Enabling a Healthier World

Lonza

PyroGene® Recombinant Factor C Assay

First in History

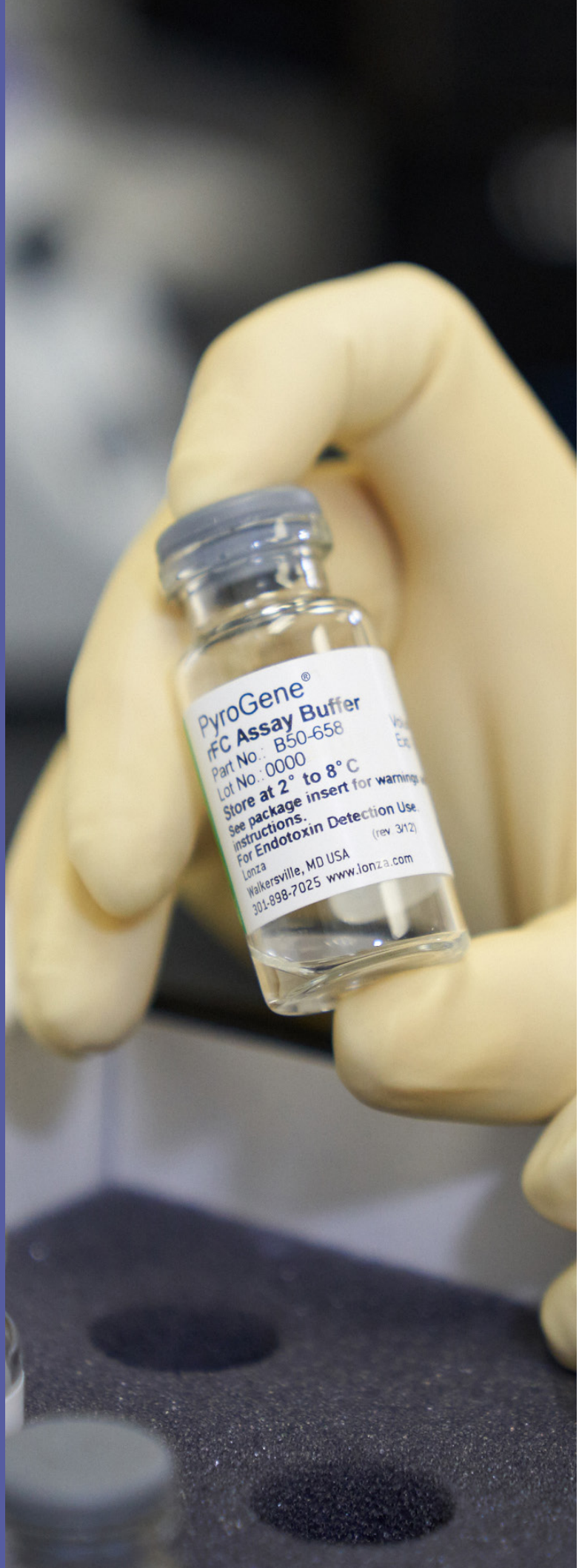
2003
rFC assay developed and brought to market

2004
rFC assay to be fully supported on true a client server endotoxin software (WinKQCL® 3.0 Endotoxin Detection Software)

2009
rFC assay to be included in U.S. FDA 510(k) clearance for use with medical devices (Securian® Tissue Reinforcement Matrix and MTA® Protective Sheet)

2018
rFC assay to be used in a U.S. FDA approved drug (Emgality®) (2018)

2021
rFC assay to be fully automated by using Lonza's PyroTec® PRO System

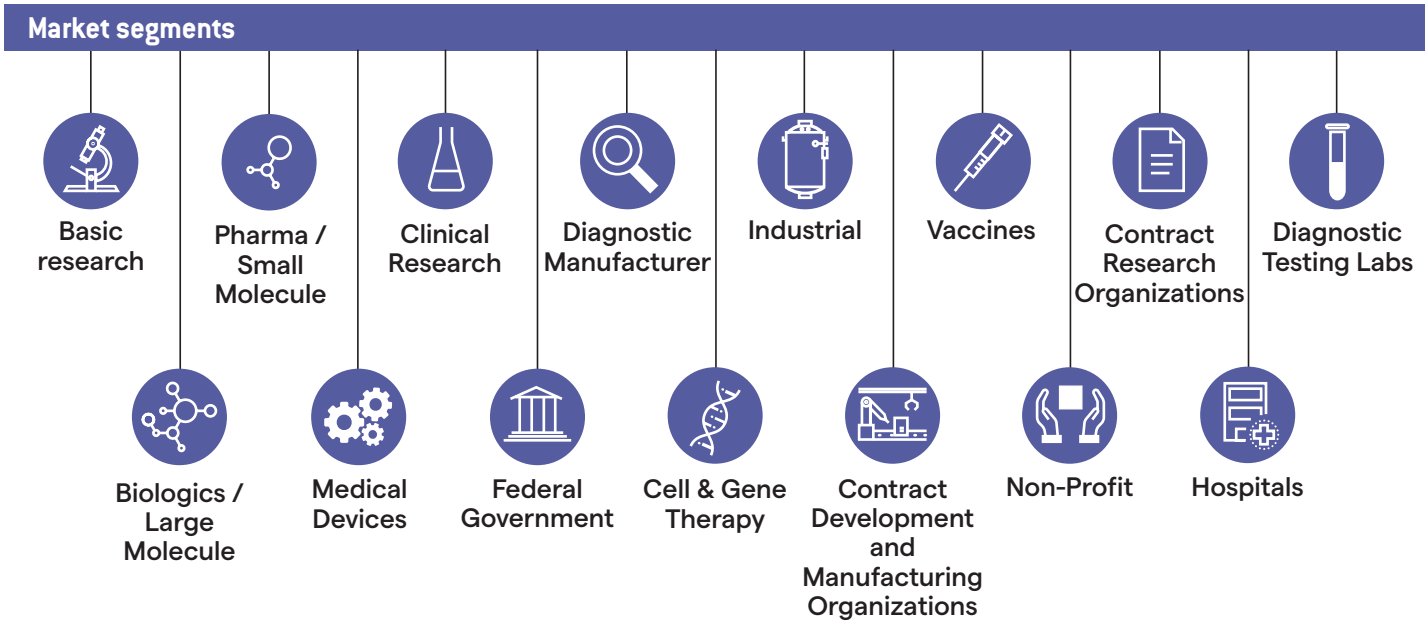


PyroGene® rFC Assay

The next source for endotoxin detection

The PyroGene® Recombinant Factor C (rFC) Assay is the evolution of the traditional limulus amebocyte lysate (LAL) test. Combining 21st century technology with the horse-shoe crab's endotoxin-sensitive Factor C protein, Lonza developed an equivalent, reliable and sustainable endotoxin detection method.

The PyroGene® rFC Assay is used by hundreds of companies, in over 30 different countries, and across many pharmaceutical industry segments.



PyroGene® rFC Enzymatic Cascade

The PyroGene® rFC contains a recombinant protein, Factor C, derived from the horseshoe crab's defensive enzyme clotting cascade which is used in traditional bacterial endotoxin tests. The recombinant Factor C (rFC) is manufactured in a lab, rather than harvested from horseshoe crab blood used in Limulus Amoebocyte Lysate (LAL) products, and is generated from the gene of Carcinoscorpius rotundicauda Factor C, which is homologous to the Limulus polyphemus Factor C gene. The PyroGene® rFC Assay works through a single enzymatic step compared to the multiple step enzymatic process necessary for LAL assays. The assay contains Factor C, and none of the other factors found in the traditional LAL cascade, so the PyroGene® Assay retains all the endotoxin reactivity of LAL, but with greater lot-lot consistency than traditional LAL. Recombinant Factor C is activated when it binds to endotoxin, it then cleaves a synthetic substrate that generates a fluorescent signal.

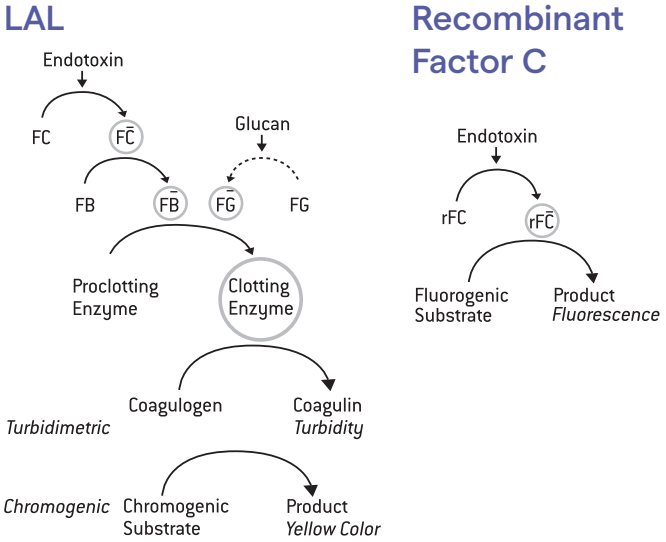


Figure 1: The traditional LAL clotting cascade can be triggered by endotoxin or glucans compared to the one-step rFC reaction, not triggered by glucans.

Benefits of the PyroGene® rFC Assay

- Sustainable resource - provides greater security of supply
- Endotoxin specific, recombinant technology eliminates false-positive glucan reactions
- Statistically more robust (when compared to LAL methods)
- High dynamic range of fluorescein provides better resolution than conventional absorbance methods
- Automation-compatible with Lonza's PyroTec® PRO System, providing significant time savings and error reduction

QC Insider® Toolbox

- Support
- Training
- Library

QC INSIDER® TOOLBOX

For more information about the PyroGene® rFC Assay including training videos, white papers and on-demand webinars, explore our QC Insider® Toolbox: www.lonza.com/qcinsider.

PyroGene® rFC Assay Timeline

2003

- PyroGene® Recombinant Factor C Assay, a sustainable alternative to quantitative LAL-based methods, is launched and commercially available.
- PyroGene® rFC is not derived from horseshoe crab blood; it is not considered a biologic and therefore, FDA licensure is not required.

2004

- Lonza's industry leading WinKQCL® 3.0 Endotoxin Detection Software is launched, offering full support for the PyroGene® rFC Assay and the first true client-server endotoxin software.

2009

- The FDA approved 510(K) applications that included Lonza's PyroGene® Assay as the final release test.
- Lonza launches WinKQCL® 4.0 Software, second generation interactive trending, Template Manager; user interface translated into 6 languages.

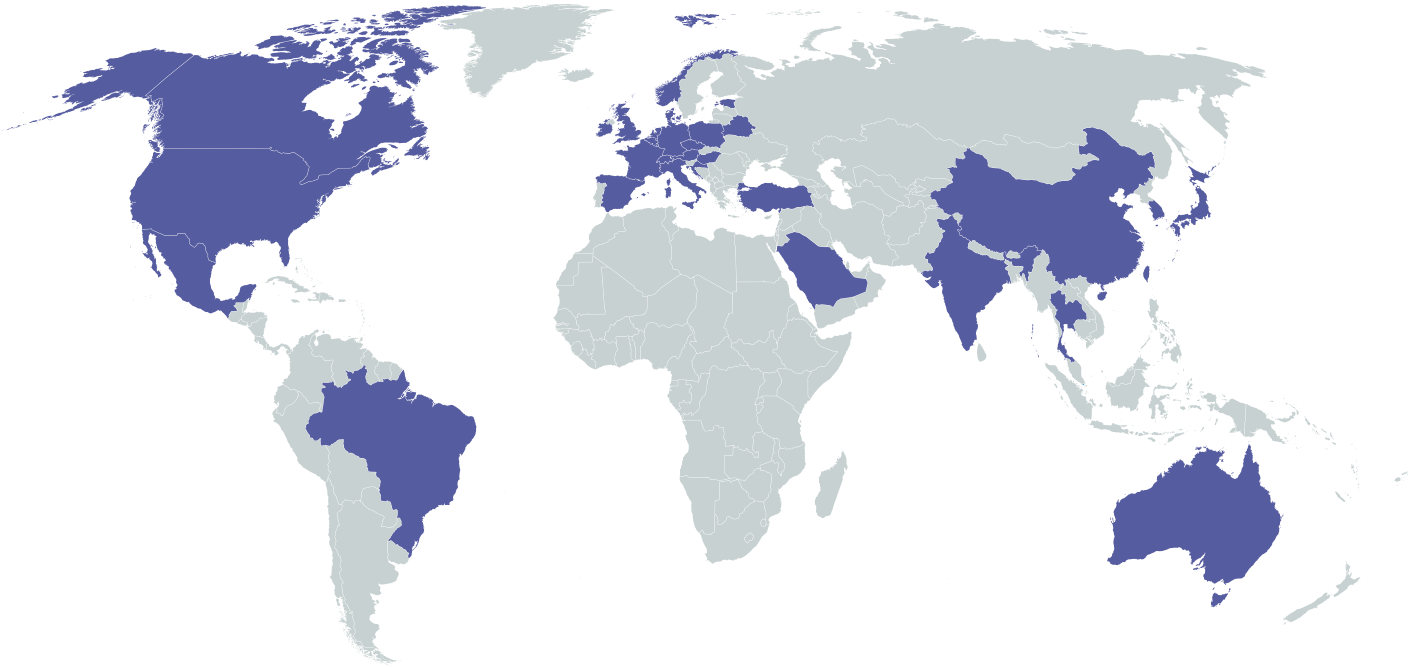
2015

- Recombinant Factor C is officially recognized by the European Pharmacopoeia (Ph. Eur.) as an alternative endotoxin detection methodology to the LAL and Rabbit Pyrogen Tests in the new draft of Chapter 5.1.10.*

2016

- European Pharmacopeia Chapter 5.1.10 officially is effective.

Global Customer Footprint



Customer Locations:

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

Figure 2: The PyroGene® rFC Assay is used by hundreds of companies, in over 30 different countries.

2018

- The U.S. Food and Drug Administration approves the first drug using a recombinant method for endotoxin testing instead of traditional LAL-based methods for a monoclonal antibody drug treatment for the prevention of migraines in adults.
- The European Pharmacopeia releases a draft of their new compendial Chapter 2.6.32 dedicated to the recombinant Factor C method.

2019

- Recombinant Factor C (rFC) is listed and described as a new compendia method for bacterial endotoxin testing in the Chinese Pharmacopeia, following the EP, JP, and FDA. The 4th version of the Chinese Pharmacopeia will be effective in 2020.

2020

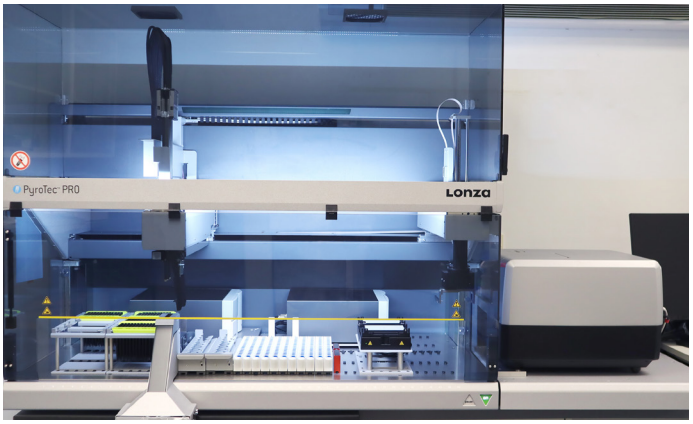
- The European Pharmacopeia's draft of their new compendial Chapter 2.6.32 dedicated to the recombinant Factor C method is approved.
- PyroGene® became the first rFC assay to be automation-compatible, using Lonza's PyroTec® PRO System.

*The rFC assay will still be considered an "Alternative Test", subject to the validation requirements of USP <1225> or ICH Q2B. Regulatory authorities will accept the test results of the recombinant Factor C assay, but a validation study must be performed for each product that will be tested using this method. (Validation studies are used to compare the alternative and compendial method, and verify the equivalence between the two methods of the assay. Post-validation, it is necessary to follow up with the appropriate regulatory filing for the drug product or device.)

Automated QC

The PyroTec® PRO Automated Solution combined with the PyroWave® XM Reader option expands the range of endotoxin testing types available on the platform to include the PyroGene® rFC Assay. The addition of the PyroWave® XM Fluorescent Reader complements the existing absorbance readers used in traditional LAL-based assays. Full integration with the WinKQCL® Endotoxin Detection and Analysis Software enables automated testing that meets data integrity requirements.

With precise and efficient endotoxin testing for the delivery of safe parenteral medicines and medical devices, and growing pressure to reduce costs and increase productivity, growing numbers of laboratories are realizing the benefits of automated testing systems. Thanks to the latest advances in robotics and integrated software, new automated endotoxin testing platforms are enhancing the accuracy, speed and efficiency of these important workflows.



Significant time savings can be achieved using Lonza's PyroTec® PRO Automated Robotic Solution.

Regulatory Status and Validation

While still considered an alternative method by the USP, rFC was designated a compendial method by the European Pharmacopoeia in 2021. For use on products destined beyond the EU, the rFC method must be validated as an alternative method according to the requirements of USP<1225> (ICH Q2b).

For more information and regulatory status updates visit: **www.lonza.com/pyrogene**.

As shown in the schematic below, one validation can be accomplished in as little as 5 days assuming that the product has been previously validated with a quantitative LAL method. The validation scheme is identical to that which would be needed for any LAL-based method with the addition of one step, "Validation of Alternative Method."

Product Validation of PyroGene® rFC Assay at a Glance

Preparation	Initial qualification	Inhibition/ enhancement testing	Validation of alternative method	Product validation
2 days	0.5 days	>0.5 days (min.)	>1.5 days (min.)	0.5 days
• IOP/Q • Sensitivity assay for rFC • Initial qualification of current and rFC method and analyst	• Not necessary if previously qualified reagent lots are used	• Only for rFC • Time depending on extent of testing required to find a suitable dilution/pre-treatment	• Tests with both current and rFC method • Good planning can save time	• 3 production lots

*We offer a full Validation Protocol that can be followed for your convenience.
For further information, please submit your Validation Protocol Request or contact our Scientific Support Team.

Ordering Information

Product Name	Sensitivity EU/mL	Description	Contents	Catalog No.
PyroGene® Recombinant Factor C Assay	0.005 to 5	192 test kit	2 x 96 tests / vial rFC enzyme solution 2 x 6 mL vial fluorogenic substrate 2 x 5 mL vial rFC assay buffer 2 x 10 ng / vial endotoxin 2 x 30 mL vial LAL Reagent Water	50-658U
PyroGene® Recombinant Factor C Assay	0.005 to 5	2880 test kit	30 x 96 tests / vial rFC enzyme solution 30 x 6 mL vial fluorogenic substrate 30 x 5 mL vial rFC assay buffer 10 x 10 ng / vial endotoxin	50-658NV
Nebula® Multimode Reader			Absorbance / Fluorescence reader	25-375S
PyroWave® XM Fluorescence Reader			Fluorescence reader	25-345S
Pyrogen-free Dilution Tubes		Without caps, 13 x 100 mm	30 / foil pack	
LAL Reagent Grade Multi-well Plates		96-well microplates	50 / case	
WinKQCL® 6 Software Package		Endotoxin Detection and Analysis Software	Software package	25-611

* Custom product which requires 6-week lead time for ordering.

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For *In Vitro* Use Only. Not intended to detect endotoxemia in man or animals, or for use in clinical diagnosis, patient management, or for the qualification of blood or blood products.

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