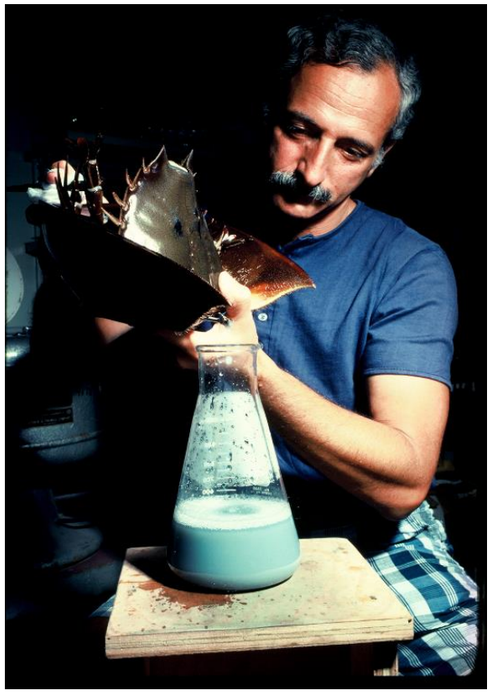
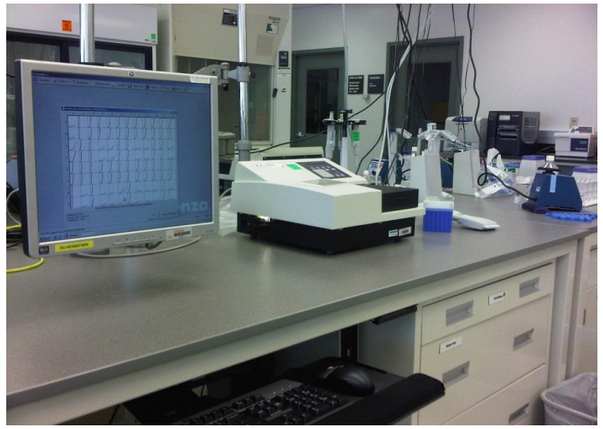


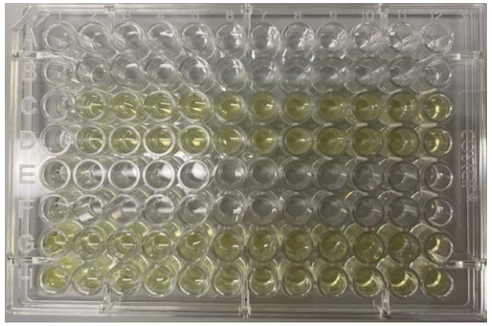


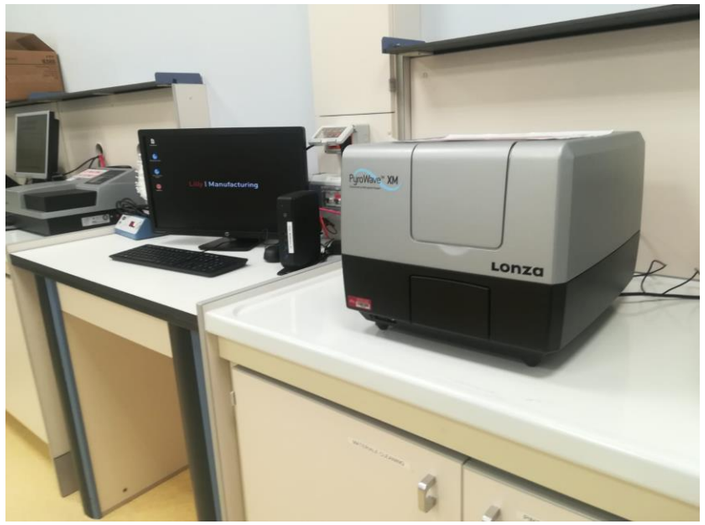

Recombinant Factor C: An Approved and Sustainable Bacterial Endotoxins Test

Evolution of Pyrogen Testing









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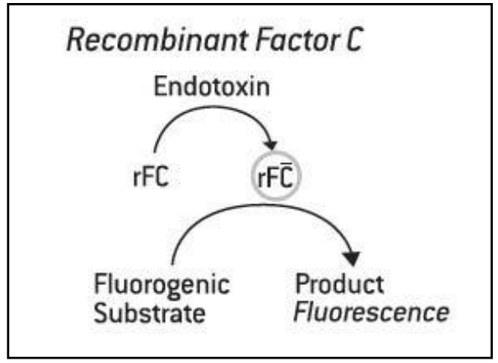
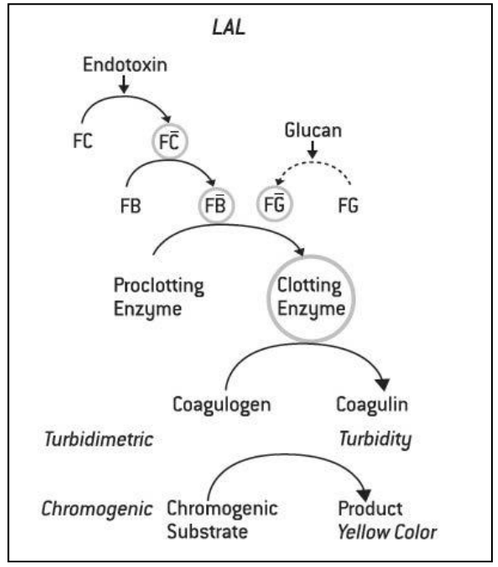
What is rFC?





Diagrams courtesy of Lonza PyroGene™ package insert

- Factor C is a natural horseshoe crab blood protein: a biosensor of bacterial endotoxins and is contained in the traditional LAL test reagent used in the bacterial endotoxins test (BET)
- recombinant Factor C (rFC) is the biotechnology-manufactured version of natural Factor C
- rFC is more specific for bacterial endotoxins because it bypasses the factor G glucan false positive pathway
- End-point fluorescence is a technique described in European and Asian pharmacopoeias to detect activation of the Factor C pathway

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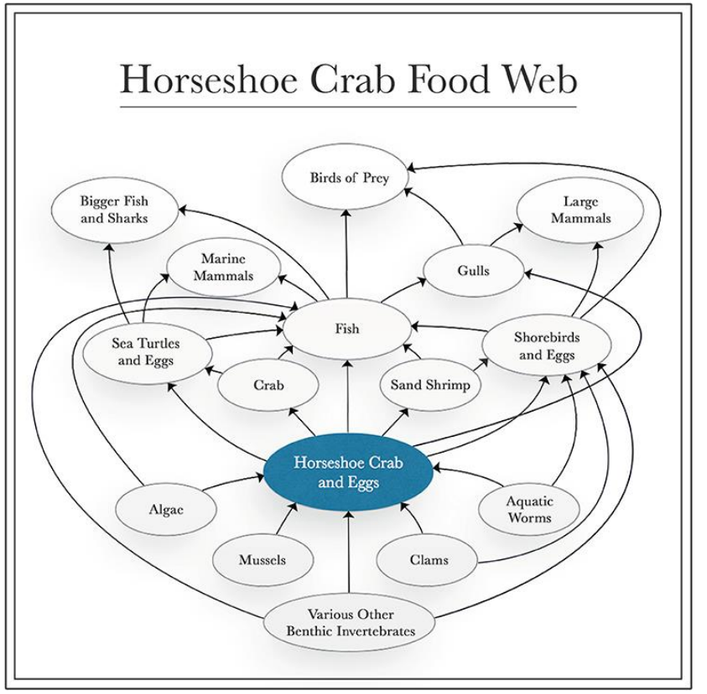
Change Rationale: Benefits

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%)



From Krisfalusi-Gannon et. al. 2018

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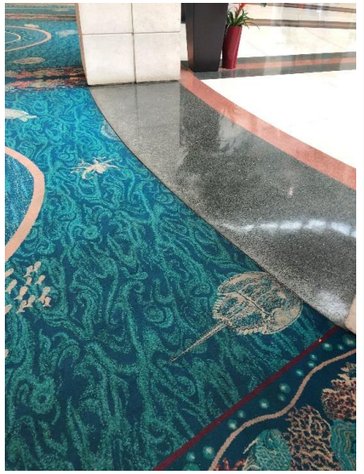
Why rFC?

- Data-driven decision: Our experience and data support the use of rFC in the BET method as equivalent or superior to using LAL
- Execution of the BET using rFC is nearly identical to the BET using LAL
  - Different reader/reagent
- The method validation is relatively easy compared to microbiological alternative methods
  - Calculation values and units do not change



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Lilly rFC Journey

Year	Milestone
2003	rFC commercially available
~2006	Initial Lilly evaluation
2012-13	FDA Guideline issued; new plant in China; USFWS lists the Red Knot as Threatened; 2nd rFC supplier entered the market
2016	Initial method implemented for water testing and clinical medicines American Horseshoe Crab declared Vunerable
2018	rFC testing implemented at 3 sites
2018	First FDA product approval: Subsequent approval by >60 health authorities
2019	rFC testing implemented at 2 sites; Tri-spine horseshoe crab declared Endangered
2020	Second FDA product approval: Subsequent approval by >8 health authorities
2020	Emergency Use Authorization for 2 COVID-19 antibody therapies
2021	rFC testing implemented at 2 sites;

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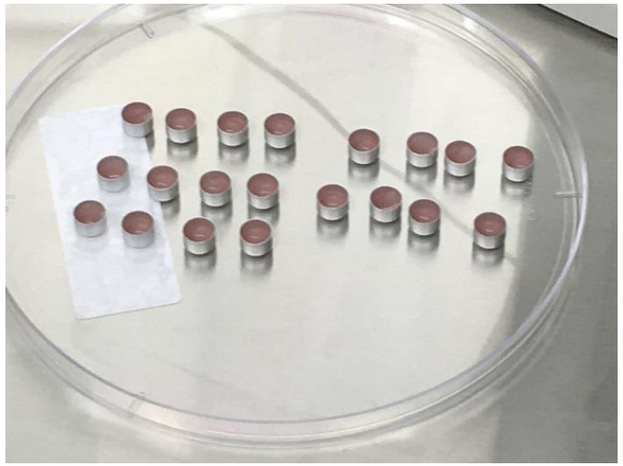
Implementation

- Line in the Sand (early 2016): All new Lilly compounds are tested by rFC and validated as a product-specific non-compendia method
  - Transitioned to product-specific verification/qualification mid-2020
- Water, raw materials, in-process, component testing are all in scope for internal change (~80-90% of all testing)



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External Activities

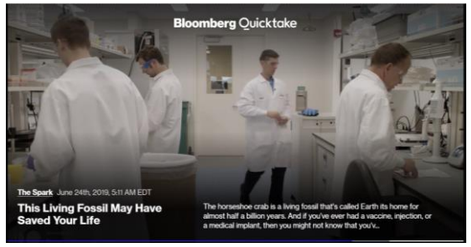
Presentations:

- 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)
- Unites States Pharmacopoeia Endotoxin Workshop (June 2019)
- United States Congressional Briefing (Oct 2020)
- Parenteral Drug Association Microbiology (Oct 2021)
- Unites States Pharmacopoeia Endotoxin Workshop (Nov 2021)

Publications

- 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

Interviews	
The Atlantic (May 2018)	The Weather Channel, Nicole Bonaccorso (July 2019)
National Audubon (May 2018)	Reuters, John Miller (June 2020)
Revive and Restore/NJ Audubon Press Event (May 2018)	NJ Spotlight, John Hurdle (June 2020)
Hakai Magazine (May 2018)	Pharmalot, Ed Silverman (June 2020)
Radiolab – NPR (July 2018)	NY Times, Jim Gorman (June 2020)
BirdWatching/South Carolina Wildlife Magazines (Sep 2018)	Lilly UN SDG Report (June 2020)
Ensia Magazine (Sep 2018)	Smithsonian Magazine (June 2020)
PDA Letter (Dec 2018)	Miami Herald (June 2020)
Top of Mind with Julie Rose, BYU Radio (Dec 2018)	Québec Science (Sep 2020)
First, Do No Harm Podcast (Feb 2018)	National Audubon (Nov 2020)
Constant Wonder, BYU Radio (Apr 2019)	Canadian Broadcasting The Current, Matt Galloway (Dec 2020)
Phil Forester, independent film maker (Apr 2019)	E&E News, Ariel Wittenberg (Jan 2020)
NY Times, John Hurdle, Science Page (May 2019)	NJ Advance Media, Michael Warrant (Jan 2020)
Problem Solved, Bloomberg Media (May 2019)	The State and The State Chiara Eisner (Feb/May 2021)
Lilly UN SDG Report (June 2019)	Lilly UN ESG Report (May 2021)
Reuters, John Miller (June 2019)	The Point – WACI, Mindy Todd (June 2021)
The Baltimore Sun, Maddy Lauria (June 2019)	Washington Post – Caren Chesler (Aug 2021)



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Challenges

- Institutional resistance to change under proactive conditions
- Competing priorities
- Impact to regulatory resources to support changes
  - Significant regulatory hurdles for multi-marketed products
- Effort required to revise a harmonized compendia chapter

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2020-2021 Compendial Updates

- Europe Ph.Eur. 2.6.32: compendia stand-alone rFC general method chapter published in Jul 2020
- China ChP 9251: guidance chapter published in 2020
- Japan JP G4-4-180: guidance chapter published in Jun 2021
- U.S.: guidance chapter proposed

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Regulatory Strategy

- New Products
  - Previous Potential Approach:
    - Dual-register non-compendia and compendia methods for internally manufactured products
  - Recommended Approach:
    - Register Ph.Eur. 2.6.32 compendia method based on:
      - Comparability is supported both by the scientific literature and experience
      - Precedence for using Ph.Eur. methods for non-endotoxin methods in global submissions
      - FDA MAPP 5310.7 suggests FDA will accept Ph.Eur. methods
      - Successful product submissions using rFC to release product

    Benefit: Streamlined regulatory and QCL activities
- Marketed Products
  - Opportunistic rFC method registration concurrent with tech transfers, new sites, open registrations, etc.

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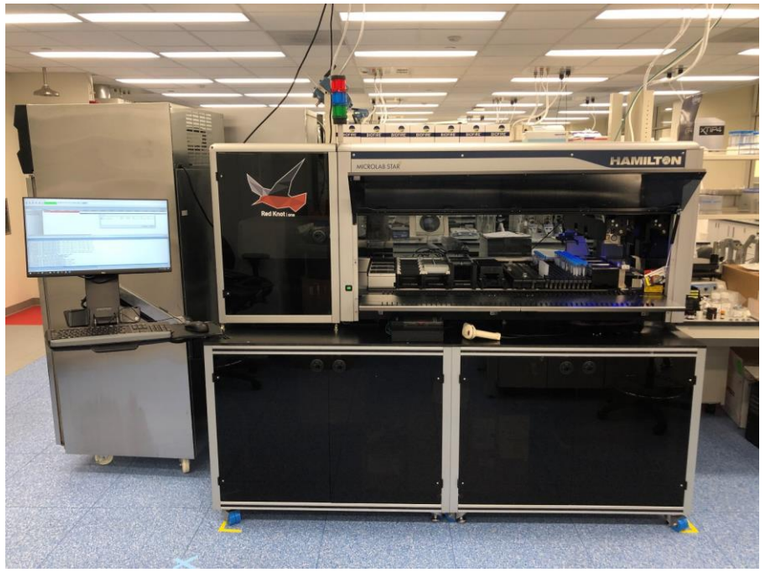
Results

- ~50 products validated; 40+ additional products qualified/verified
- >92,000 samples tested
- 5 medicines approved/authorized by multiple global health authorities
- Automation efforts underway
- Beta tests of rCR (rLAL) reagents
- Efforts supported our first Sustainability Bond (€600M) – Investor Relations / ESG



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Possible Next Steps for Consideration

- Mutual recognition of major compendia method chapters and/or
- Fast track to compendial harmonization and/or
- Declaration of “annual reportable” mechanism by health authorities to switch from LAL to rFC

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Questions

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