BACTERIAL ENDOTOXIN TESTING: PROGRESSIVE SCIENCE AND SUSTAINABILITY

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DECEMBER 2025

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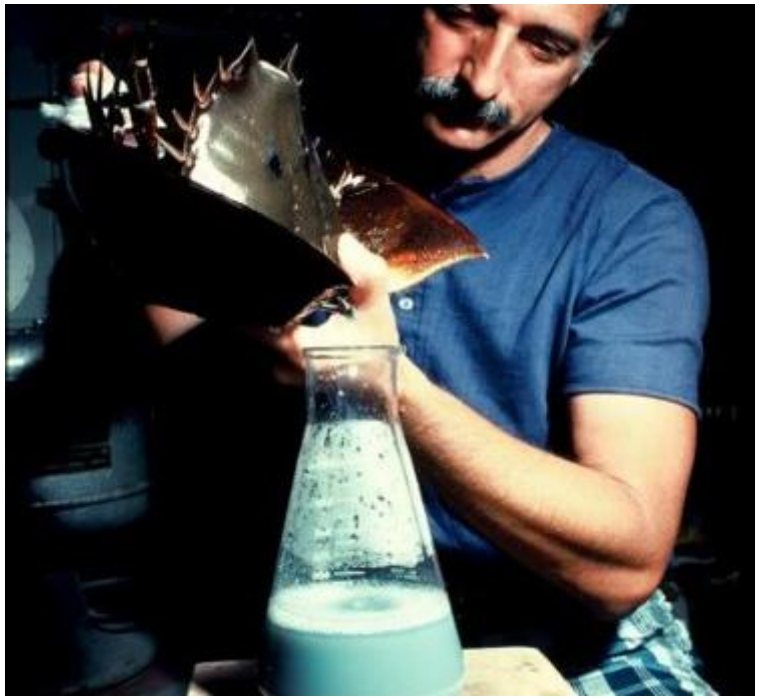
Jay Bolden is a member of the USP Microbiology Expert Committee. The views expressed are the author's alone and not those of USP, including the USP Microbiology Expert Committee.

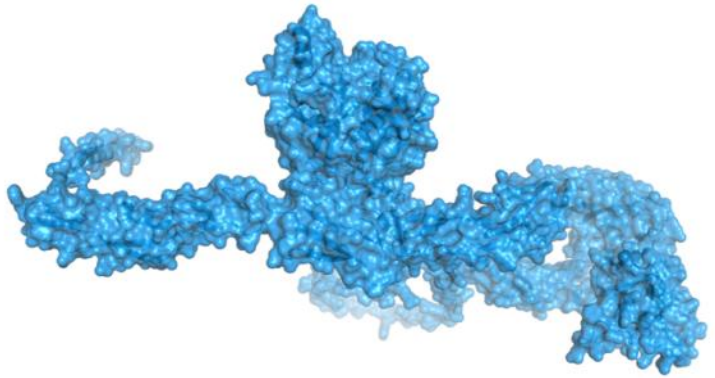
Evolution of Bacterial Endotoxins Testing

Rabbit Pyrogen Test



Bacterial Endotoxins Test: LAL & TAL

Image courtesy of Jack Levin

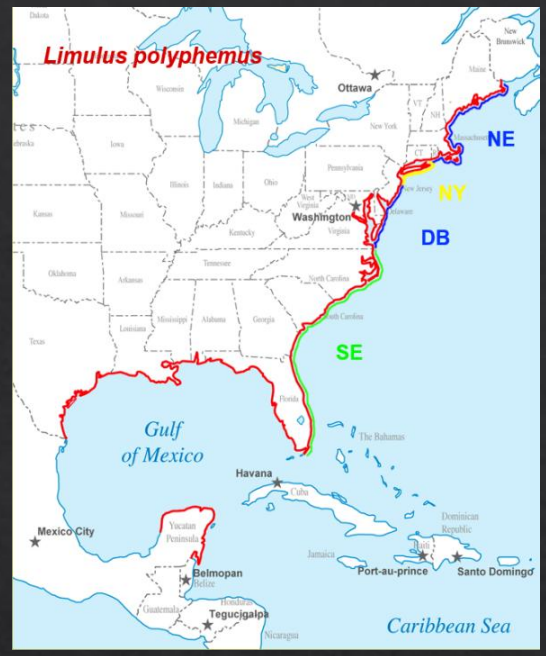


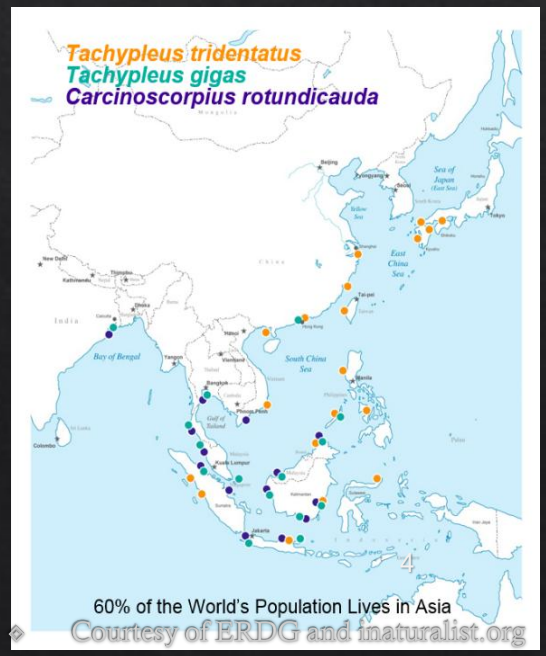
Recombinant Bacterial Endotoxins Test

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

Horseshoe Crab Distribution

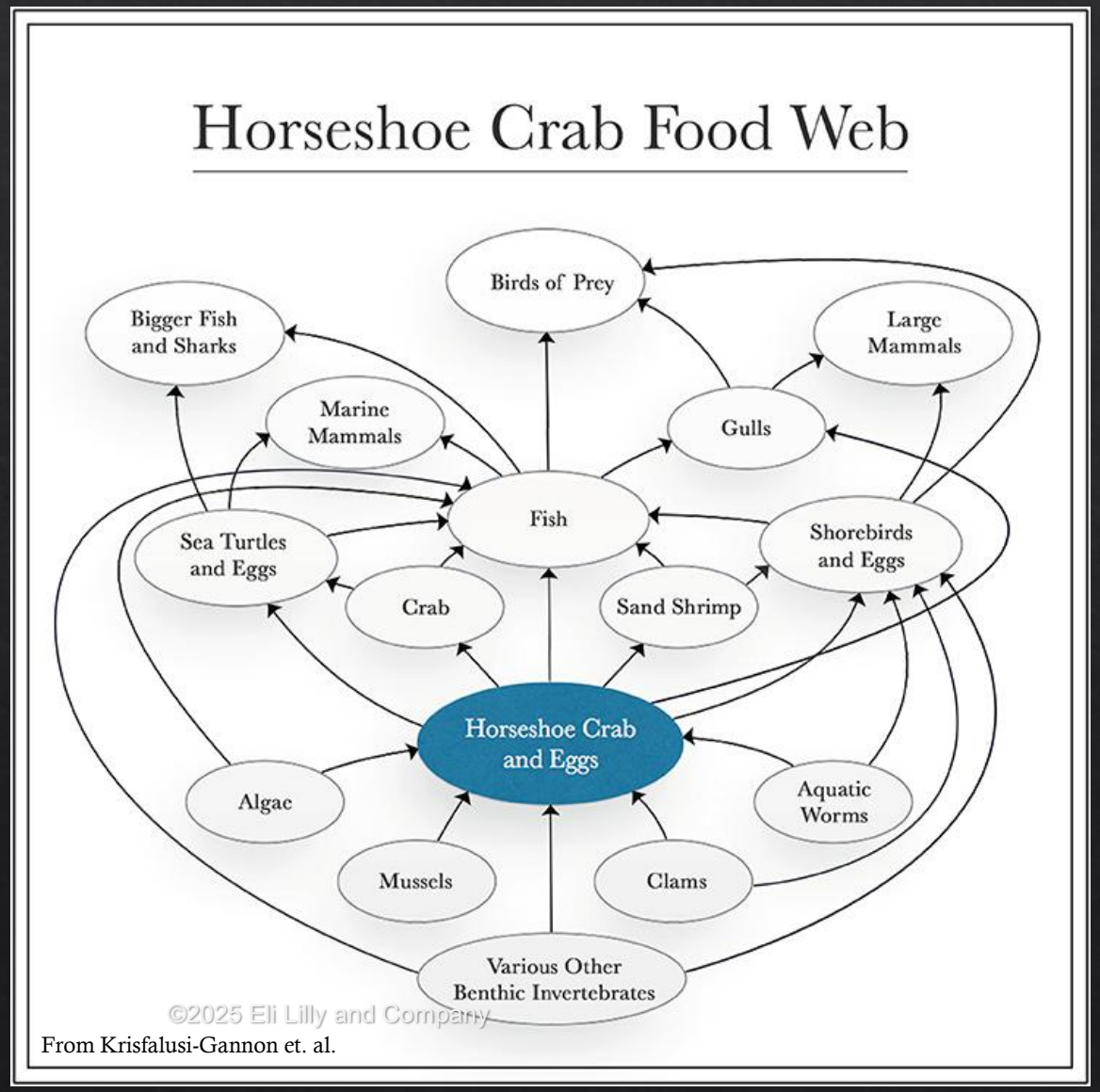
Genus / Species	Common Name	IUCN Status
Limulus polyphemus	American horseshoe crab	Vulnerable
Carcinoscorpius rotundicauda	Mangrove horseshoe crab	Data Deficient*
Tachypleus gigas	Indo-Pacific horseshoe crab	Data Deficient*
Tachypleus tridentatus	Tri-spine horseshoe crab	Endangered





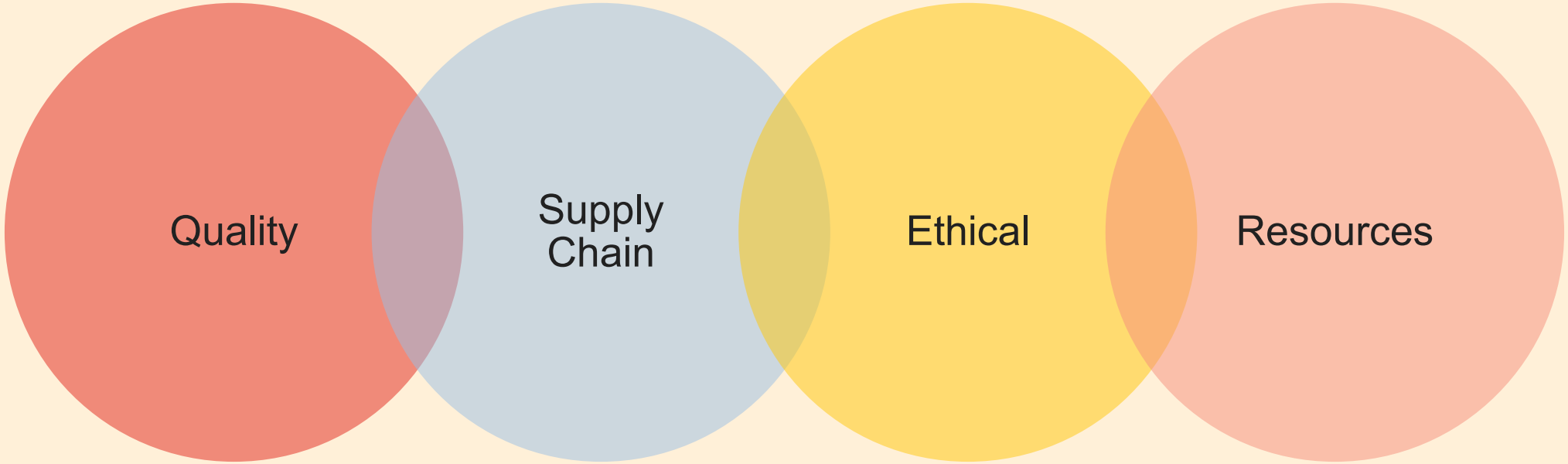
*2024-2025-iucn-ssc-horseshoe-crab-sg_publication_r2.pdf

Many Species Depend on Horseshoe Crabs



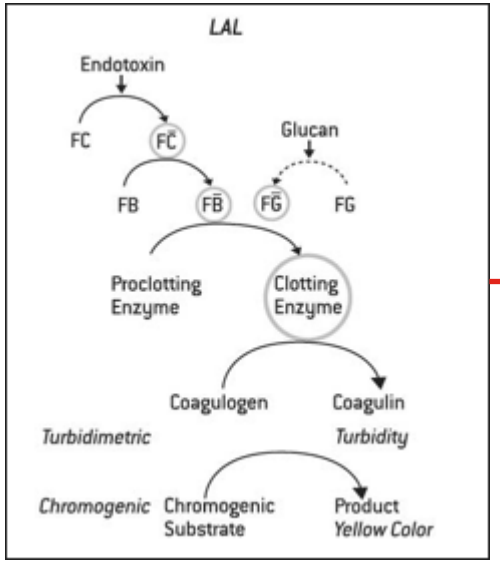
From Krisfalusi-Gannon et. al.

Change Rationale

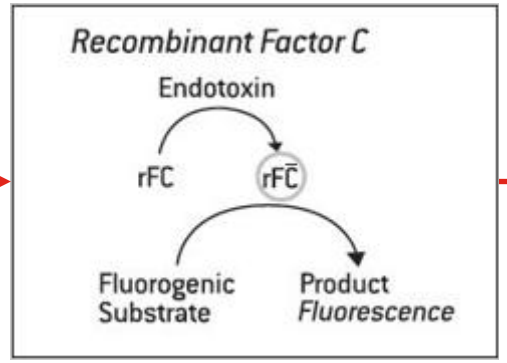


Recombinant Reagents are Biochemical Equivalents to LAL

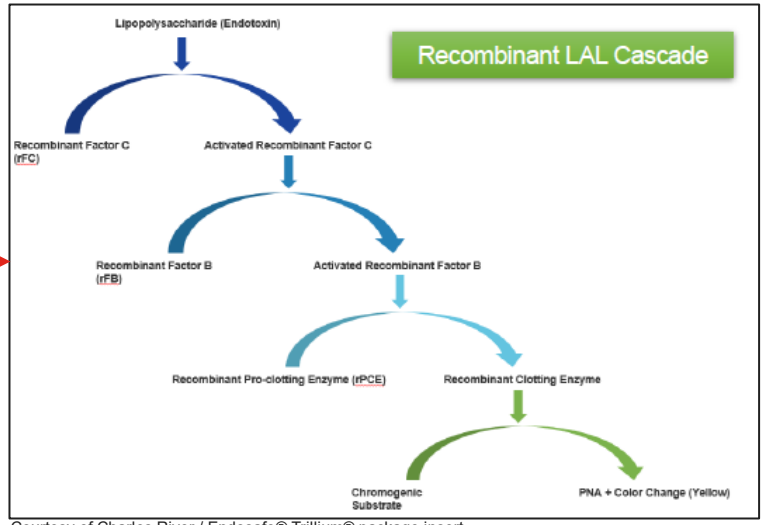
Mechanism of Action



Courtesy of Lonza PyroGene™ package insert



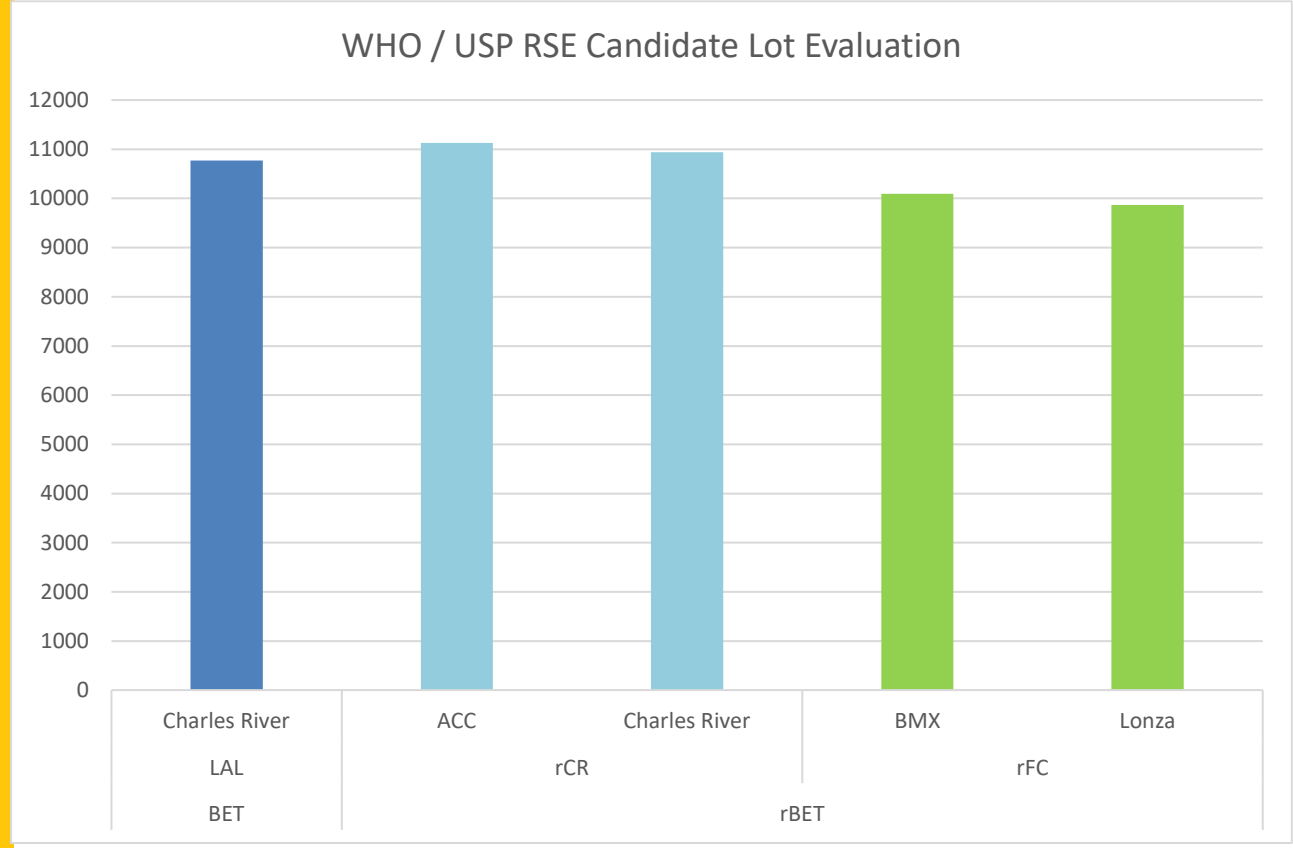
Courtesy of Lonza PyroGene™ package insert



Courtesy of Charles River / Endosafe® Trillium® package insert

Recombinant Comparability

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%

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
- rCR applications

Challenges

- Widespread use of Gel Clot LAL in developing countries
- Overcoming Technology Barriers
- Potential Supply Chain Impacts
- Compendial Harmonization
- Regulatory Pressure

Opportunities

- Cease use of TAL
- Reduce LAL use
- Implement rBET


PSCI

THE USE OF HORSESHOE CRABS IN THE PHARMACEUTICAL SECTOR

A position paper from the PSCI

Context and background

- Converge Compendial Methods → de facto Harmonization
- Regulatory Innovation and Consistency

Real World Impacts

Anti-smuggling operation in October 2019, the Riau Police successfully intercepted a smuggling attempt of 1,500 horseshoe crabs

Horseshoe crabs from Palembang, South Sumatra to Medan, North Sumatra in February 2020.

460 *Tachypleus gigas* were confiscated from the local fisherman in Medan by special crime branch department in March 2020

"Global Trade Data and Conservation Challenges for Asian Horseshoe Crabs"
Akbar John, IUCN Horseshoe Crab SSC 2024

WARNING LETTER

Amneal Pharmaceuticals, LLC

MARCS-CMS 709894 — AUGUST 27, 2025

2. Your firm failed to establish laboratory controls that include scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity (21 CFR 211.160(b)).

During our inspection, we observed the preparation and reading of bacterial endotoxin tests and identified numerous deficiencies:

- Failure to completely discharge the micropipette solution during preparation of water for injection samples, which may compromise the accuracy and reproducibility of the test results.
- Transfer of gel-clot sample tubes from the incubator to the waste container in a single rapid motion during microbiological analysis, without the crucial pause required to inspect the firmness of the gel for endotoxin detection.
- The analyst responsible for the **(b)(4)** verification of the gel-clot assessment was unable to adequately observe or verify the process due to the primary microbiologist's rapid assessment. Despite this basic deficiency, the secondary analyst signed off on the verification.
- Inadequate documentation practices when recording sample results of bacterial endotoxin tests for water for injection, including non-contemporaneous documenting of negative sample results and assigning a passing result to a positive control sample prior to the actual observation and confirmation of the result.

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

What is in your control to change?