




Bacterial Endotoxins Testing – Progressive Science and Sustainability

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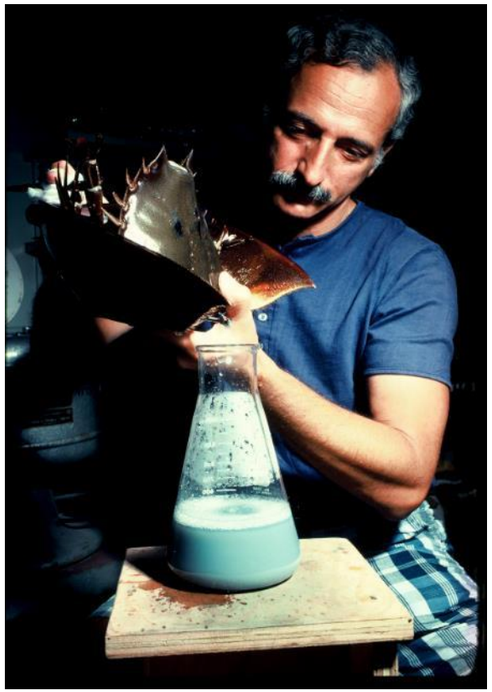
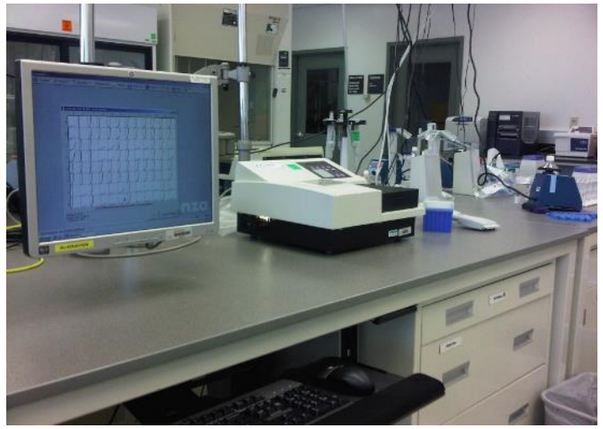
Disclosure

- Disclosures: 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.

BACKGROUND

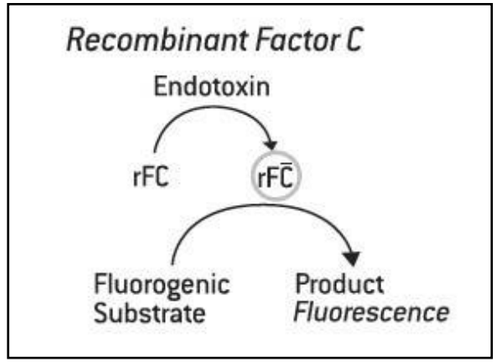
Evolution of Pyrogen Testing

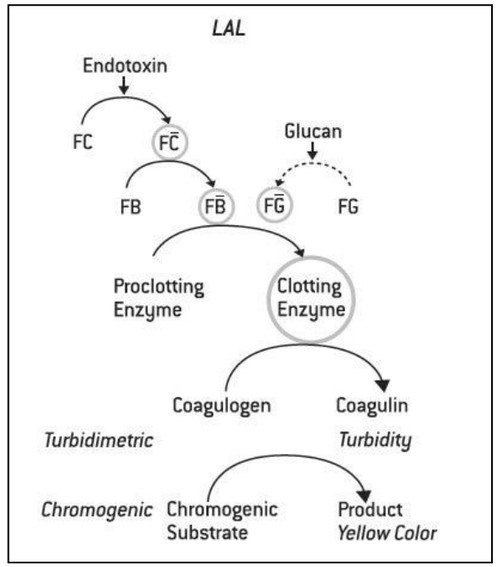




RECOMBINANT FACTOR C

What is rFC?





Diagrams courtesy of Lonza PyroGene™ package insert

- Factor C is a natural horseshoe crab blood protein: a biosensor of bacterial endotoxins, the activation of which is the mechanism of action in the bacterial endotoxins test (BET) using LAL
- recombinant Factor C (rFC) is the biotechnology-manufactured version of natural Factor C and relies on the same mechanism of action
- rFC is more specific for bacterial endotoxins because it bypasses the factor G glucan false positive pathway

Change Rationale: Benefits

Impact	Benefit
Quality	• Improved assay performance • Opportunity to align global sites • Not sensitive to Factor G false positive pathway compared to LAL
Supply Chain	• Proactive steps against potential reagent shortages and subsequent impact to our ability to test and release our products to the market

Ethical	• Replaces the use of an animal-derived reagent, consistent with the 3Rs principle to Replace-Reduce-Refine
	• Aligned with
	  • Lilly Corporate Animal Care and Use Principle
	  • ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)
	  • European Union Directive 2010/63/EU

Cost	• Waste factor reduction • Low regulatory impact testing (water, etc.) constitutes largest test volume (80-90%)

The Road to Implementation

History

1997 – Patented
2003 – Commercially Available
2006 – Lilly Evaluation

Success

2018 – First product approval

2020 – 2nd product approval; 3 COVID-19 antibodies; Tri-spine HSC declared endangered; Ph.Eur. 2.6.32

Groundwork

2012-2013: New Lilly China plant; FDA Guideline; USFWS lists Red Knot; 2nd rFC supplier

By 2016: generated evaluation data, validated method, drew a line in the sand

The Future

Proposed USP <86>

Convert remaining 20%

rCRs

rFC Equivalency: Literature

- 2020 PDA Literature Review:
  - 14 peer reviewed studies from 2010 to 2018
  - 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
  - 1087 sample containing real world endotoxin

❖ LAL and rFC are comparable for endotoxin detection

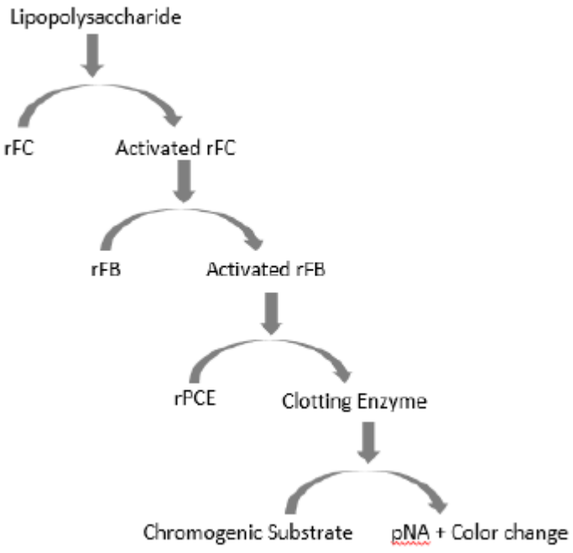
❖ rFC is superior to LAL for endotoxin specificity

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		

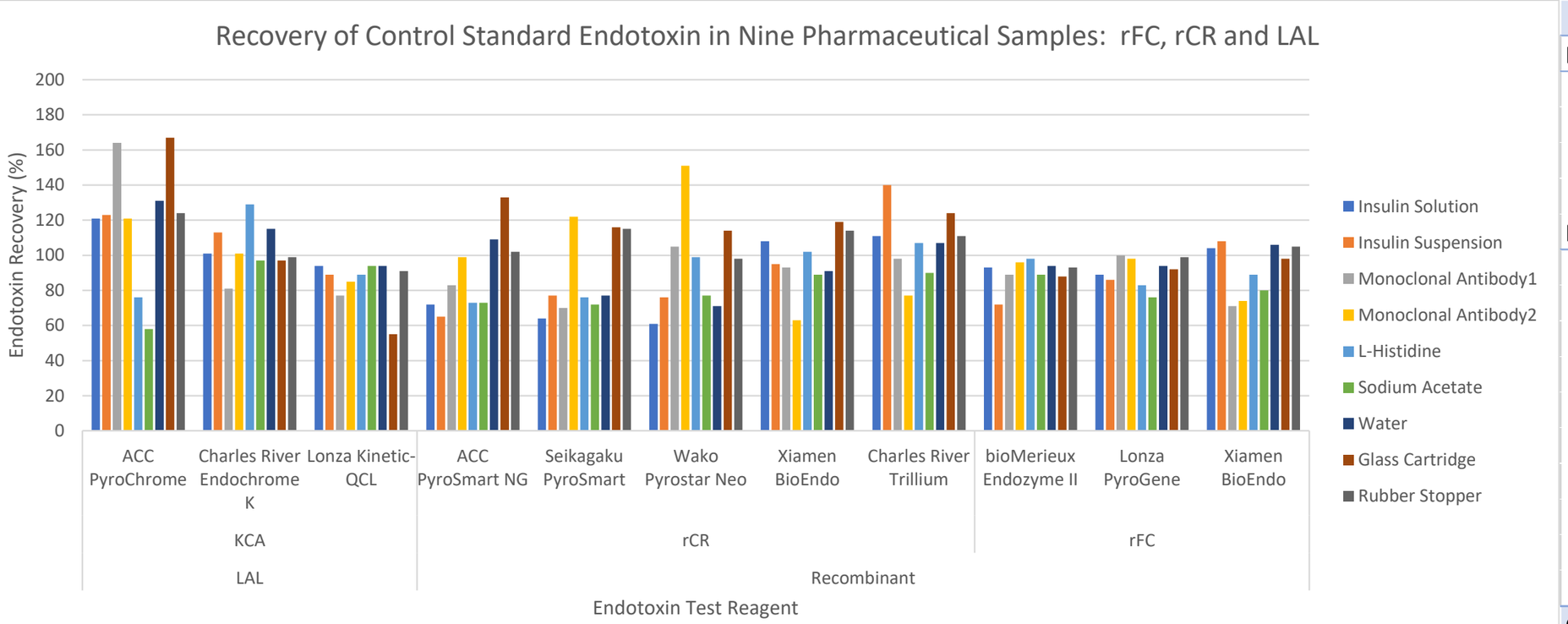
rCR

- Recombinant Cascade Reagent
  - recombinant Factor C + recombinant Factor B + recombinant proclotting enzyme
- More limited public comparability data set
- 4 commercially available + 1 known in development
- Internal evaluation: LAL, rFC and rCR are broadly equivalent for accuracy and detection limits



Courtesy of Endosafe® Trillium® package insert

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

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%

External Activities

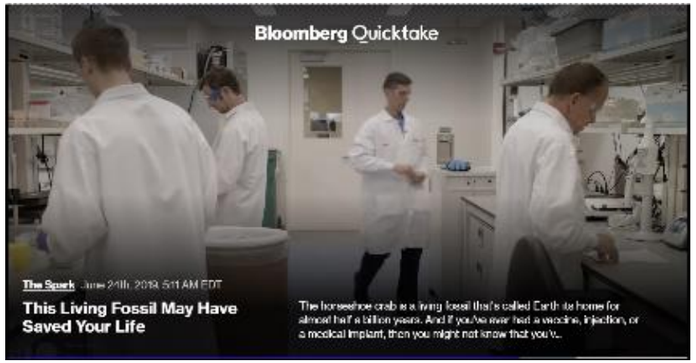
- Presentations:
  - Lonza Endotoxin Summit (May 2016)
  - Parenteral Drug Association Microbiology (US – Oct 2016)
  - Pharmaceutical Microbiology Forum Bacterial Endotoxin Summit (Feb 2017)
  - Parenteral Drug Association Microbiology (EU – Feb 2017)
  - Lonza Endotoxin Summit (June 2018)
  - Parenteral Drug Association Endotoxin Workshop (Oct 2018)
  - Lonza Endotoxin Summit (June 2019)
  - United States Pharmacopoeia Endotoxin Workshop (June 2019)
  - United States Congressional Briefing (Oct 2020)
  - Parenteral Drug Association Microbiology (Oct 2021)
  - United States Pharmacopoeia Endotoxin Workshop (Nov 2021)
  - CASSS/WBCP (Jan 2022)
  - BPOG Sustainability (Jun 2022)
  - PSCI Annual General Meeting (Jul 2022)
  - Korean MFDS and KoBIA Trade Association (Oct 2022)
  - PDA New England Chapter (Dec 2022)
  - FDA Seminar (Dec 2022)
  - PDA Micro poster (Oct 2023)
  - PDA APAC Regulatory (Nov 2023)

- Peer-reviewed Literature
  - Bolden, Jay S, and Kelly R Smith. 2017. "Application of Recombinant Factor C Reagent for the Detection of Bacterial Endotoxins in Pharmaceutical Products." PDA J Pharm Sci and Tech (71): 405-412.
  - Bolden, Jay S, Michael Knight, Share Stockman, and Bastian Omokoko. 2017. "Results of a harmonized endotoxin recovery study protocol evaluation by 14 BioPhorum Operations Group (BPOG) member companies." Biologicals 48: 74-81.
  - Bolden, Jay S, Mark E Claerbout, Matthew K Miner, Marie A Murphy, Kelly R Smith, and Rob E Warburton. 2014. "Evidence against a bacterial endotoxin masking effect in biologic drug products by limulus amebocyte lysate detection." PDA J Pharm Sci and Tech 68 (5): 472-477.
  - Chapter 13: Recombinant Factor C. Endotoxin Detection and Control in Pharma, Limulus, and Mammalian Systems. Kevin Williams, ed. 2019. Springer Publishing, ISBN 978-3-030-17148-3
  - Bolden, Jay. “Application of Recombinant Factor C Reagent for the Detection of Bacterial Endotoxins in Pharmaceutical Products and Comparability to Limulus Amebocyte Lysate” USP Pharmacopoea Forum. 46(3), 2020.
  - Bolden, Jay; Knutsen, Chris; Levin, Jack; Milne, Catherine; Morris, Tina; Mozier, Ned; Spreitzer, Ingo; von Wintzingerode, Friedrich; Currently available recombinant alternatives to horseshoe crab blood lysates: Are they comparable for the detection of environmental bacterial endotoxins? A Review. PDA Journal of Pharmaceutical Science and Technology. 74 (5): 602-611
  - Baker E, Ponder J, Oberdorfer J, Spreitzer I, Bolden J, Marius M, Bonnevay T, Sullivan K. Barriers to the Use of Recombinant Bacterial Endotoxins Test Methods in Parenteral Drug, Vaccine and Device Safety Testing. Altern Lab Anim. 2023 Oct 19:2611929231204782. doi: 10.1177/02611929231204782. Epub ahead of print. PMID: 37855095.

External Media Attention

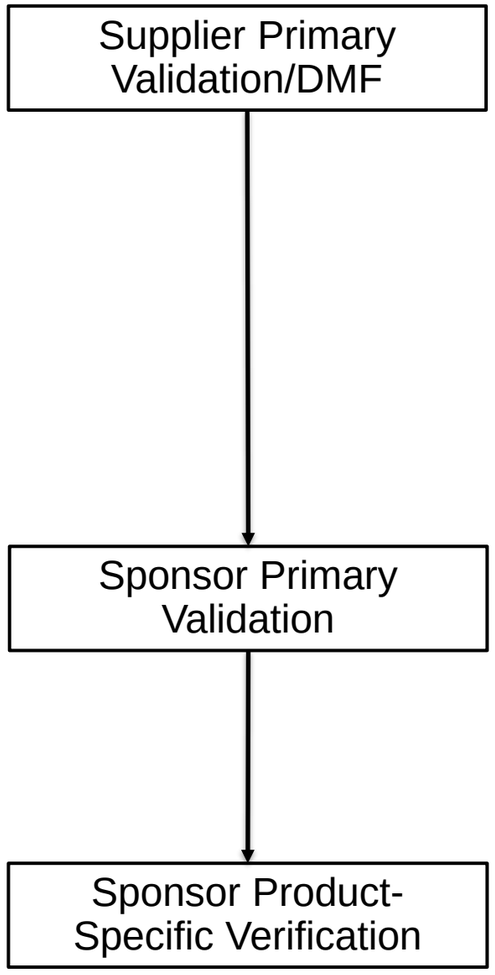
The Atlantic (May 2018)
National Audubon (May 2018)
Revive and Restore/NJ Audubon Press Event (May 2018)
Hakai Magazine (May 2018)
Radiolab – NPR (July 2018)
BirdWatching/South Carolina Wildlife Magazines (Sep 2018)
Ensia Magazine (Sep 2018)
PDA Letter (Dec 2018)
Top of Mind with Julie Rose, BYU Radio (Dec 2018)
First, Do No Harm Podcast (Feb 2018)
Constant Wonder, BYU Radio (Apr 2019)
Phil Forester, independent film maker (Apr 2019)
NY Times, John Hurdle, Science Page (May 2019)
Problem Solved, Bloomberg Media (May 2019)
Lilly UN SDG Report (June 2019)
Reuters, John Miller (June 2019)
The Baltimore Sun, Maddy Lauria (June 2019)
The Weather Channel, Nicole Bonaccorso (July 2019)
Reuters, John Miller (June 2020)
NJ Spotlight, John Hurdle (June 2020)
Pharmalot, Ed Silverman (June 2020)
NY Times, Jim Gorman (June 2020)

Lilly UN SDG Report (June 2020)
Smithsonian Magazine (June 2020)
Miami Herald (June 2020)
Québec Science (Sep 2020)
U.S. Congressional Briefing (Oct 2020)
National Audubon (Nov 2020)
Canadian Broadcasting The Current, Matt Galloway (Dec 2020)
E&E News, Ariel Wittenberg (Jan 2020)
NJ Advance Media, Michael Warrant (Jan 2020)
The State and The State Chiara Eisner (Feb/May 2021)
Lilly UN ESG Report (May 2021)
The Point – WACI, Mindy Todd (June 2021)
Washington Post – Caren Chesler (Aug 2021)
National Geographic – Dina Maron (Aug 2022)
The Economist – Rebecca Jackson (Aug 2022)
NY Times – Deborah Cramer (Feb 2023)
Long Now Talk – Ryan Phelan (April 2023)
NPR Weekend Edition – Chiara Eisner (June 2023)
Financial Times – Jamie Smythe (Aug 2023)
Medpage – Kristina Fiore (Sep 2023)
IamBIO Podcast and Bio.News-Theresa Brady and Tom Popper (Sep 2023)
Philadelphia Magazine – Mike Weilbacher (May 2024)

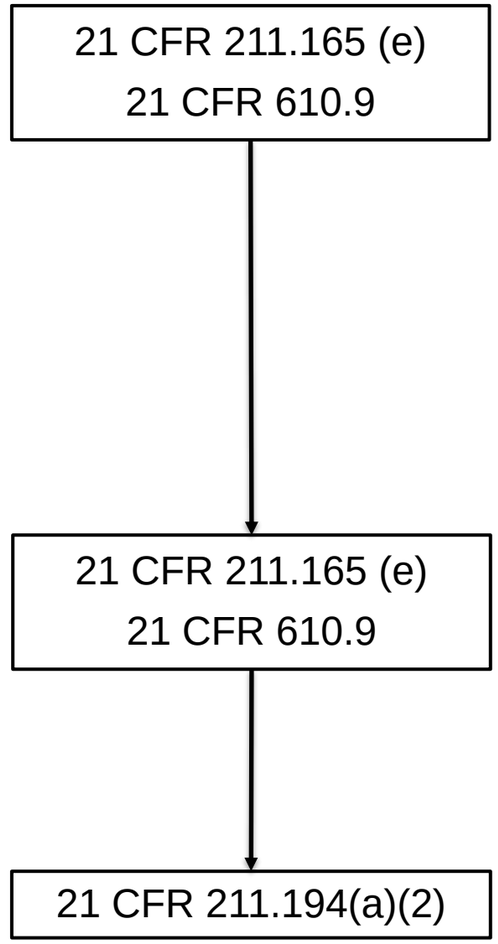


REGULATORY

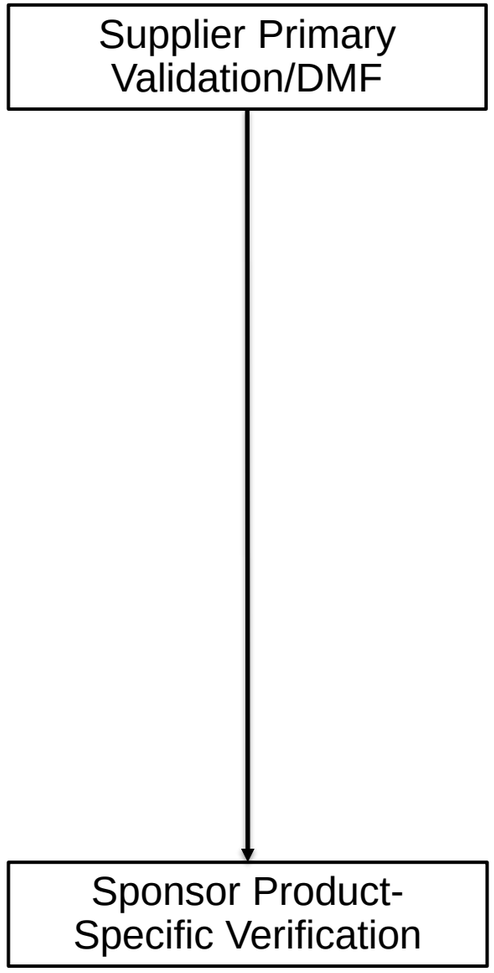
Alternative Method Validation <1225>



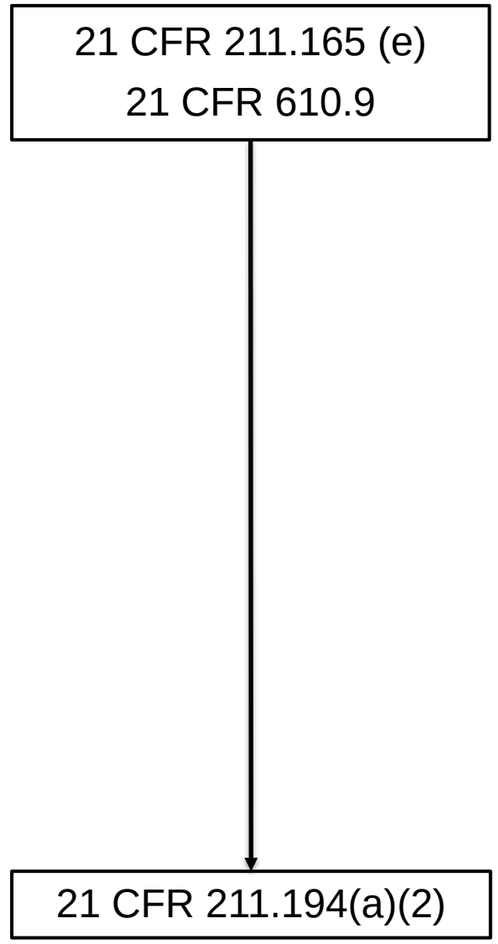
1. Lonza published a Stimuli to the Revision Process in the USP Pharmacopoeia Forum 36(1) demonstrating equivalency and/or superiority to the existing USP kinetic methods per USP 1225
2. Lilly performed a non-product (water) validation using both rFC suppliers + equivalency
3. Lilly executed 60+ more validations prior to switching to verification



Alternative Method Verification <1226>



1. A compendial method should remove the need for an end-user primary non-product specific validation
2. Level playing field: recombinant data package = LAL data package
3. Understand your compendia, e.g. USP General Notices, <1225>, <1226>, etc.

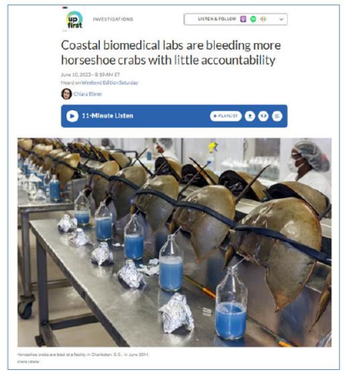


Regulatory Status: rFC

- 9 Lilly products approved
  - FDA CDER and 65+ health authorities
- 1 known medical device approved – Xtant medical via FDA CDRH
- FDA recombinant reagent characterization presentations (2022, 2023)

2023 ISPE Biotechnology Conference | 26 – 27 June 2023
Dublin, Ireland and Virtual

Status quo for sourcing LAL is not a secure position



State of competing priorities and interests

- Harvest/husbandry regulations exist though limited, ambiguous authorities
- Implementation varied
- Industry expanding
- Compliance inconsistent
- Enforcement challenging

5

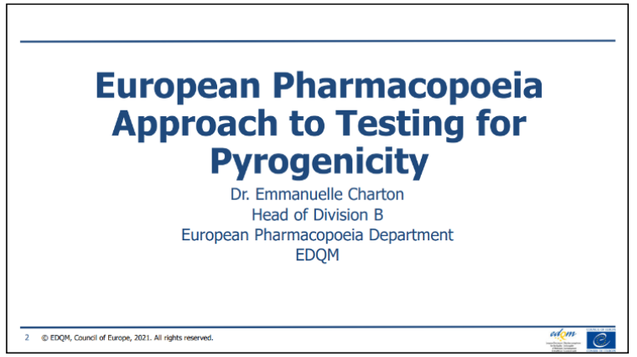
C1: Endotoxins and Pyrogens: Next Generation Testing Platforms
Moderator: Simleen Kaur, MSc, Biologist, Team Lead, OCBQ, CBER, *U.S. FDA*

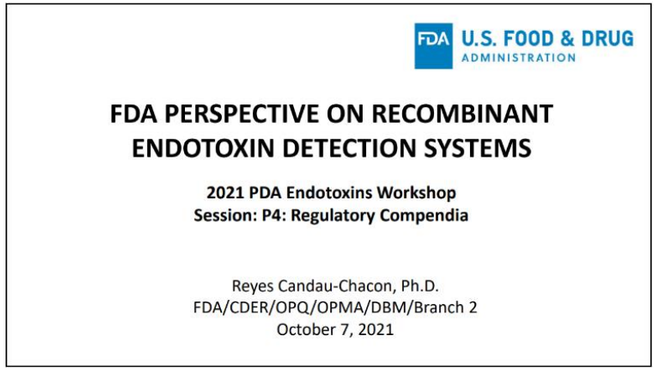
It is time to remove barriers and adopt innovative platforms for detection of Endotoxins and Pyrogens during production and testing products. The proven efficacy of the modern methods provides an opportunity for the pharmaceutical industry to modernize traditi contribute significantly to the conservation of animals. This session will focus on evaluation of Recombinant Factor C and Monocyte testing during manufacturing and release of products.

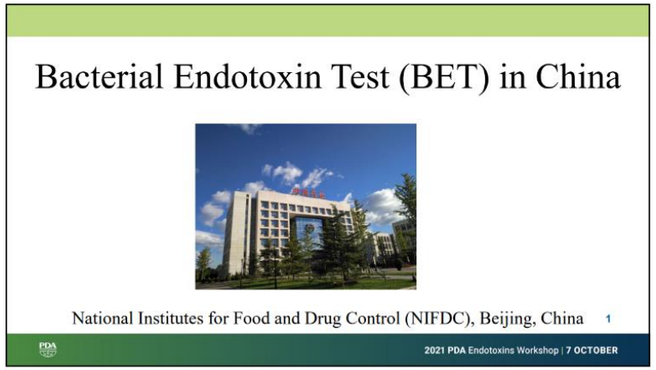
10:45 | Characterization of Endotoxin-Detecting rFC Products for Evaluation of Manufacturing Quality and Assay Performance
Talia Faison, MS, CQA, Biologist, Product Quality Research-Reviewer, OPQ, CDER, *U.S. FDA*

Compendial Status - Global

- Compendial Method
  - 2020: Ph.Eur. 2.6.32
  - 2022: EAEU (proposed)
  - 2024: BP XIV C (proposed)
  - 2024: USP <86> (proposed)
- Compendial Monograph
  - 2023: Ph.Eur. WFI
- Compendia Guidance Chapter
  - 2020: ChP 9251
  - 2021: JP G4-4-180
  - 2023: KP







Results

- 150+ products qualified/verified/validated
- >300,000 samples tested
- 9 medicines approved/authorized by multiple global health authorities
- Compendial method status mid-2020 (Ph.Eur.)

- Corporate level commitments:
  - 2019-2024 corporate UN SDG & ESG Integrated reports, including CEO highlights
  - 80% converted
  - Our rFC initiative helped ESG support a €600M corporate sustainability bond in 2021
  - All 8 of our original manufacturing sites have implemented rFC
  - 6 new Lilly sites coming on line
  - 11 external partners implemented or implementing

Questions