Enabling a Healthier World

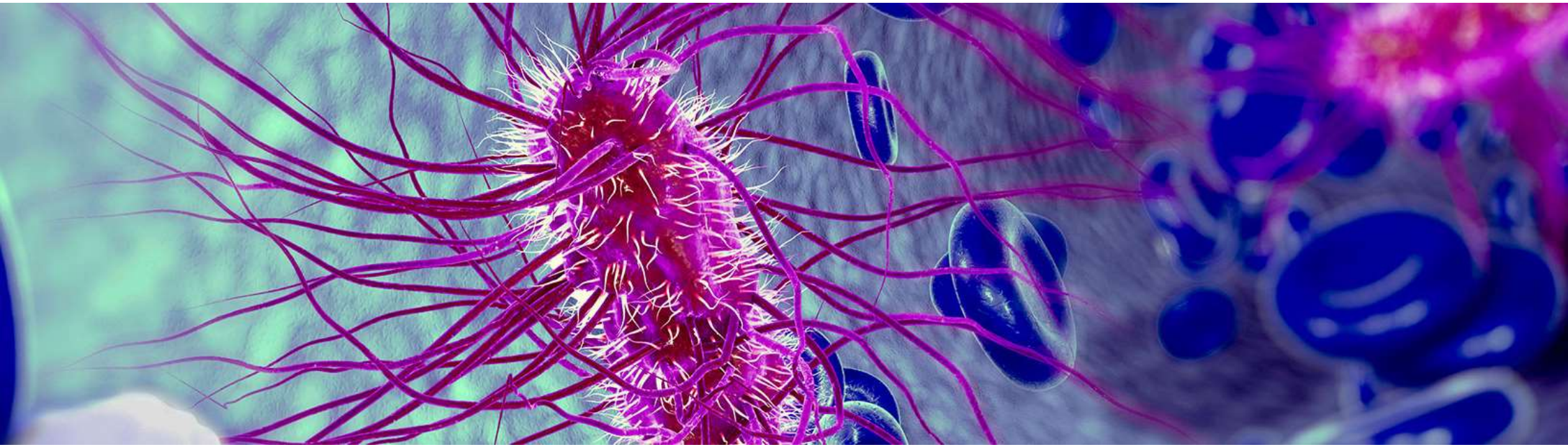
Lonza

Moving Towards Sustainable BET Testing

PyroGene® rFC Assay

Katrin Pauls | 22 August 2024

Business Use Only



Presenter

Speaker:

Katrin Pauls

Market Development and Scientific Affairs Manager

Katrin studied Microbiology and Virology at the University in Duesseldorf, Germany and has over 15 years‘ experience in QC Microbiology for Pharma, Food and aviation industries. In her current role as Market Development and Scientific Liaison Manager for Testing Solutions at Lonza’s Bioscience Cologne site Katrin is responsible for developing markets and leading communicating on technical aspects of sustainable testing solutions.

Lonza's QC Testing Solutions

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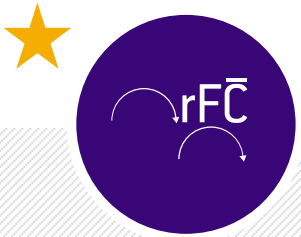
Traditional Bacterial Endotoxin Tests (BET)

- PYROGENT® Gel Clot Assays
- PYROGENT® 5000 Turbidimetric Assays
- Kinetic-QCL® Chromogenic Assays



Sustainable Pyrogen & Endotoxin Tests

- PyroGene® Recombinant Factor C (rFC) Assays
- PyroCell® Monocyte Activation Test (MAT) Rapid Systems

Instrumentation, Software, & Accessories

- Multimode, Absorbance and Fluorescence Readers
- PyroTec® PRO Automated Robotic Solution
- WinKQCL® Endotoxin Analysis Software
- Accessories and Consumables

Training & Support

- e-Learning Certification Modules
- QC Insider® Toolbox
- Testing Services
- Field Support Services
- Scientific Support

Lonza has been providing endotoxin detection solutions and services since the 1970s

Traditional BET Tests Are Based on a Wild Animal Resource

The horseshoe crab – a "sustainable" creature

- By fossil records over 445 million years old
- Occupied a niche in the ecosystem, 4 species are still around today
- Mandatory actions to stabilize the population, e.g.
  - Quota for Fishing/ Bait industry
  - Measures against erosion, habitat shifting and alteration due to coastline development
  - Actions against climate change and severe weather
  - **Use of recombinant methods**

ASMFC[1] – *Limulus polyphemus (LAL)*

- Overall neutral trend in population.
- Multiple challenges from pressure on habitats – action plans for protection

IUCN[2] – *Tachypleus gigas, T. tridentatus and Carcinoscorpius rotundicauda*

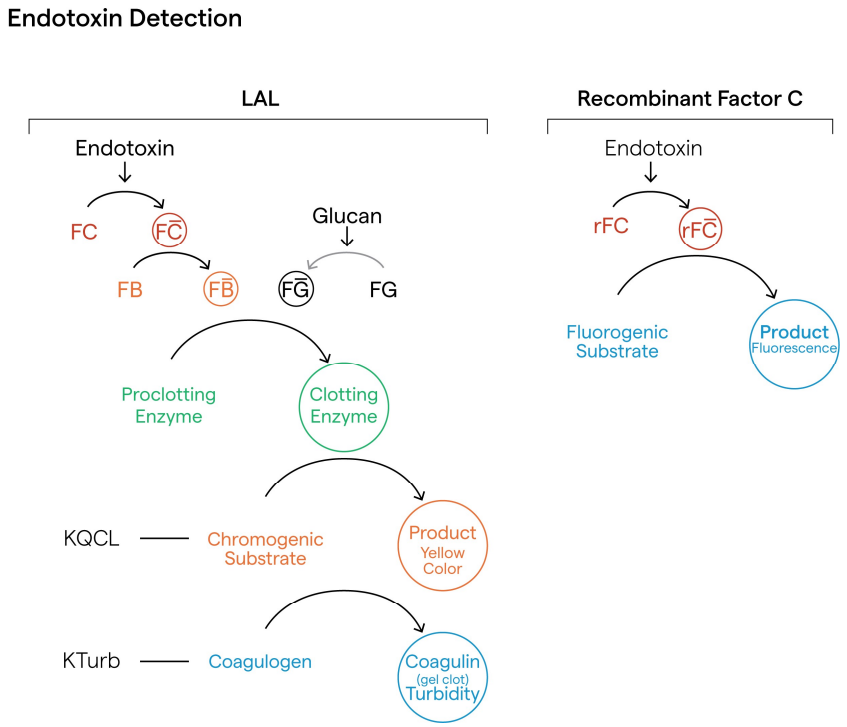
- Endangered and data deficient
- No action plan in place

1. http://www.asmfc.org/species/horseshoe-crab
2. https://www.iucnredlist.org

Recombinant Factor C Assay – A Sustainable BET Test?

A simple answer... Yes!

- Eliminates the dependency on LAL and TAL as a natural resource
- Secures the supply chain – addresses the constant growth in the number of annual BET tests
- Absence of biological variability, higher lot-to-lot consistency
- No false positive results - Glucan path eliminated
- Comparability of LAL/TAL and rFC demonstrated by a large number of studies



Recombinant Factor C Assay - Supported by Regulations

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European Pharmacopeia	United States Pharmacopeia	Japanese Pharmacopeia	Chinese Pharmacopeia
Compendial	→ Compendial (New)	Alternative	Compendial

- Ph. Eur. 2.6.32 general analytical chapter
- Ph. Eur. 5.1.10 Guidance for using BET tests
- New (2025): Ph. Eur. 5.1.13 Guideline for selecting a pyrogenicity test (→ monograph methods)

- USP <1085> general chapter
- New (2025): USP <86> general analytical chapter "recombinant methods"

- JP G4-4-180 BET & alternative methods using recombinant proteins

- ChP 1143 Guideline for BET applications

FDA

Guidance for Industry: Questions and Answers
"If a manufacturer chooses to use a recombinant factor C based assay method validation should be in accordance with the requirements of USP <85> , "BET" and USP <1225>, Validation of compendial procedures."

[1]Pharmacopeial Forum 36:1 Jan/Feb 2010

History of PyroGene® Recombinant Factor C Assay

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PyroGene® rFC Assay history

2003

- PyroGene® rFC Assay is launched and made commercially available

2004

- PyroGene® rFC is fully supported on a true client server endotoxin software (WinKQCL® 3.0 Endotoxin Detection Software)

2009

- The U.S. FDA approves 510(K) applications that include PyroGene® rFC as the final release test (Securian® Tissue Reinforcement Matrix and MTA® Protective Sheet)

2012

- The U.S. FDA recognizes rFC as an acceptable alternative to the BET in their 2012 Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers

2018

- The U.S. FDA approves the first pharmaceutical drug using a rFC method for release testing (Emgality®)

History of PyroGene® Recombinant Factor C Assay (2)

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PyroGene® rFC Assay history

2020/ 2021

- The PyroGene® rFC Assay became the first rFC assay to be automation-compatible, using Lonza's PyroTec® Pro System
- The European Pharmacopeia released compendial Chapter 2.6.32 dedicated to recombinant Factor C

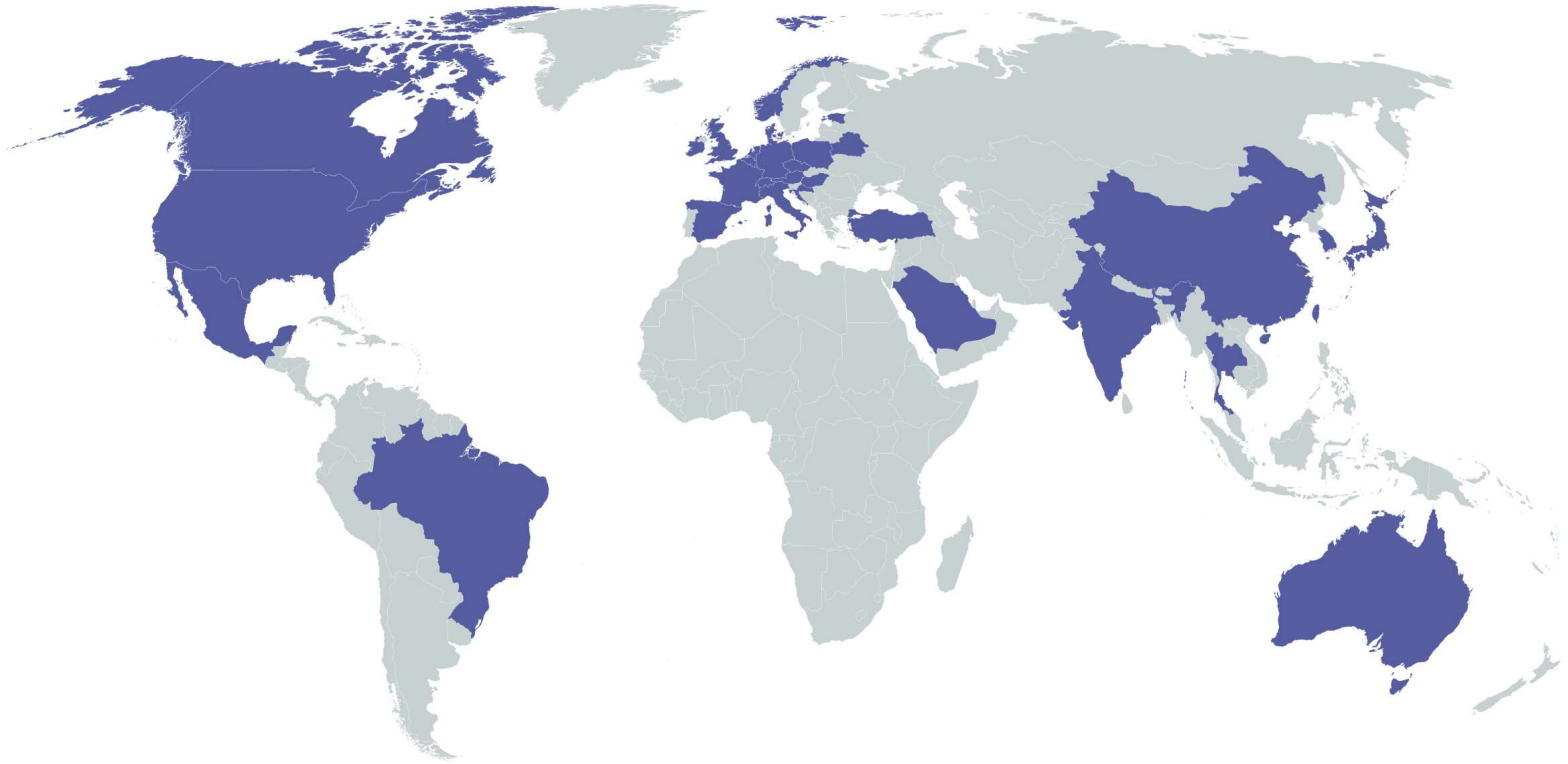
2022

- Lonza's Nebula® Multimode Reader is optimized for use with the PyroGene® rFC Assay, allowing customers to use rFC or traditional LAL assays in one reader

2023 - 2025

- In course of their pyrogenicity strategy the European Pharmacopeia will revise > 60 monographs for reference to rFC
- The United States Pharmacopeia launches chapter <86> on recombinant BET methods

PyroGene® Recombinant Factor C Assay – Global Footprint



Customer Locations:

Australia	Italy
Austria	Japan
Belarus	Luxembourg
Belgium	Mexico
Brazil	Netherlands
Canada	Norway
China	Poland
Croatia	Saudi Arabia
Czech Republic	Singapore
Denmark	South Korea
Estonia	Spain
France	Switzerland
Germany	Taiwan
Hong Kong	Thailand
Hungary	Turkey
India	United Kingdom
Ireland	United States of America

PyroGene® Recombinant Factor C Assay – An Overview

The sustainable Bacterial Endotoxins Test

- A quantitative, single-step, endpoint photometric method with sensitivity down to 0.005 EU/mL
- Assay uses liquid reagents, takes 1 hour at 37° C
- Follows BET methodology and guidelines, the assay is performed in a standard 96-well microplate
- The assay read-out is performed in a Fluorescence Reader (PyroWave®, Nebula® → Ex/Em 380/440 nm) controlled by WinKQCL® Software

The PyroGene® Recombinant Factor C Assay – Standards and Controls

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The sustainable Bacterial Endotoxins Test

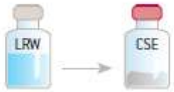
- Uses standards calibrated to RSE to produce a standard curve
- Uses a PPC at or near the middle of the standard curve
- Follows validation protocols as described
- Routine testing is as per quantitative methods
- Assessment of assay data as per USP/EP/JP
- The primary difference between the rFC and LAL methods is that the rFC reagent is not sourced from HSC blood

PyroGene® Recombinant Factor C Assay - QuickGuide

This is a step-by-step guide depicting how to perform the PyroGene™ Recombinant Factor C assay. Prior to initiating the assay procedure, allow reagent vials to equilibrate to room temperature. The incubating microplate reader should also be turned on and a plate template created in the WinKQCL™ Software.

Step 1

Reconstitute Control Standard Endotoxin (CSE) with LAL Reagent Water (LRW) to yield a solution containing 20 EU/ml.



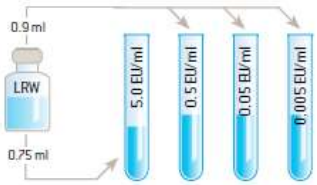
Step 2

Vortex for 15 minutes.

Step 3

Label the tubes with the appropriate endotoxin concentration and add 0.75 ml of LRW to the 5.0 EU/ml tube.
Add 0.9 ml of LRW to each of the remaining tubes.

(Example based on a test with an operating standard curve of 0.005–5.0 EU/mL)



Step 4

Prepare a series of endotoxin standards.

Step 5

Dispense 100 µl of the LRW blank, endotoxin standards, product samples, positive controls, etc. into the appropriate wells of the microplate.

Step 6

Pre-incubate the plate for ≥ 10 minutes at 37°C ± 1°C in the microplate reader.

Step 7

During incubation period, prepare the working reagent, which consists of fluorogenic substrate, assay buffer and rFC enzyme solution in a 5:4:1 ratio, respectively. Add enzyme last to the buffered substrate.

- PyroGene® rFC Assay is a standard photometric assay, remarkably like kinetic chromogenic or kinetic turbidimetric LAL
- rFC is the same binding protein operating in the LAL assay
- The activated recombinant Factor C enzyme cleaves a substrate directly instead of activating another enzyme in a series (the LAL cascade)
- The substrate has a fluorescent tag, which gives a wide dynamic range with better resolution

Phased Approach for Adoption

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Bacterial Endotoxin Testing (BET) is an essential quality control test for raw materials, in-process samples and final product release

When adopting a recombinant assay into your endotoxin testing workflow a conservative, phased approach could be considered

- **Phase 1** – raw material and in-process samples (such as in-process water). Other samples such as dialysate and research samples should be considered
- **Phase 2** – final products
- **Phase 3** – automation

PyroGene® rFC Assay Validation

Validation Protocol

Please contact your local scientific support team for validation protocol and support!

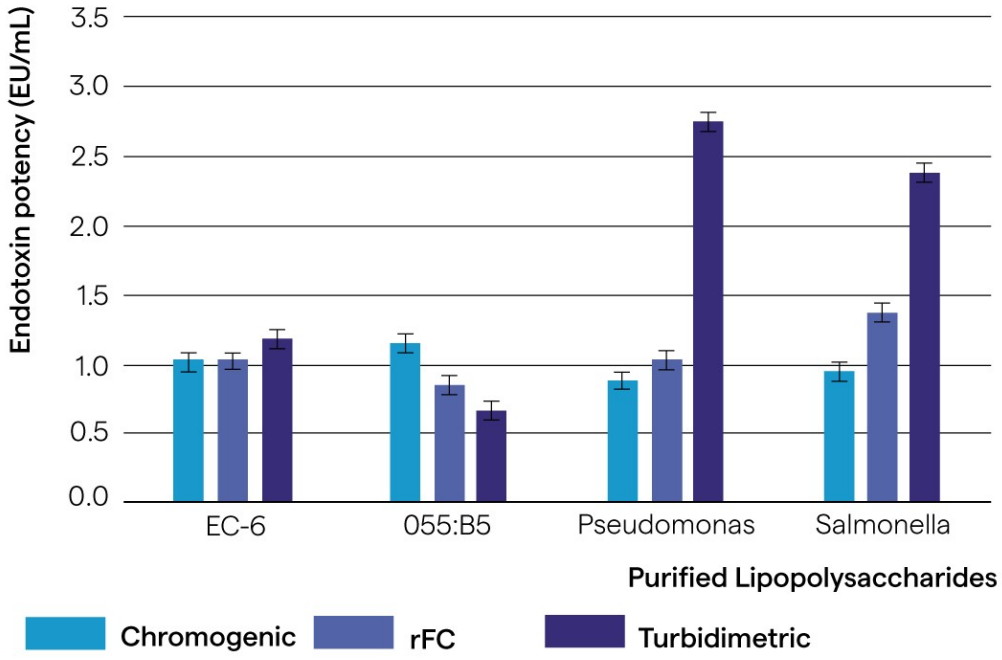
Preparation	Initial Qualification	Inhibition/ Enhancement testing	Validation of Alternative Method/ Comparability	Product Validation
IOPQ	Not necessary if previously qualified reagent lots are used	rFC & test product	Compare current LAL and rFC method	3 production lots
Sensitivity assay for rFC	0.5 days	Time depending on extent of testing required to find a suitable dilution or pretreatment	Good planning can save time	0.5 days
Initial qualification of rFC method and analyst		0.5 days (minimum)	1.5 days (minimum)	
2 days				

Validation Strategy for New Recombinant Factor C Users

Method Comparability – PyroGene® Assay Validation

A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin
Pharmacopeial Forum, Vol. 36 (1), Jan.-Feb. 2010

- Multi-center study – sites in US and Europe
- Set-up followed requirements of USP Chapter <1225>
- Ten different pharmaceuticals or related products
- Tested at MVD, 1/2 MVD and 1/10 MVD
- **The rFC method was comparable to LAL procedures but offered more specificity (non-reactive to glucans)**



Results of Pyrogene® rFC Assay and LAL Assays Are Comparable

Comparison of four endotoxin detection reagents in measuring naturally contaminated endotoxin levels in four representative parenteral products

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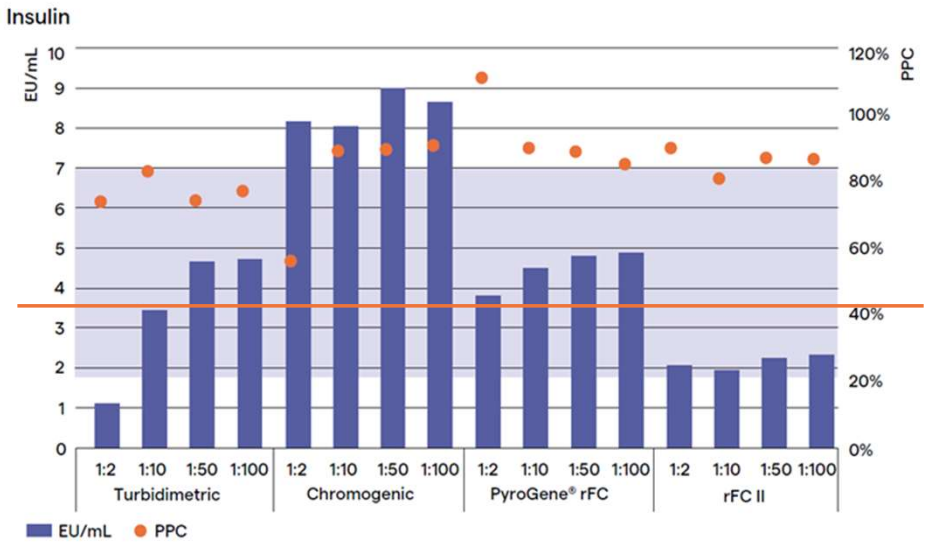
- Four parenteral products (Saline, Insulin, Acyclovir, Gentamicin) spiked with natural water to simulate a breach in the purification system
- Products were tested using a kinetic turbidimetric method, a kinetic chromogenic method, and two rFC methods
- All three assay types safely detected the contaminants
- **There is no "best method" for every parenteral product**



Example:
Comparison of different BET and rFC methods on Insulin contaminated with natural water. The light purple background indicates the valid detection range of the natural contaminant.

Why PyroGene® Recombinant Factor C Assay?

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In Summary

- Lonza pioneered the commercialization of an equivalent, reliable recombinant endotoxin detection method
- The PyroGene® rFC Assay has been gaining acceptance world-wide
- With the recombinant Factor C assay, you can meet the demands of supply security while protecting a natural resource
- A phased implementation of the rFC assay offers a conservative approach
- Reliable lot-to-lot consistency, statistically more robust than LAL methods
- Adopting recombinant reagents provides many benefits and further coupling those reagents with automation helps to reduce errors in the BET assay process and prepare for the future

PyroGene® rFC Assay – Kit Size

Catalogue Number	No of tests/ reactions	Components	Sensitivity (EU/mL)
50-658U	192 tests	2 x 96 tests/vial rFC Enzyme 2 x 6mL vials Fluorogenic Substrate 2 x 5mL vials rFC Assay Buffer 2 vials E. coli Endotoxin 2 x 30mL vials LAL Reagent Water	0.005 to 5
50-658NV	2880 tests	30 x 96 tests/vial rFC Enzyme 30 x 6mL vials Fluorogenic Substrate 30 x 5mL vials rFC Assay Buffer 10 vials E. coli Endotoxin	0.005 to 5

Become a QC Insider

QC Insider Toolbox - Endotoxin Expertise at Your Fingertips®

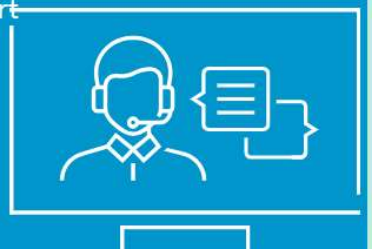
An online suite of comprehensive resources from beginner to advanced support tools, training modules, videos, webinars and a library of information that helps QC professionals perform classic and recombinant bacterial endotoxins test.

Visit: www.lonza.com/qcinsider

Support

Access to guidance and detailed information about software support, recertification and more.

- Recertification Services
- Reader Install & Maintenance
- Software/IT Support
- Scientific Support
- Testing Services
- Automation



Training

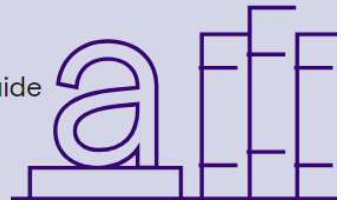
Self-directed resources that will help you build on your bacterial endotoxins test expertise.

- e-Learning Modules
- Videos
- Webinars
- Seminars

Library

Technical resources that will help ensure your success with the bacterial endotoxins test.

- Certificates of Analysis
- Package Inserts
- Quick Guides
- Scientific Posters
- Tech Tips
- White Papers
- Assay Selection Guide
- Presentations



Questions?

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Any questions?

Please submit your questions using the questions pane at the bottom of your screen

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Thank you!

Katrin Pauls | 22 August 2024