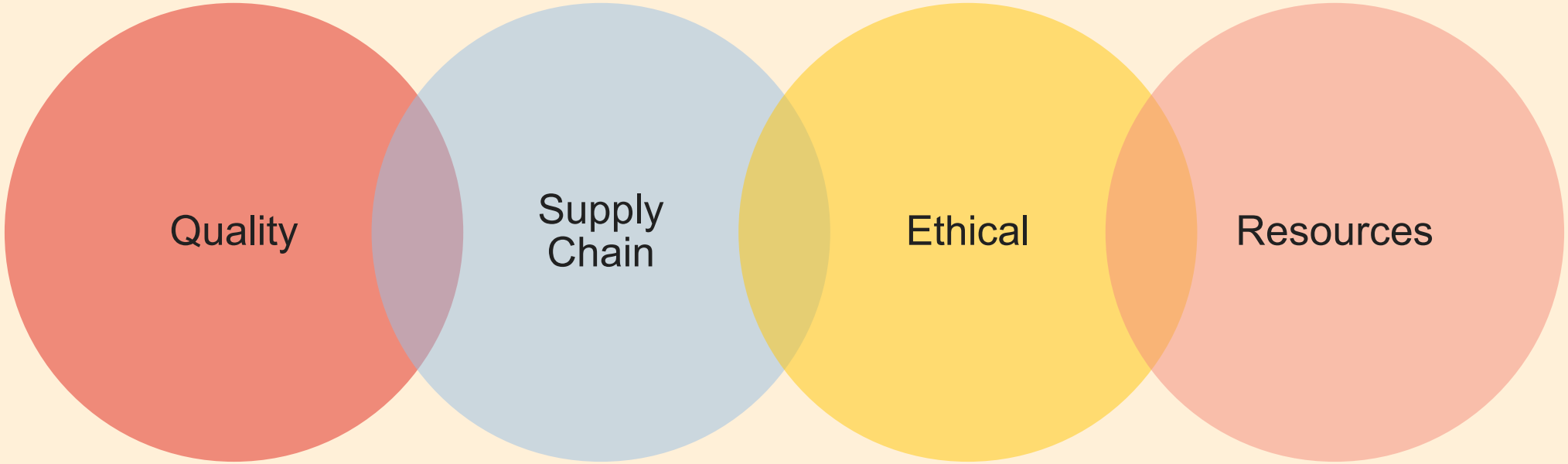
*2024-2025-iucn-ssc-horseshoe-crab-sg_publication_r2.pdf

Many Species Depend on Horseshoe Crabs



From Krisfalusi-Gannon et. al.

Change Rationale



Timeline

HISTORY

1997: Patented
2003: Commercially Available
2006: Lilly Evaluation

THE START

2012-2013:

- New Lilly China plant
- FDA Guideline
- USFWS lists Red Knot
- 2nd rFC supplier

THE LINE

2016:

- Generated evaluation data
- Validated method
- Line in the sand

SUCCESS

2018: First product approval

2020:

- Additional product approvals
- Ph.Eur. 2.6.32

- Tri-spine horseshoe crab declared endangered

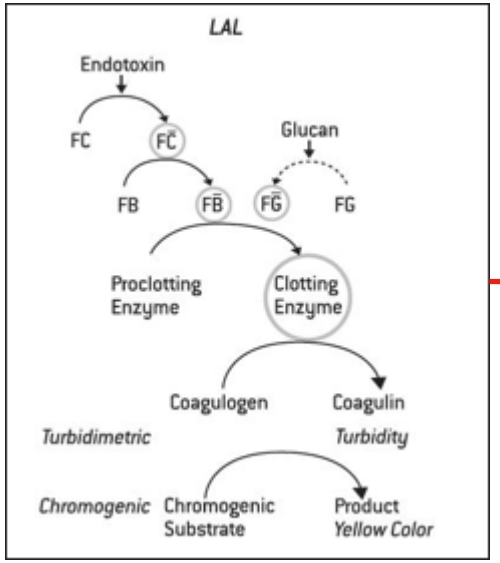
FUTURE

- PDG Harmonization
- PSCI Principles
- Automation
- 2021+: rCR

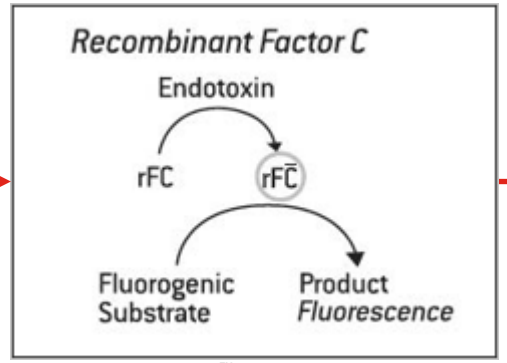
The fundamental biotechnology supports the change

Recombinant Reagents are Biochemical Equivalents to LAL

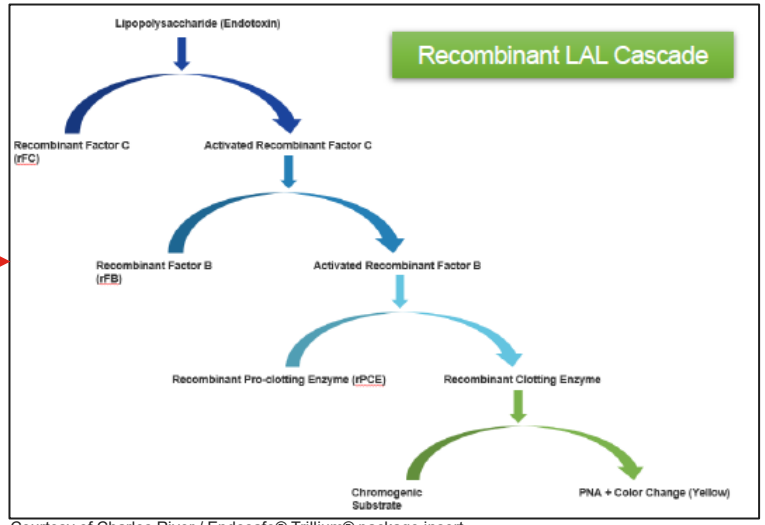
Mechanism of Action



Courtesy of Lonza PyroGene™ package insert



Courtesy of Lonza PyroGene™ package insert



Courtesy of Charles River / Endosafe® Trillium® package insert

The data supports the change

Literature

Author	Title	Journal	Year	Vol	Pages
Kikuchi et al	Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides Part 1	Pharm & Med Device reg Science	2017	40(4)	252-260
Kikuchi et al	Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides Part 2	Pharm & Med Device reg Science	2018	49(10)	708-718
Mizumura et al	Genetic engineering approach to develop next-generation reagents for endotoxin quantification	Innate Immunity	2017	23(2)	136-146
Muroi et al	Application of a Recombinant Three-Factor Chromogenic Reagent, PyroSmart, for Bacterial Endotoxins Test Filed in the Pharmacopeias	Biological and Pharmaceutical Bulletin	2019	42 (12)	2024-2037
Stevens et al	Advanced Recombinant Cascade Reagent PyroSmart NextGen® for Bacterial Endotoxin Test as Described in the Pharmacopoeias	BPB Reports	2022	5 (5)	105-114
Shapovalova O V et. al.	New direction in the determination of bacterial endotoxins: Analysis using recombinant Factor C.	Pharmaceutical chemistry journal	2022	56	1133–1139
Kikuchi et al	Collaborative Study on the Bacterial Endotoxins Test Using Recombinant 1 Factor C-Based Procedure for Detection of Lipopolysaccharides (Part 3)	Pharm & Med Device reg Science	2023	54 (4)	341-351
P. Cliffe, K. Capper	Feasibility assessment of recombinant reagents for the application of endotoxin testing of pharmaceutical waters	EJPPS	2025	304	

Recombinant Comparability 2023-2024

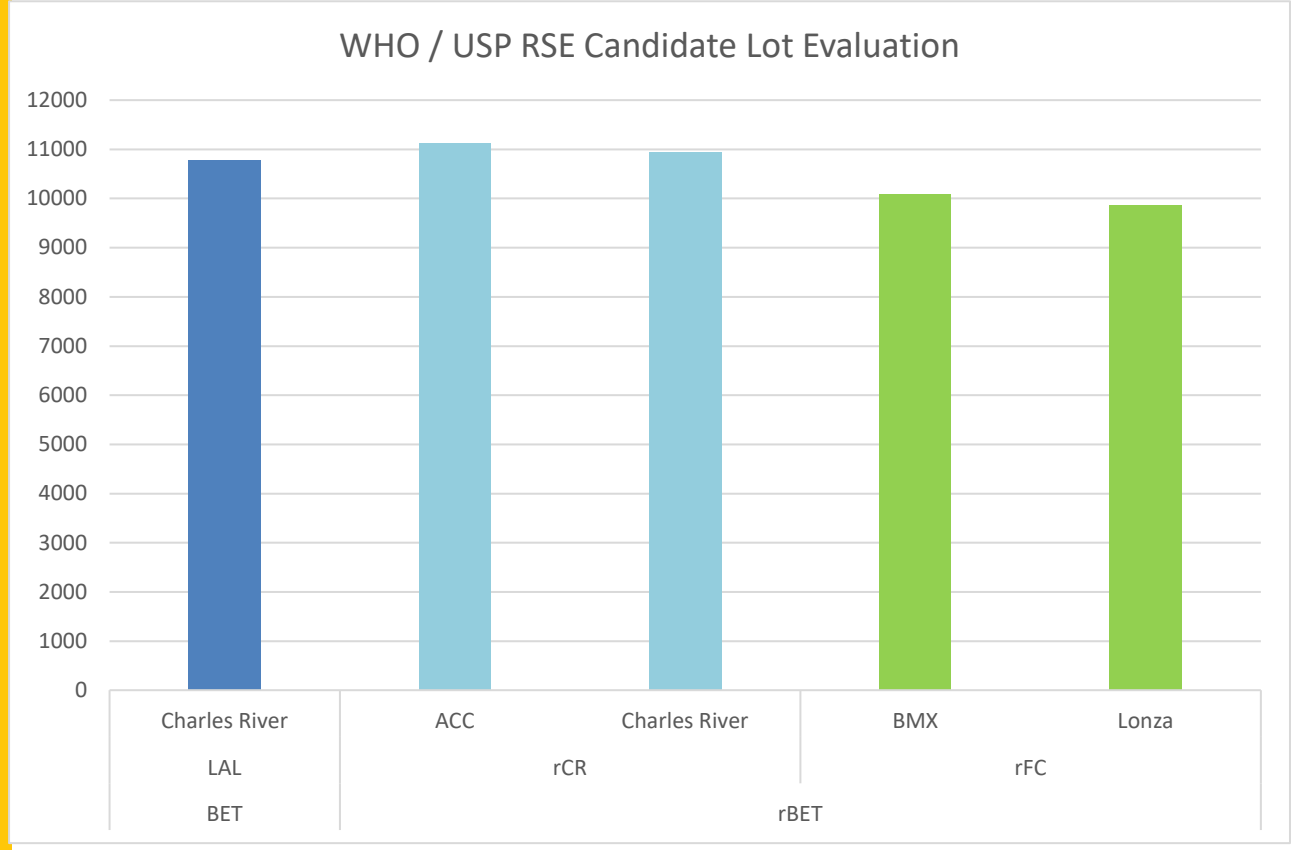
Recombinant reagents are equivalent or superior to LAL using 10,000 EU/vial national Reference Standard candidate lot endotoxin

Reagent	Average (EU/vial)	%CV
CR Gel Clot	1.58E+04	25%
CR KTA	1.20E+04	24%
Lonza LAL	1.15E+04	22%
Lonza rFC	1.05E+04	18%
BMX rFC	1.10E+04	19%
Xiamen rFC	9.90E+03	9%
Wako rCR	1.06E+04	28%
Xiamen rCR	9.99E+03	3%

Reagent	Average (EU/vial)	%CV
LAL	1.23E+04	27%
rFC	1.05E+04	17%
rCR	1.03E+04	21%
Recombinant (rFC + rCR)	1.04E+04	19%

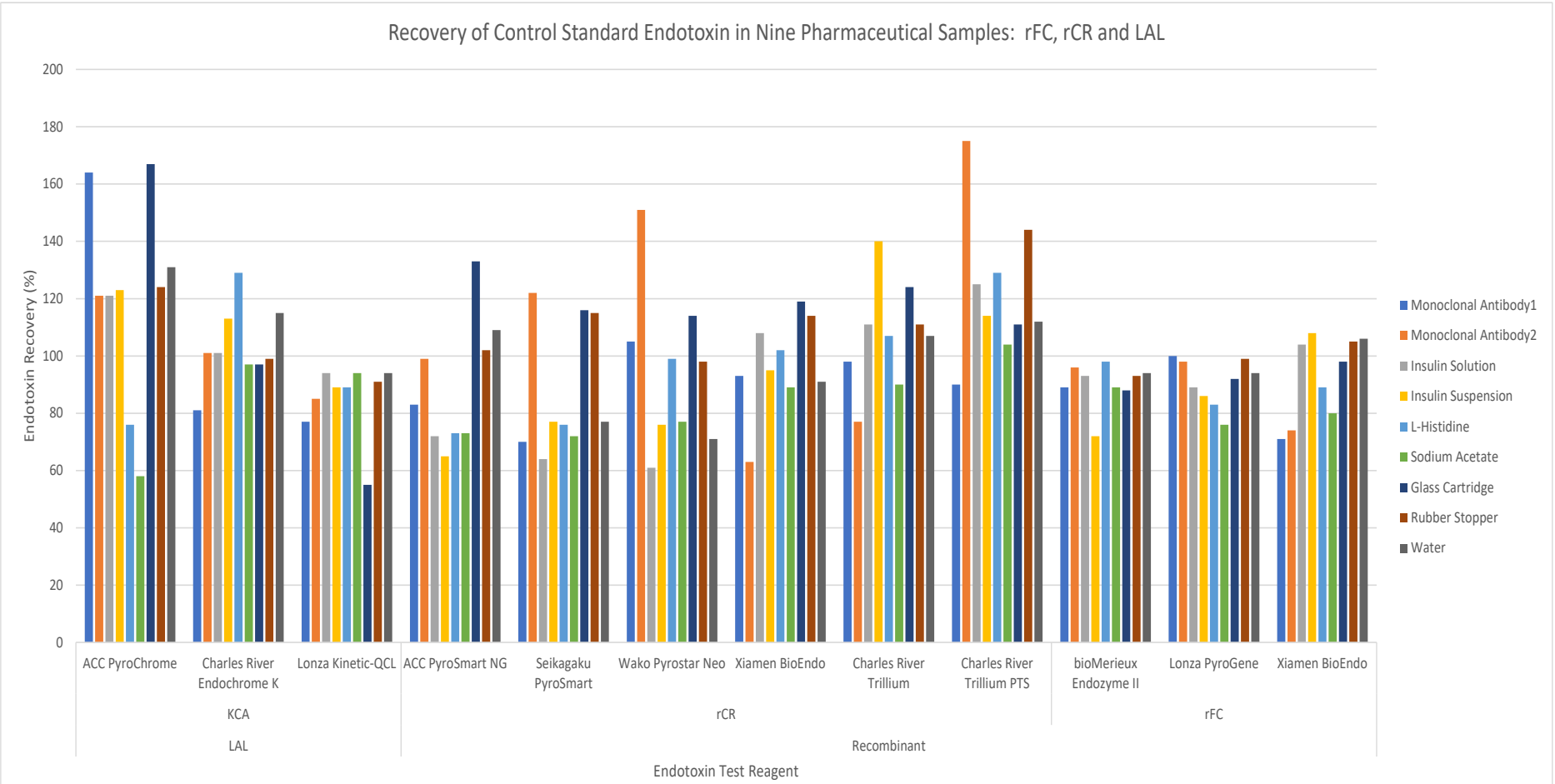
Recombinant Comparability 2025

Recombinant reagents are equivalent or superior to LAL using 10,000 EU/vial international WHO Reference Standard and USP RSE candidate lot



Row Labels	Average of Result (EU/vial)	%CV
BET	10771	14%
LAL	10771	14%
Charles River	10771	14%
rBET	10514	10%
rCR	11030	8%
ACC	11130	9%
Charles River	10942	8%
rFC	9988	10%
BMX	10091	5%
Lonza	9866	13%
Grand Total	10568	11%

Recombinant Comparability



Average of %PPC	Grand Total
LAL	**104**
KCA	**104**
ACC PyroChrome	121
Charles River Endochrome K	104
Lonza Kinetic-QCL	85
Recombinant	**97**
rCR	**100**
ACC PyroSmart NG	90
Seikagaku PyroSmart	88
Wako Pyrostar Neo	95
Xiamen BioEndo	97
Charles River Trillium	107
Charles River Trillium PTS	123
rFC	**91**
bioMerieux Endozyme II	90
Lonza PyroGene	91
Xiamen BioEndo	93
Grand Total	**99**

- Compendial recovery criteria is 50-200%; product dilutions were based on equivalent LOQs, not necessarily optimized for PPC recovery
- Theoretical recovery target is 90.9% based on a 10 µL “hot spike” into 100 µL sample

Lilly rCR Validation Data

rCR Technology	Accuracy (%, H/M/L) Criteria 50-200%			Precision (%, H/M/L)			Inhibition/Enhancement (%)			pH
Microfluidics 1	125	86	68	12	8	10	124-145	96-123	108-141	7.46-7.87
96 well plate supplier 1	92	89	79	8	14	15	84-97	85-109	89-95	
96 well plate supplier 2	100	99	117	8	7	16	90-104	95-115	93-103	
Microfluidics 2 supplier 1	81	85	91	2	2	9	83-88	82-84	84-86	
Microfluidics 2 supplier 2	84	88	90	5	5	23	84-89	92-94	82-90	

1. Supplier validations
2. Lilly validation
3. Lilly product-specific verifications → Enabled one approved commercial submission

At Line: Single Sample Microfluidics

Material	Dilution	Sample Value	Sample CV	Spike Value	Spike Recovery	Spike CV
Viral filtration starting material	1:50	<0.598 EU/mL	5.3%	0.174 EU/mL	134%	4.6%
TFF final material	1:50	<0.500 EU/mL	0.0%	0.207 EU/mL	159%	1.0%
TFF final material	1:50	<0.500 EU/mL	0.0%	0.168 EU/mL	129%	2.2%
Low pH Viral Inactivated Intermediate	1:50	<1.69 EU/mL	46.0%	0.066 EU/mL	51%	11.4%
ProA Column Pack Effluent	1:100	<1.00 EU/mL	0.0%	0.165 EU/mL	127%	1.6%
CEX Column Pack Effluent	1:100	<1.00 EU/mL	0.0%	0.177 EU/mL	136%	4.9%
Histidine Arginine-HCl Buffer	1:5	0.067 EU/mL	1.9%	0.102 EU/mL	78%	1.1%
Sodium Acetate Buffer	1:5	<0.05 EU/mL	0.0%	0.186 EU/mL	143%	0.0%
Sodium Acetate, NaCl, Sucrose Buffer	1:5	<0.05 EU/mL	0.0%	0.101 EU/mL	77%	1.9%
Histidine Arginine-HCl Buffer	1:5	<0.05 EU/mL	0.0%	0.152 EU/mL	117%	0.3%
Histidine Sucrose Buffer 1	1:5	<0.05 EU/mL	0.0%	0.172 EU/mL	107%	1.0%
Histidine Sucrose Buffer 2	1:5	<0.05 EU/mL	0.0%	0.154 EU/mL	119%	0.3%

- rFC is our platform, however we have specific use cases for rCR: e.g. at line critical buffer testing and rFC-interfering samples

Lilly rCR Experience Summary

- 55+ unique pharmaceutical items
  - drug product
  - drug substance
  - excipient
  - component
- 3 technology platforms
  - 96 well plate reader
  - single sample microfluidics
  - 21 sample microfluidics disc
- 6 different rCR reagents / formats
- 10 sites implemented
  - 3 internal sites (EU / USA)
  - 7 external sites (EU / USA / Asia)
- USP <86> registered in approved INDs and BLAs

The regulatory and pharmacopoeia landscape support the change

Compendial Status – Momentum & Convergence

Compendial Method

2020: Ph.Eur. 2.6.32
2024: BP XIV C
2024: EAEU 2.1.6.12
2024: USP <86>
2025: Brazil & WHO (proposed); PDG Statement

Compendial Monograph & Guidance Chapters

2023: Ph.Eur. Water for Injection Monograph

2020: ChP 9251
2021: JP G4-4-180
2023: KP

Appendix: PDG position

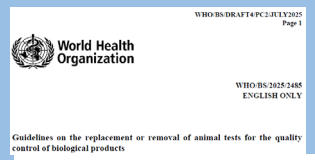
PDG is committed to making efforts to develop and revise existing test methods, for example, the test for Bacterial Endotoxins (BET), to decrease the use of animals or animal derived reagents.

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.


WHO/BS/DRAFT4/PC2/JULY2025
Page 1

World Health Organization

WHO/BS/2025/2485
ENGLISH ONLY

Guidelines on the replacement or removal of animal tests for the quality control of biological products

Risk assessments should take into account the nature of the product, the starting/raw materials and the product-related impurities. Careful consideration should be given to the selection and implementation of pyrogenicity/endotoxin assays at the appropriate stages of product development, manufacture and quality control. In all cases, the use of the RPT is no longer recommended, and it should be replaced with the alternative pyrogenicity/endotoxin tests described above. Where there is a risk of non-endotoxin pyrogens being present, the use of the MAT is recommended. In cases where non-endotoxin pyrogens are unlikely to be present, endotoxin testing using the rFC assay or rCR assay is recommended.

Global Health Authority Approvals (rFC)

Argentina	Hong Kong	Pakistan
Australia	Hungary	Peru
Austria	Iceland	Philippines
Bahrain	India	Poland
Bangladesh	Indonesia	Portugal
Belgium	Ireland	Qatar
Bosnia And Herzegovina	Israel	Romania
Brazil	Italy	Russian Federation
Brunei Darussalam	Japan	Saudi Arabia
Bulgaria	Kazakhstan	Serbia
Canada	Korea, Republic Of	Singapore
Chile	Kuwait	Slovakia
China	Latvia	Slovenia
Colombia	Lebanon	South Africa
Croatia	Liechtenstein	Spain
Cyprus	Lithuania	Sweden
Czechia	Luxembourg	Switzerland
Denmark	Malaysia	Taiwan
Ecuador	Malta	Thailand
Egypt	Mexico	Turkey
Estonia	Netherlands	United Arab Emirates
Finland	Netherlands, Kingdom Of The	United Kingdom (Great Britain)
France	New Zealand	United Kingdom (Northern Ireland)
Germany	Norway	United Kingdom Of Great Britain And Northern Ireland
Greece	Oman	United States Of America

Overcoming Challenges and Outcomes

Considerations and challenges in the adoption of recombinant reagents as an alternative to traditional LAL-based methods

Barrier	Current Status
Suppliers	6 known commercial rFC suppliers 5 known commercial rCR suppliers
Equipment	Fluorescence (rFC) or Absorbance (rCR) readers required; Improves Data Integrity position
Pharmacopoeia	General Chapters in Europe (2020); UK, Eurasia, USA (2024) Guidance Chapters in China, Japan, Korea WHO, PDG and Brazil proposals
Data Equivalence	Pharmacopoeia Expert Committees have advanced chapters based on the underlying biotechnology science and available comparability data
Regulatory Acceptance	More than 10 products approved using rBET in at least 75 countries

Outcomes

- 10 products approved since 2018
- 30+ internal / external labs enabled
- 75 markets approved
- 300,000+ samples tested
- ~80% internal conversion
- Annual integrated report disclosures since 2019

Acknowledgements

Kelly Smith

Ben Claywell

Carlos Figueroa

Matt Bedwell

Rob Warburton

Sophie Rhinn

Noopur Bindal

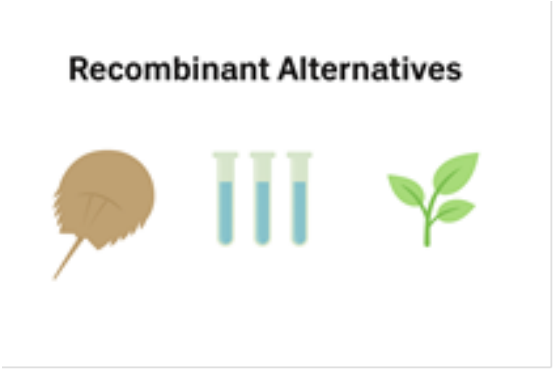
Regulatory Perspectives on Recombinant Alternatives (rFC & rCR) and Their Path to European Acceptance

Barbora Ladinová, State Institute For Drug Control, Czechia and BWP (EMA) member

02 March 2026

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
- Ph. Eur. encourages data generation to support future inclusion of rCR, Pharmeuropa 2026 introduction of a reference to rCR
- 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

  - Request to downgrade variation category from Type II

  - Need for harmonization between USP and Ph. Eur.

- 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

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)
- 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 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, **exploring Q&A** to support **downgrade** as a Type IB variations, currently under the discussion (Q.I.b.2. (z), Q.II.c.2. (z), Q.II.d.2 (z))
- rCR remains Type II until compendial status changes

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
- Lack of global harmonization and 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

Advantages & Sustainability Impact

- 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. rFC 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.
- rFC 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.

SÚKL

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
- 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

SÚKL

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
- Platform knowledge needs to be substantiated by data submissions to regulatory authorities
- Further regulatory evolution should be based on shared data, not variation category debate

SÚKL

THANK YOU FOR YOUR ATTENTION

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Recombinant reagents for Bacterial Endotoxin Testing (BET)

Regulatory and pharmacopoeia acceptance of recombinant reagents in Europe and worldwide

Position of the Ph. Eur., the USP and the Pharmacopoeial Discussion Group

Dr. Leslie Furr, Senior Scientist, US Pharmacopeia (USP)

Dr. Emmanuelle Charton, Head of Division B, European Pharmacopeia Department, EDQM, Council of Europe

Thursday, 26 February 2026

The Pharmacopeial Discussion Group (PDG)

- **Began as an informal group in 1989**; participants include USP, Ph. Eur., IPC, and JP
  - ★ IPC joined as member in 2023
  - ★ WHO joined as observer in 2001
- Focuses on selected official, broad-impact General Chapters and excipient monographs
- Eliminates/minimises need to perform multiple tests and procedures and to comply with multiple acceptance criteria for the same article
- Detailed process, with specific stages and terminology
- One face-to-face meeting a year, with a video conference in the interim

Ph. Eur. (EDQM)

IPC

JP (MHLW /PMDA)

USP

Candidate Participant

PDG Mission

To harmonize pharmacopeial standards while maintaining a constant level of science with the shared goal of protecting public health.

Position of the USP

Endotoxins Chapters in USP

Chapters

- <85> Bacterial Endotoxins Test
- <86> Bacterial Endotoxins Test Using Recombinant Reagents (New – Official 01-Apr-2025)
- <1085> Guidelines for Bacterial Endotoxins Testing (Revised – Official 01-Feb-2026)
- *<1085.1> Use of Recombinant Reagents in the Bacterial Endotoxins Test – Photometric and Fluorometric Methods Using Recombinantly Derived Reagents* (never published)

Endotoxin Reagents

USP proposals to add recombinant reagents

2019

Original proposal to add rFC to <85>

- Published data did not support comparability (only suitability)
- Approach was abandoned in favor of an informational chapter

2020

<1085.1>

PF 46(5)

- Included all recombinant reagents (one recombinant cascade reagent was near commercialization, and another was in late-stage development)

2021

<1085.1>

future publication

- Modified to include ALL ASSAYS that are replacements for the BET
- Never published

2023

<86> drafted

PF 49(6)

Include recombinant reagents

rFC

rCR

Endotoxin Reagents Timeline

July 2024 New chapter <86> Approved

Apr 2025
USP announces call for data for all product types

Jan 2026
PF52(1)
USP Water monographs
proposed revision to add <86>

2024

2025

2026

Nov 2024
USP publishes comments, FAQs, and additional materials

May 2025
<86> Official

Feb 2026
<1085> revised to include <86>

Recombinant Reagents

Next steps

- Review data submissions
- Water EP to review comments on proposal to add <86> in 7 water monographs (Comments open until 31-Mar-2026)

SPECIFIC TESTS

[NOTE—Required for bulk and packaged forms of *Water for Injection*.]

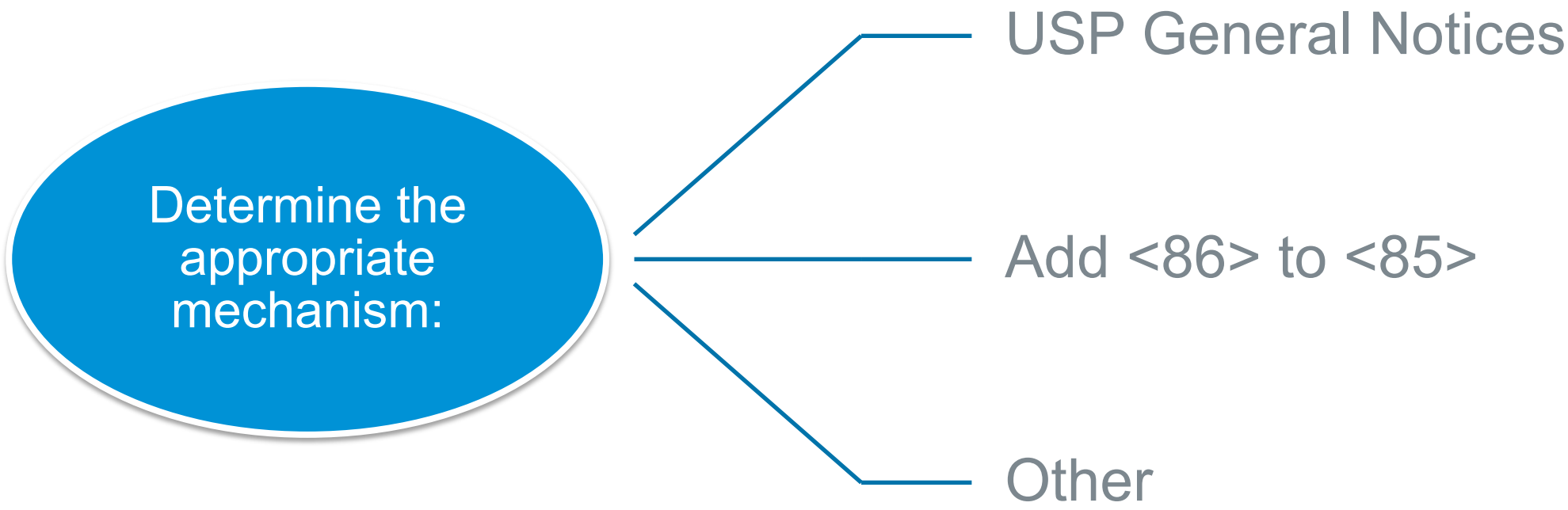
Change to read:

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩ ▲or **BACTERIAL ENDOTOXINS TEST USING RECOMBINANT REAGENTS** ⟨86⟩:▲ (USP 1-APR-2027) It contains less than 0.25 USP Endotoxin Units/mL.

Recombinant Reagents

Next steps

- Enable implementation of <86> for monograph products and products filed citing <85>



Position of the Ph. Eur.

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

A major step towards animal-free testing for the test for bacterial endotoxins

★Press release published on the EDQM website on 18 December 2025

The European Pharmacopoeia (Ph. Eur.) is moving into a new era of innovation and sustainability. Starting **1 January 2026**, the rabbit pyrogen test (general chapter *2.6.8. Pyrogens*) will disappear from Ph. Eur. texts, ending decades of reliance on animal-based methods. This change reflects a clear commitment: protecting animal welfare while ensuring the highest standards of patient safety and scientific rigour.

From that date onwards, pyrogen testing will rely on modern, animal-free, science-driven approaches. Manufacturers will choose methods based on the specific risks of the substance or product containing non-endotoxin pyrogens, using advanced alternatives to animal testing such as the monocyte-activation test or the bacterial endotoxins test (BET). For the latter, the European Pharmacopoeia Commission (EPC) is proud to announce that **recombinant Factor C (rFC)** – a synthetic, validated, animal-free solution – will be fully integrated as of Issue 13.1 of the Ph. Eur. as one of the seven methods that can be used to test for bacterial endotoxins (general chapter *2.6.14*). Users retain method choice guided by risk assessment and suitability under *Pyrogenicity (5.1.13)*. This marks a significant milestone in reducing dependence on natural resources like the

We are also looking ahead. New technologies such as recombinant Cascade Reagents (rCR) show great promise, and while they are not yet included in the Ph. Eur., the EPC is encouraging data generation to pave the way for their future consideration. This will maintain global harmonisation and support innovation across the pharmaceutical industry.

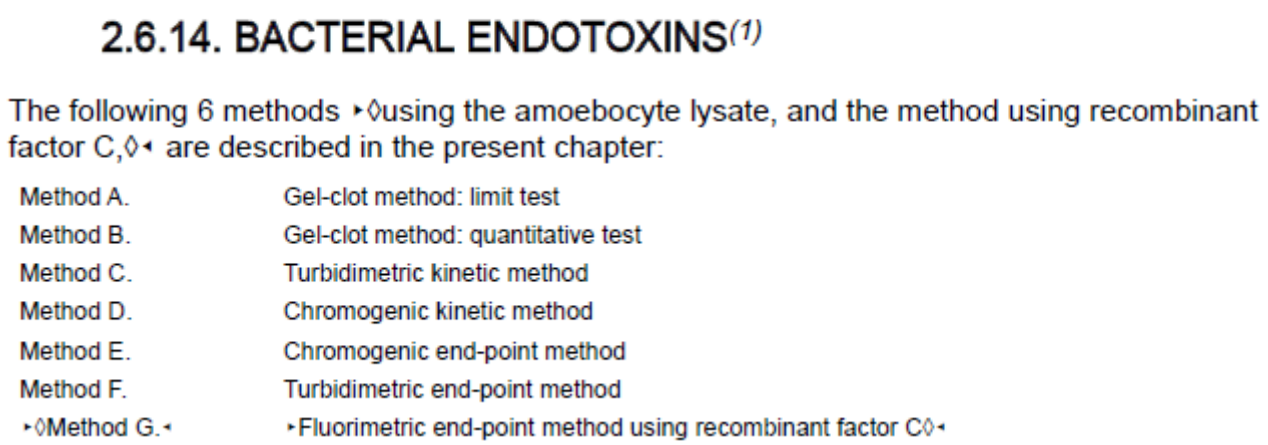
To help stakeholders navigate this transition, the EDQM and the European Partnership for Alternative Approaches to Animal Testing (EPAA) will hold a free joint symposium in **February 2026** to share the roadmap, discuss global approaches and answer questions. This symposium will be of particular interest to professionals from the pharmaceutical and biopharmaceutical industries, contract laboratories and health authorities.

Together, we are building a future where science and sustainability go hand in hand.

https://www.edqm.eu/en/-/a-major-step-towards-animal-free-testing-for-the-test-for-bacterial-endotoxins-1

Bacterial endotoxin test: inclusion of rFC as new method G in general chapter 2.6.14

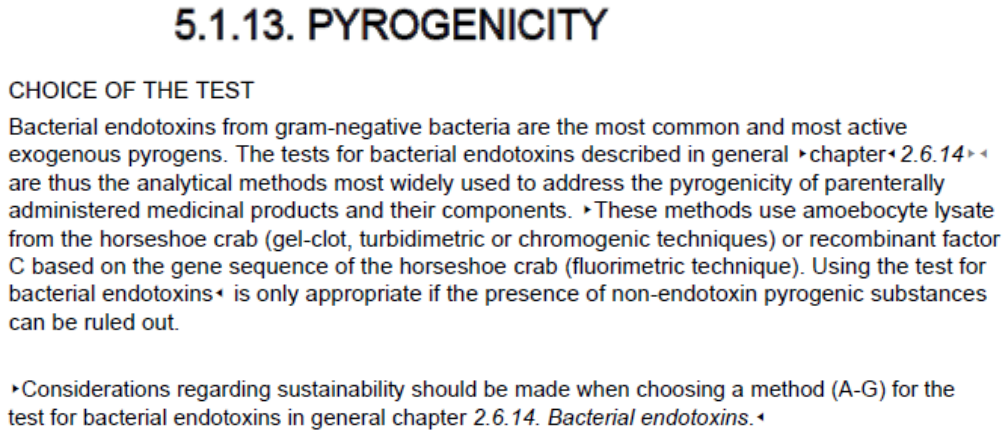
- At its March 2025 Session, the Ph. Eur. Commission decided to **revise chapter 2.614** *Bacterial Endotoxins* **to include** the test using recombinant factor C (**rFC**) as the 7th BET method (i.e. as new method G)
- This revision gives the possibility to use rFC in all Ph. Eur. texts referring to chapter 2.6.14 (~500 texts) and gives full recognition of the **equivalence of rFC with** all the **LAL** methods
- In addition, chapter 5.1.13 was revised to reflect the 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 were approved by the Ph. Eur. Commission at its November 2025 Session, and will be published in Issue 13.1 (April 2026, with an implementation date of 1 January 2027)



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◊◂



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 ▸chapter◂ *2.6.14*▸◂ 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◂ 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.*◂

European Pharmacopoeia Commission clarifies rFC integration and future innovation pathways

★ **Press release published on the EDQM website on 26 January 2026**

The European Pharmacopoeia Commission (EPC) has taken a significant step toward modern, animal-free testing by integrating recombinant Factor C (rFC) – a synthetic, validated solution – as one of the seven methods in general chapter *2.6.14. Bacterial endotoxins* of the European Pharmacopoeia (Ph. Eur.). This milestone reinforces the commitment of the European Directorate for the Quality of Medicines & HealthCare (EDQM) and of the EPC to patient safety, scientific rigour and sustainability under the 3Rs principle (replace, reduce, refine) and provides another valid alternative for users now that the rabbit pyrogen test has been suppressed (as of 1 January 2026).

To support stakeholders during the transition to rFC, the EDQM has published a comprehensive FAQ addressing common questions about rFC inclusion and future developments. The FAQ explains that rFC was adopted based on mature, evidence-backed data demonstrating equivalence to traditional limulus amoebocyte lysate (LAL) methods. Laboratories and manufacturers retain full method choice, guided by risk assessment and product suitability under chapter *5.1.13. Pyrogenicity*.

The EDQM also clarifies its position on recombinant cascade reagents (rCR): while promising, these methods are still in the data-generation phase. The *EPC encourages data generation to pave the way for future consideration of rCR*. This approach ensures that innovation is embraced responsibly, balancing scientific progress with patient safety.

Harmonisation remains a priority. The inclusion of rFC aligns with ongoing dialogue within the Pharmacopoeial Discussion Group (PDG), enabling users to select methods that fit their regulatory and market requirements.

For more details, **consult the full FAQ on the EDQM website**. This resource aims to provide clarity, transparency and practical guidance as Europe leads the way in implementing validated, *synthetic, animal-free solutions for bacterial endotoxin testing*.

See also:

- Joint EDQM-EPAA Symposium: 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)
- Outcome of the 183rd session of the European Pharmacopoeia Commission
- European Pharmacopoeia publishes first individual monoclonal antibody medicinal product monograph

https://www.edqm.eu/en/-/european-pharmacopoeia-commission-clarifies-rfc-integration-and-future-innovation-pathways

Recombinant factor C (rFC)

Does the Ph. Eur. favour limulus amoebocyte lysate (LAL)-based methods over the recombinant factor C (rFC)-based method?

General chapter *5.1.13. Pyrogenicity* does not recommend LAL-based methods (general chapter *2.6.14*) over the rFC-based method (previously general chapter *2.6.32*, in general chapter *2.6.14* as of Issue 13.1) or vice versa. Users are responsible for selecting the most appropriate method based on risk assessment and their assessment of the potential presence of pyrogens. General chapter *5.1.13* (Issue 13.1, publication date of April 2026, with an implementation date of 1 January 2027) also mentions: "*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*." It is the user's responsibility to decide on a testing strategy and limits.

Recombinant factor C (rFC)

I want to use recombinant factor C (rFC) for my bacterial endotoxins test (BET): do I have to validate its equivalence against Methods A-F?

As of 1 January 2027, method G will be fully recognised as an official Ph. Eur. method for the BET and it will no longer be necessary to demonstrate equivalence with limulus amoebocyte lysate (LAL)-based methods (methods A-F).

Recombinant cascade reagents (rCR)

What is the status of recombinant cascade reagents (rCR) in the Ph. Eur.?

rCR, an animal-free solution that also reduces dependence on natural resources, are not yet included in the Ph. Eur. To pave the way for their future consideration in the Ph. Eur., the European Pharmacopoeia Commission is encouraging data generation: 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.

Inclusion of rFC: 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 – see 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.

Inclusion of rFC: 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.

Thank you for your attention!

More information

www.edqm.eu

https://go.edqm.eu/Newsletter

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