Transition from LAL test for endotoxin to rFC methods

Animal Health Industry Perspective

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Context

- **Endotoxins**: heat–stable lipopolysaccharides in the outer membrane of Gram-negative bacteria
- Contaminant that can be found in:
  - Raw materials
  - Medical devices
  - Parenteral products: injectable pharmaceuticals, sterile solvent, vaccines, MAb…
- It can cause severe reactions in **humans and animals**: fever, inflammation, and even septic shock
- Endotoxin-free products are critical for safety across human and animal health sectors

History & Concerns

- Historical tests used rabbits to run a rabbit pyrogen test (RPT).
- Current tests use Limulus Amoebocyte Lysate (LAL) extracted from Horseshoe Crabs.
- Factor C in LAL triggers a cascade of reactions (coagulation) in presence of endotoxins allowing detection and measurement.
- However, the horseshoe crab population is declining up to a point where the species is endangered.

  More broadly, this destabilises the whole ecosystem bound to these animals.

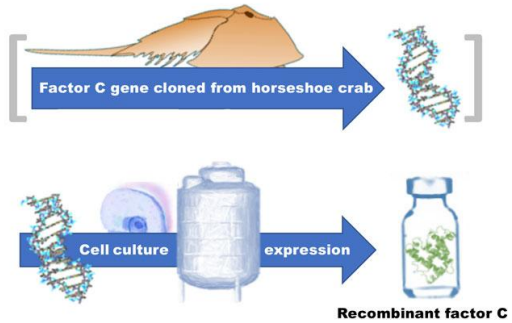
Source: The Declining Population of Horseshoe Crabs

History & Concerns

- **Case for change**
  - Ethical concerns : Pharmaceutical industry may have played a part in the decline in the horseshoe crab population
  - Supply at risk as relies on one endangered species only
  - Reliability of the test impacted by animal variability

Source: MAT Research - LAL test

- **Work on alternatives started more than two decades ago**
  - Non-animal-derived reagent recombinant Factor C (rFC)
  - More recently : Recombinant Cascade Reagent (rCr)



Source: Specificity in the recombinant factor C test for endotoxin

Regulatory landscape

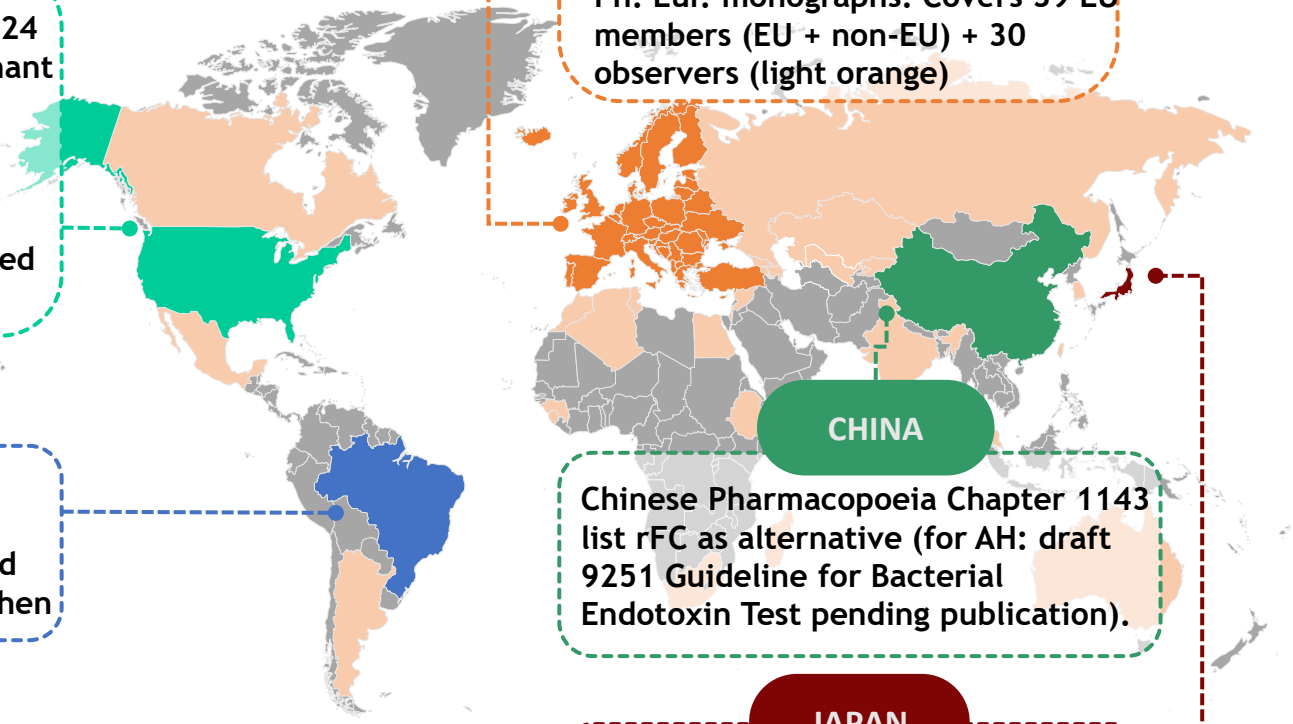
- EU: Compendial test
- Outside EU: Alternative to Compendial test
- Current regulatory context is rather favorable to implement non-animal alternatives and has been addressed by key markets
- Concern: The absence of harmonization across regions is presenting significant challenges for developing a coherent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

USA

USP Chapter <86> published 2024 includes rFC and rCR (recombinant Cascade Reagent).
No reference in individual USP monographs. Recombinant methods are still considered alternative and must be validated as such.

BRAZIL

CONCEA consultation done, rFC only mentioned. In AH, will need further endorsement by MAPA then



EUROPE

Ph. Eur. introduced rFC in 2015 with a dedicated monograph 2.6.32 published in 2021. Edition 11.8 (effective 01JUL2025) implemented 2.6.32 in individual Ph. Eur. monographs. Covers 39 EU members (EU + non-EU) + 30 observers (light orange)

CHINA

Chinese Pharmacopoeia Chapter 1143 list rFC as alternative (for AH: draft 9251 Guideline for Bacterial Endotoxin Test pending publication).

JAPAN

Japan Pharmacopeia 18th edition.

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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 by regulatory bodies globally, complicates choice of equipment and method.
- **Contract Manufacturing Organizations (CMO) constraints:**
  - Challenge for equipment standardization and planning.

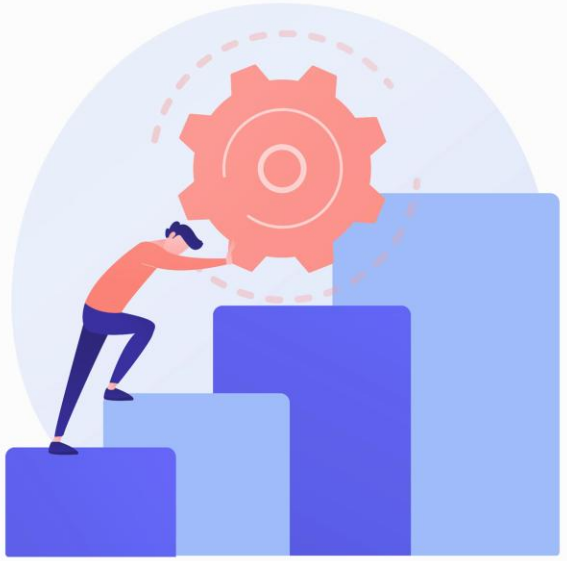
Industry transition - Validation Challenges

Significant validation effort required across regions

Equipment, validation methods & data may differ according to the regions.

Examples 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 consistent test results
- Raw materials: as many validations as suppliers



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

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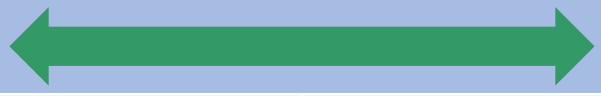
Validation Matrix

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: suitability confirmation	Full Method validation *(in theory)*	rFC alternative method
rCR alternative	Full method validation	Full method validation	rCR alternative method

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*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:**
  - Recent dossiers have anticipated and mentioned the possibility to use both Ph.Eur. 2.6.14 & 2.6.32.
  - Other cases: rFC alternative method may require full method validation. Requirements and acceptability for rFC need to be checked one by one with regulators internationally.

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

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 (API End of Shelf Life in FP method validation)?

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

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Conclusion

- Industry progressing toward recombinant reagent adoption for BET.
- Large scope, multi-year effort, with investment, training, SOP updates.
- Validation burden may be high; pragmatic range-based approach suggested.
- Burden due to dual testing.
- Regulatory clarity needed for rCR acceptance and compendia harmonization.

International collaboration across agencies for harmonized regulatory approach:

horseshoes crabs' population at stake

THANK YOU

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