BACTERIAL ENDOTOXIN TESTING: PROGRESSIVE SCIENCE AND SUSTAINABILITY

JANUARY 2025

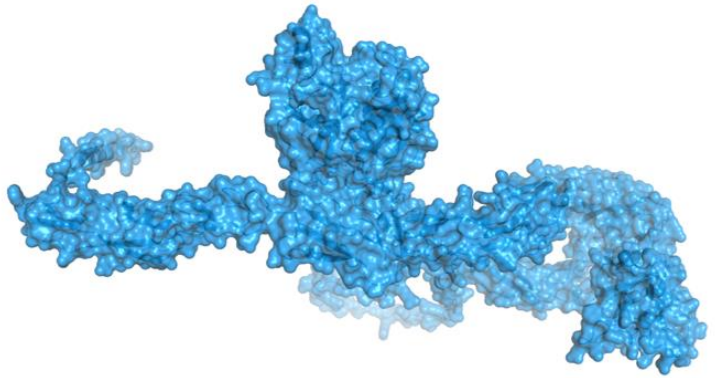
Lilly

Evolution of Bacterial Endotoxins Testing

Rabbit Pyrogen Test

Bacterial Endotoxins Test: LAL & TAL

Image courtesy of Jack Levin

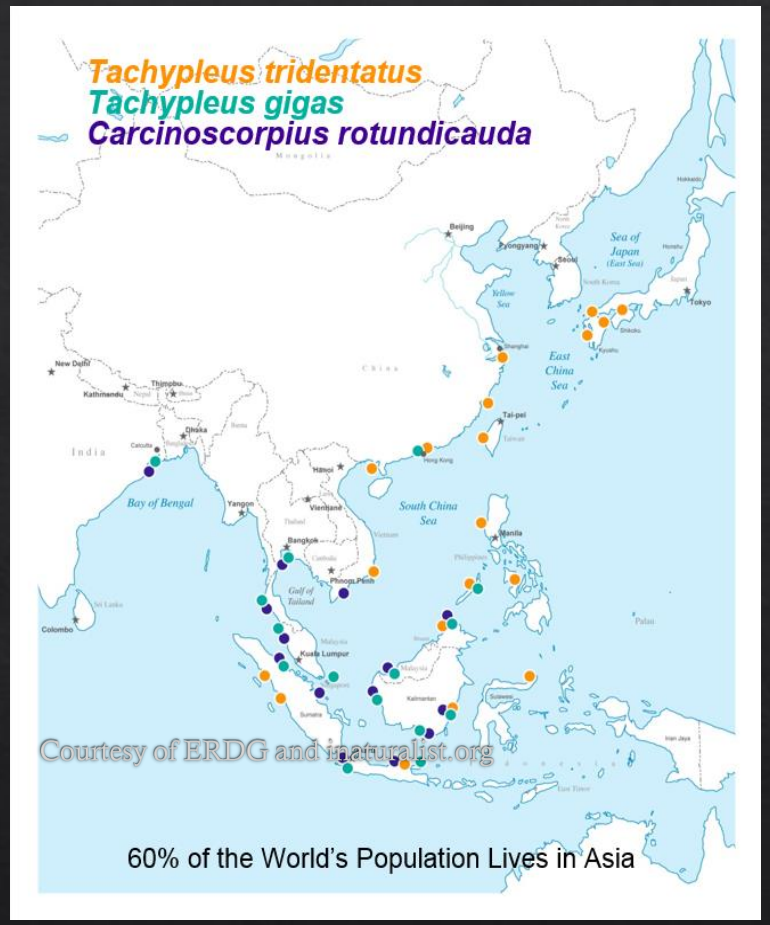


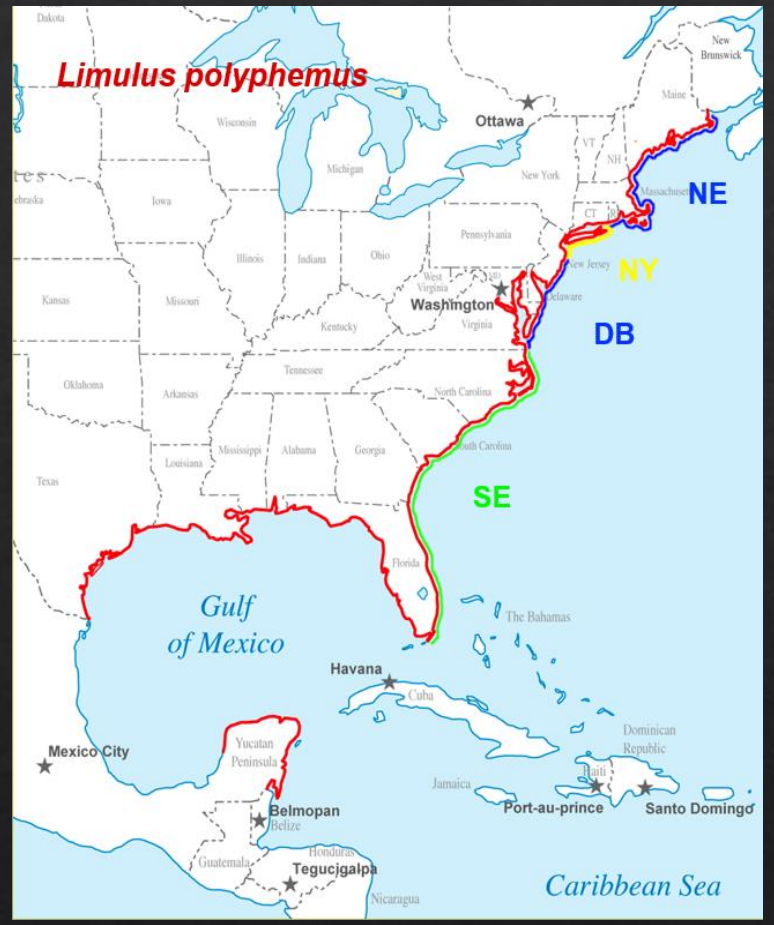
Recombinant Bacterial Endotoxins Test

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

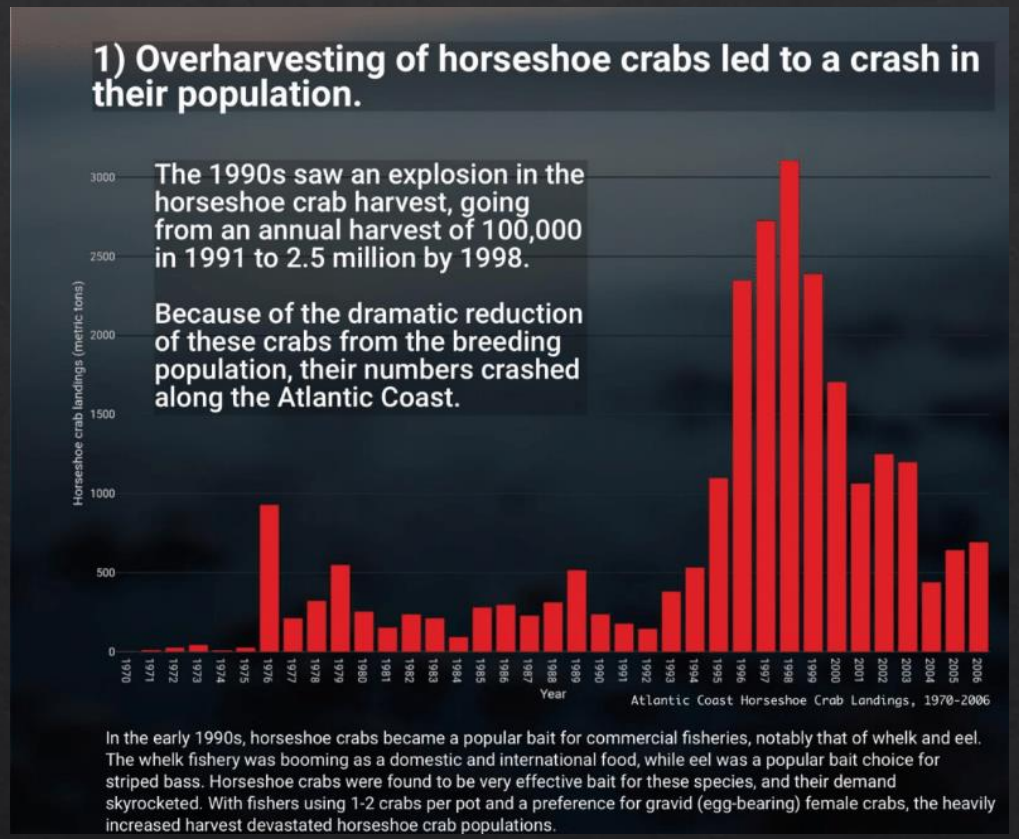
Horseshoe Crab Distribution

- *Carcinoscorpius rotundicauda*, the mangrove horseshoe crab
- *Limulus polyphemus*, the Atlantic or American horseshoe crab
- *Tachypleus gigas*, the Indo-Pacific, Indonesian, Indian or southern horseshoe crab
- *Tachypleus tridentatus*, the Chinese, Japanese or tri-spine horseshoe crab





The Limulus Crash



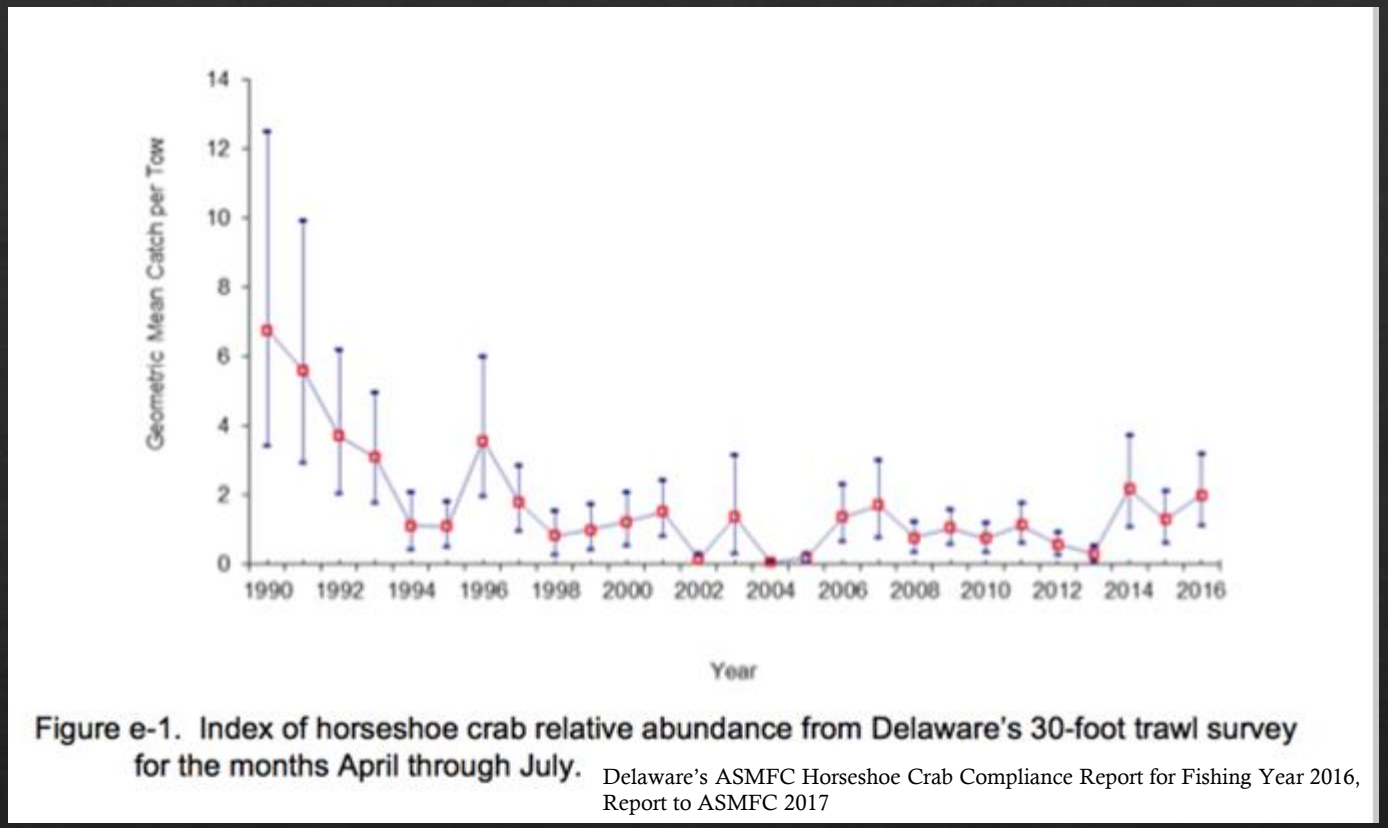
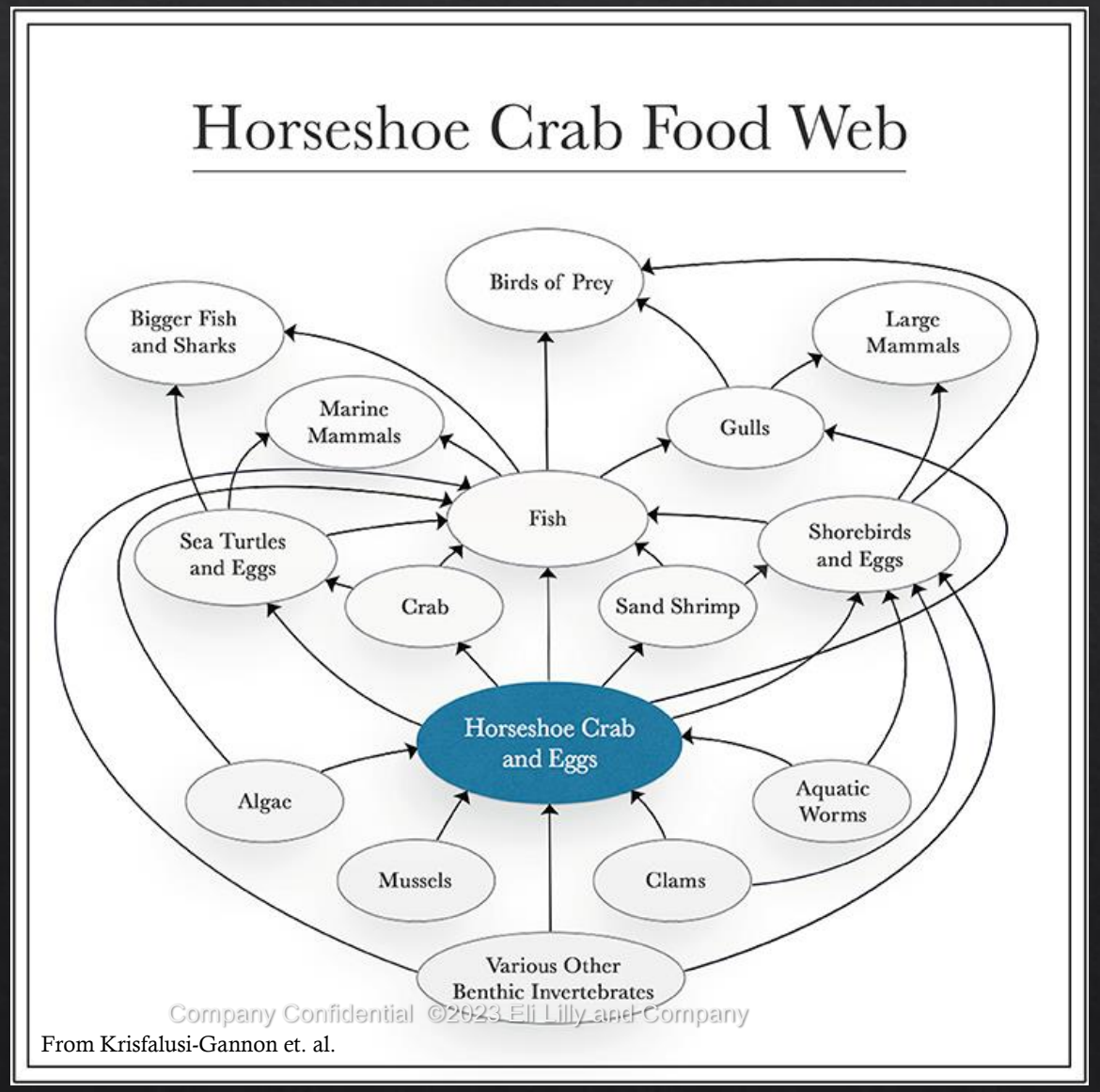


Figure e-1. Index of horseshoe crab relative abundance from Delaware's 30-foot trawl survey for the months April through July.

Delaware's ASMFC Horseshoe Crab Compliance Report for Fishing Year 2016, Report to ASMFC 2017

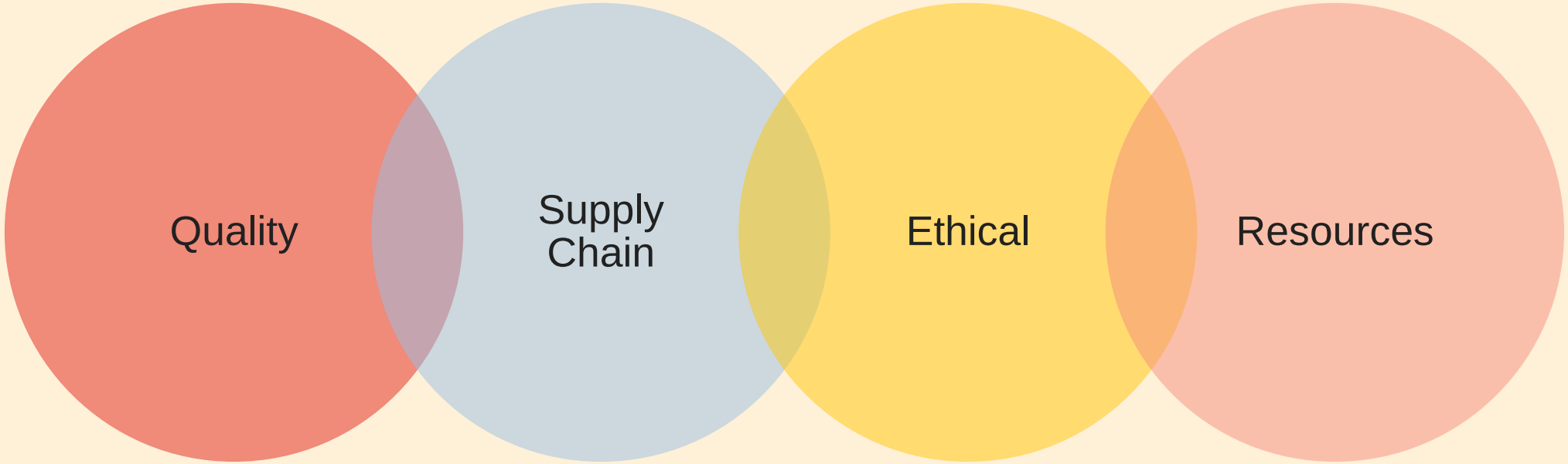
- The collapse of the Delaware Bay Horseshoe crab population in the late 1990's created an unprecedented corresponding collapse of shore bird populations which ultimately precipitated the Federal Listing of the Red Knot.
- By 2002 Horseshoe Crab numbers fell to historic lows

Shorebirds Depend on Horseshoe Crabs



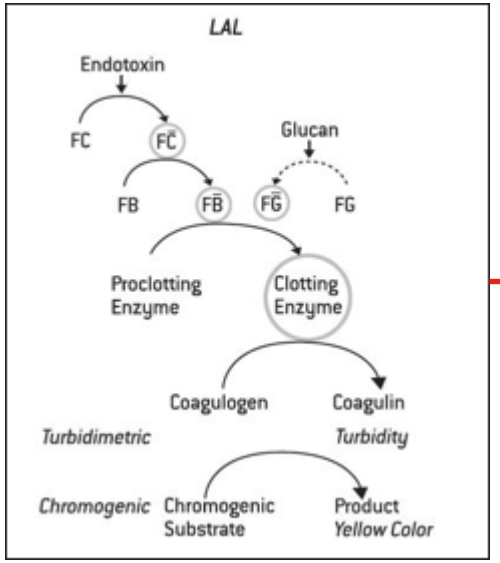
From Krisfalusi-Gannon et. al.

Change Rationale

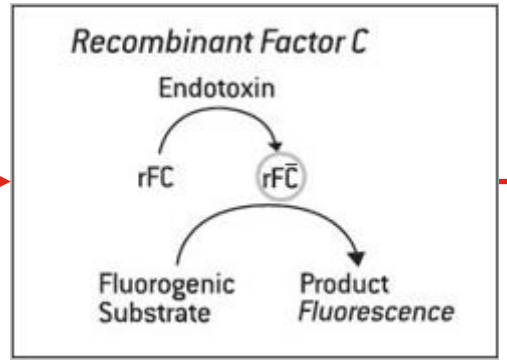


Recombinant Reagents are Biochemical Equivalents to LAL

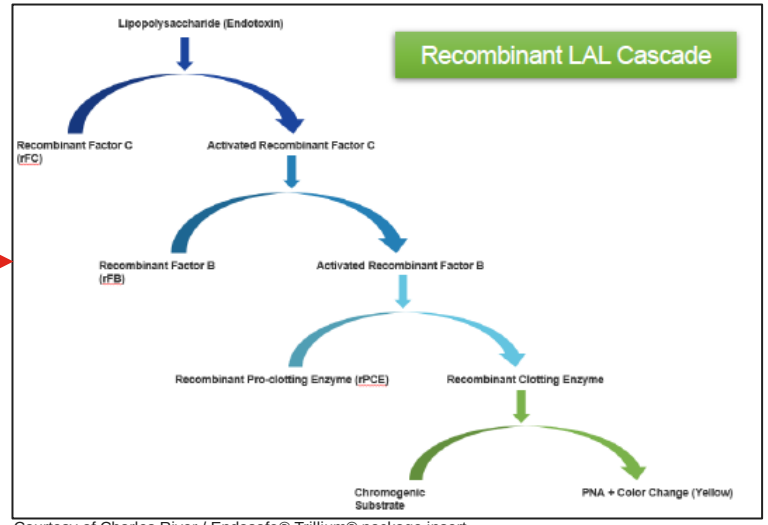
Mechanism of Action



Courtesy of Lonza PyroGene™ package insert

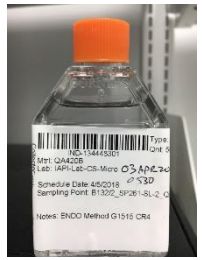


Courtesy of Lonza PyroGene™ package insert



Courtesy of Charles River / Endosafe® Trillium® package insert

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

2024:

- USP <86>
- Strive for full conversion
- Automation

rFC Equivalency: 2020 PDA Literature Review

- 14 peer reviewed studies from 2010 to 2018
- 1087 sample containing real world endotoxin
- 213 different relevant pharmaceutical products in 7 categories:
  - Large molecule/Peptide
  - Small molecule
  - Vaccine
  - Excipient / raw material
  - Container component
  - Device
  - Buffer / Water
  - Clinical
  - Plant Extract

❖LAL and rFC are comparable for endotoxin detection

❖rFC is superior to LAL for endotoxin specificity

Jay Bolden, Chris Knutsen, Jack Levin, Catherine Milne, Tina Morris, Ned Mozier, Ingo Spreitzer, Friedrich von Wintzingerode. Currently available recombinant alternatives to horseshoe crab blood lysates: Are they comparable for the detection of environmental bacterial endotoxins? A review. 2020. PDA Journal of Pharmaceutical Science and Technology, Volume 74, Issue 5, Pages 602-611

Environmental Endotoxin	Type	Source
3	Live Bacteria/Pharma	Bolden 2017
13	Pharma	Mozier
6	Environment	Kikuchi 2017
20	Environment	McKenzie
5	Pharma	Schwarz
912	Environment	Thorne
60	Environment	Alwis
1	Pharma	Marius
2	Pharma	Bolden USP
65	Clinical	Strachan
1087		

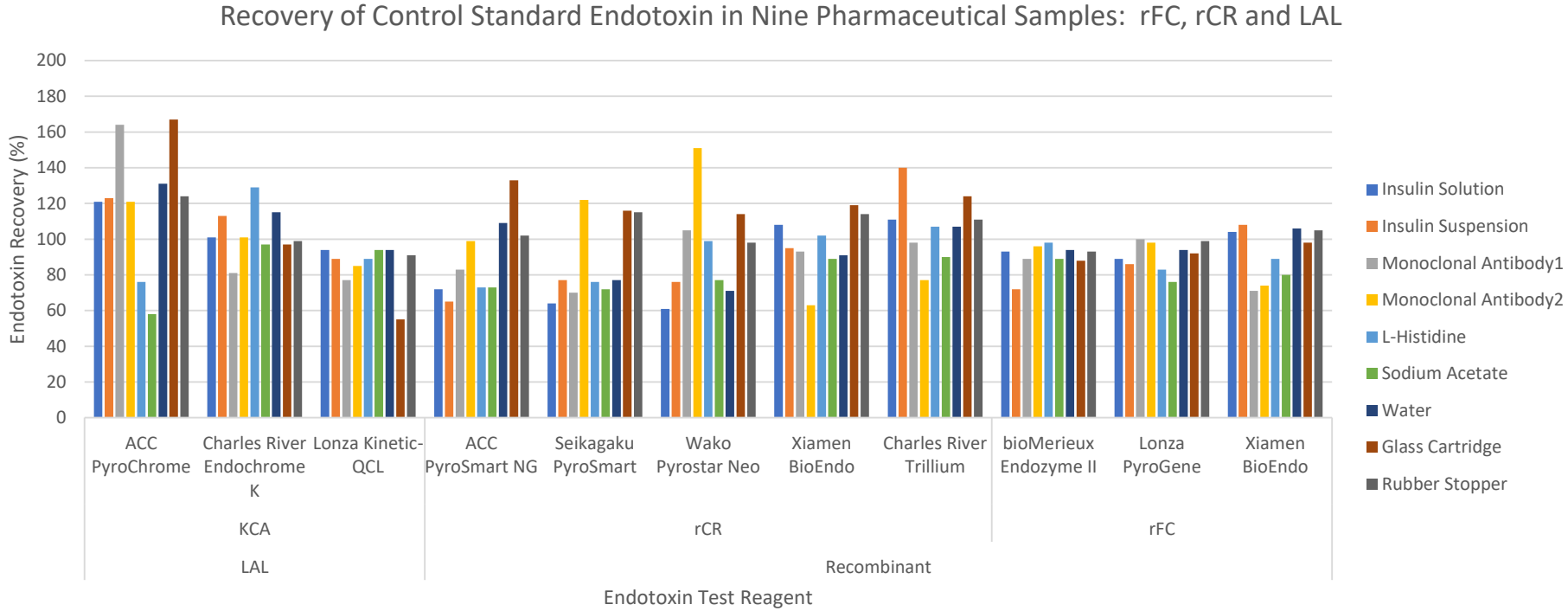
Recombinant Comparability

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



	Average of %PPC
LAL	**105**
KCA	**105**
ACC PyroChrome	121
Charles River Endochrome K	106
Lonza Kinetic-QCL	90
Recombinant	**93**
rCR	**94**
ACC PyroSmart NG	91
Seikagaku PyroSmart	88
Wako Pyrostar Neo	91
Xiamen BioEndo	95
Charles River Trillium	107
rFC	**90**
bioMerieux Endozyme II	89
Lonza PyroGene	89
Xiamen BioEndo	93
Grand Total	**96**

- 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

Compendial Status - Global

Compendial Method

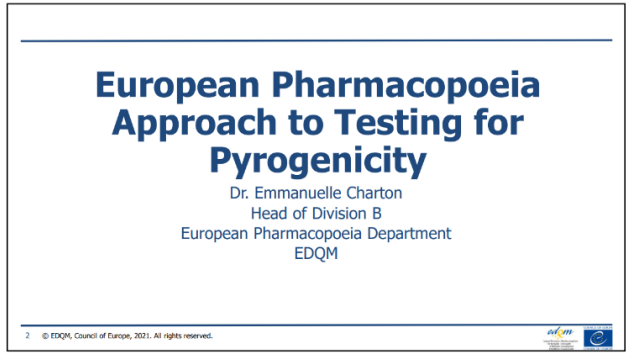
- 2020: Ph.Eur. 2.6.32
- 2022: EAEU (proposed)
- 2024: BP XIV C
- 2024: USP <86>

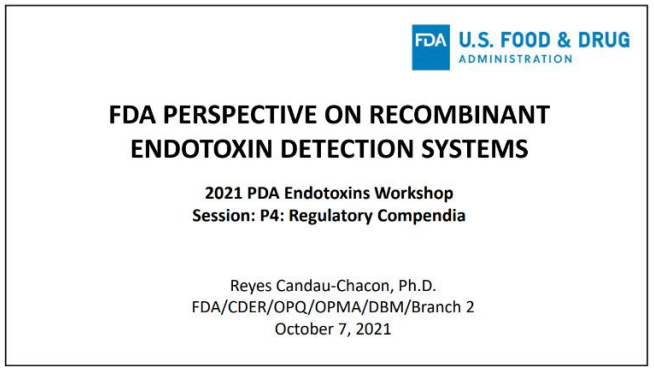
Compendial Monograph

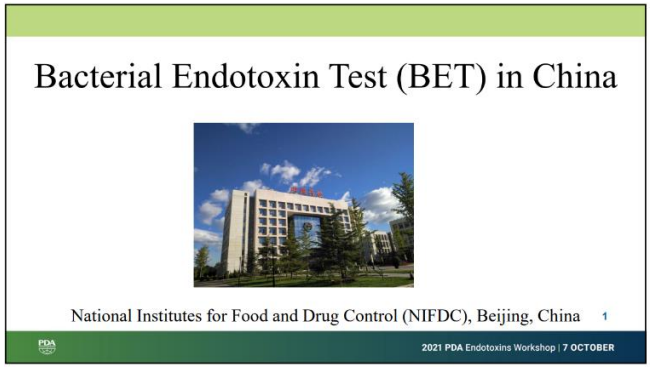
- 2023: Ph.Eur. WFI

Compendia Guidance Chapter

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







USP <86>

2019

- Proposed revision to <85> to include recombinant reagents

2020

- Proposed revision withdrawn based on stakeholder feedback
- Proposed guidance chapter <1085.1>

2021

- Published <1085.1> stakeholder feedback
- Proposed USP comparability study

2023

- New USP Microbiology Expert Committee
- Proposed <86> rBET

2024

- Announced approval of <86> and early adoption date for **01-Nov-2024**
- Proposed revision to <1085>

Challenges

Widespread use of Gel Clot LAL in developing countries

Overcoming Technology Barriers

Potential Supply Chain Impacts

Compendial Harmonization

Regulatory Pressure

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

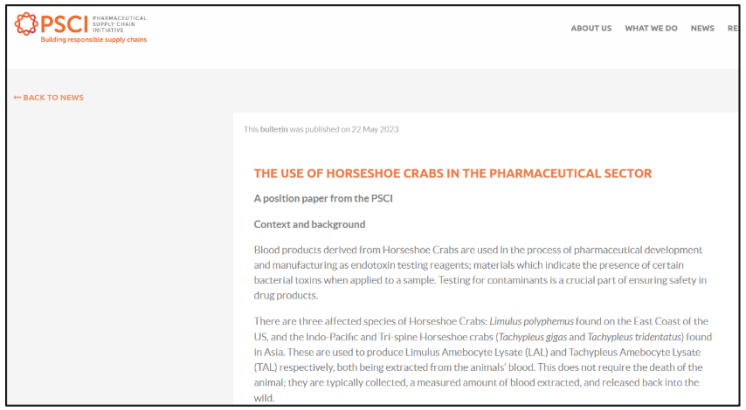
"Global Trade Data and Conservation Challenges for Asian Horseshoe Crabs" Akbar John, IUCN Horseshoe Crab SSC 2024

Opportunities

Cease use of TAL

Reduce LAL use

Implement rBET



Compendial Methods → de facto Harmonization

Regulatory Innovation and Consistency

Courtesy of Charles River

What is in your control to change?