Enabling a Healthier World

Lonza

Measuring Autochthonous Endotoxin Levels in Parenteral Products

The PyroGene® Recombinant Factor C Assay is the evolution of the LAL test. Due to its recombinant origin, the PyroGene® rFC Assay does not use horseshoe crab blood as the raw material. Therefore, it offers a reliable, equivalent, and sustainable method for endotoxin detection. A recent study comparing four different endotoxin detection reagents was coordinated by Lonza. This study was based on requests by the United States Pharmacopeia (USP) to consider the inclusion of the recombinant Factor C (rFC) assay in that compendia.

Pharmaceutical companies often rely on internal water systems to deliver filtered, endotoxin-free water for product manufacturing. When these water systems fail, they can introduce autochthonous (formed or originating in the place where found) endotoxin into intermediates or products. The parenteral products tested in this study were acyclovir, gentamicin, insulin, and intravenous (IV) saline. Each product was spiked with a water sample containing a known amount of autochthonous endotoxin. These products were then tested for autochthonous endotoxin using a kinetic turbidimetric method, kinetic chromogenic method, and two rFC methods (one of which was PyroGene® rFC Assay). The endotoxin detection methods were carried out using a PyroWave® Fluorescence Reader, a BioTek™ ELx808™ Optical Density Reader, and WinKQCL® Software.

The contaminated water sample used in this study was collected after an activated carbon bed in Lonza's

Walkersville, MD water purification system. Collecting a sample from this location simulates a breach to the water purification system, contaminating downstream processes with autochthonous endotoxin. Once collected, this sample was tested for endotoxin contamination levels in Walkersville, MD. The sample was then frozen for long term storage and shipped to the contract testing lab where it was again measured for endotoxin levels. As the endotoxin and glucan values for the post carbon water sample were unknown, those levels were first determined at the contract test lab using several dilutions in a screening before use as the inoculant. The samples were tested in duplicate to determine a non-interfering dilution for further water testing within the study.

Acyclovir

Acyclovir is an antiviral drug active against herpes viruses. Acyclovir has an endotoxin release limit (ERL) of 12.5 EU/mL. The acyclovir sample was prepared with a nominal spike of 3.5 EU/mL. The acceptable detection range is 1.75 – 7 EU/mL indicated by the shaded area on the chart. Endotoxin detected in dilutions of 1:50 and 1:100 were compared using the four test methods. The acyclovir data shown was collected without the use of beta glucan blocker. Of the four methods tested, turbidimetric, chromogenic, and PyroGene® rFC Assays were able to detect endotoxin in the acceptable range.

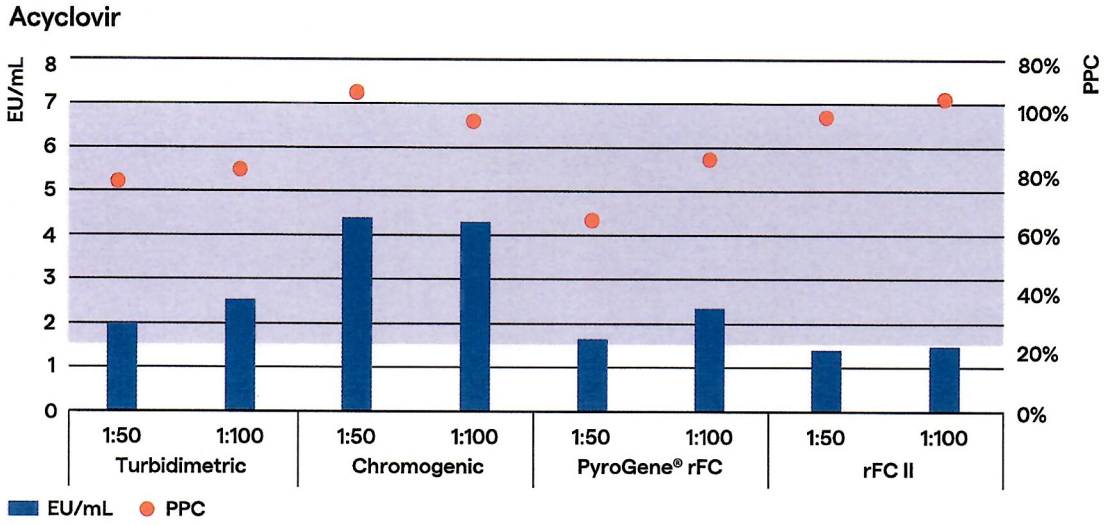


Figure 1.
Endotoxin (EU/mL) measured by four different assays in Acyclovir.

Gentamicin

Gentamicin is an injection used to treat certain serious infections that are caused by bacteria such as meningitis. It is in a class of medications called aminoglycoside antibiotics. Gentamicin has an endotoxin release limit (ERL) of 1.42 EU/mL. The gentamicin sample was prepared with a nominal spike of 0.7 EU/mL. The acceptable detection range is 0.35 – 1.4 EU/mL indicated by the shaded area on the chart. The gentamicin sample was prepared with and without beta glucan blocker. Dilutions of 1:50, 1:100, 1:1000, and 1:10000 were compared between the four test methods. Of the four methods tested, only turbidimetric and PyroGene® rFC Assays were able to detect endotoxin in the acceptable range.

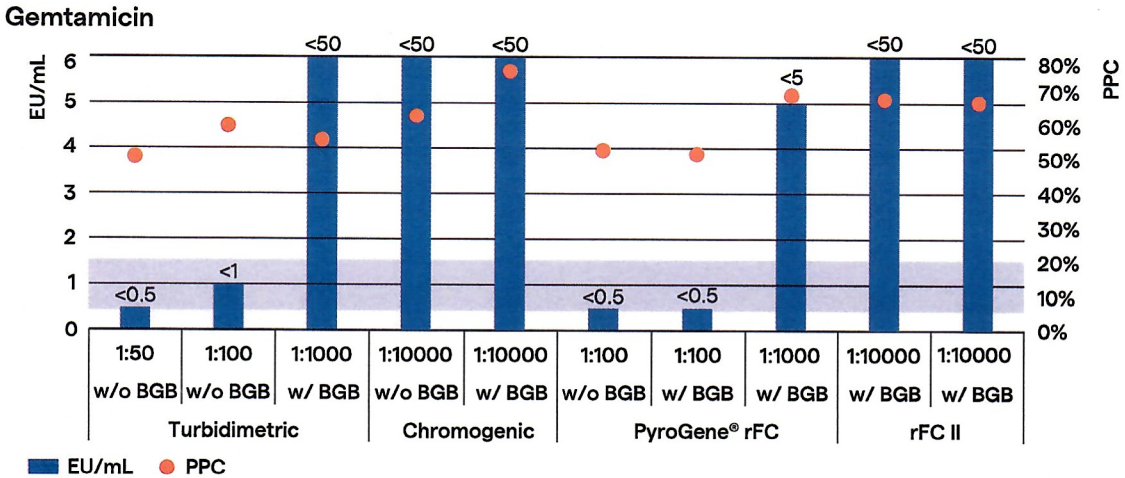


Figure 2.
Endotoxin (EU/mL) measured by four different assays in Gemtamicin.

Insulin

Human insulin is used to control blood sugar in people who have type 1 or type 2 diabetes. Insulin has an endotoxin release limit (ERL) of 330 EU/mL. The insulin sample was prepared with a nominal spike of 3.5 EU/mL. The acceptable detection range is 1.75 – 7 EU/mL indicated by the shaded area on the chart. Dilutions of 1:2, 1:10, 1:50, and 1:100 were compared between the four test methods. The Insulin data shown was collected without the use of beta glucan blocker. Of the four methods tested, turbidimetric, PyroGene® rFC, and rFC II assays were able to detect endotoxin in the acceptable range.

Insulin

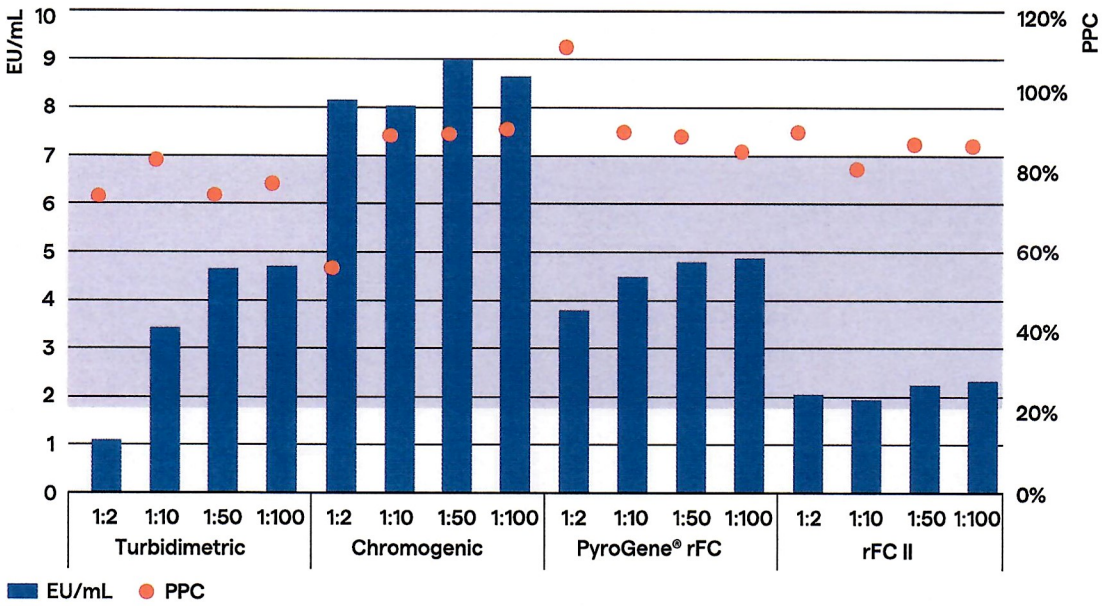


Figure 3.
Endotoxin (EU/mL) measured by four different assays in Insulin.

IV Saline

IV saline is a cornerstone of intravenous solutions commonly used in the clinical setting. It is a crystalloid fluid administered via an intravenous solution. IV saline has an endotoxin release limit (ERL) of 0.5 EU/mL. The IV saline sample was prepared with a nominal spike of 0.125 EU/mL. The acceptable detection range is 0.0625 – 0.25 EU/mL indicated by the shaded area on the chart. Dilutions of 1:1, 1:2, 1:5, and 1:10 were compared between the four test methods. The IV saline data shown was collected without the use of beta glucan blocker. Of the four methods tested, turbidimetric, chromogenic, and PyroGene® rFC Assays were able to detect endotoxin in the acceptable range.

IV Saline

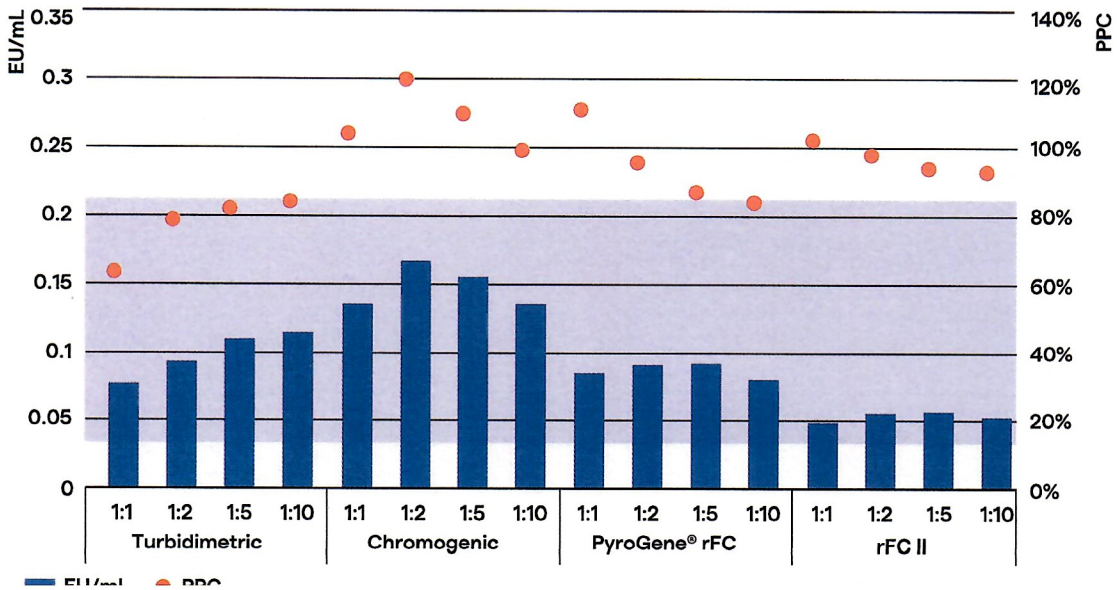
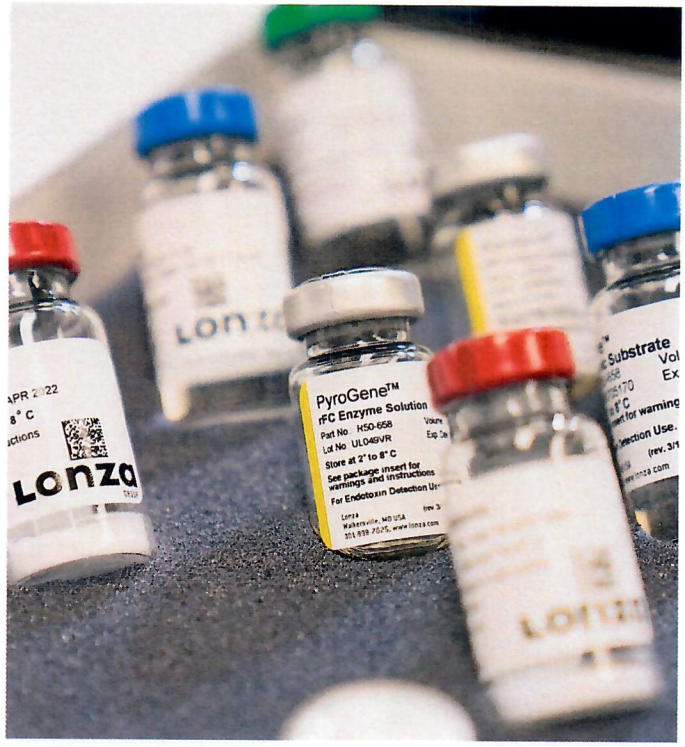


Figure 4.
Endotoxin (EU/mL) measured by four different assays in IV Saline

Summary

Results showed that for all products tested, the rFC methods provided comparable results to the LAL-based methods. PyroGene® rFC Assay's endotoxin detected and positive product control (PPC) recoveries were all within acceptable limits for all parental products tested. Due to the various physical/chemical differences of the products, some methods performed better than others on certain products. Variability is common when comparing different LAL-based methods. This is even true when comparing different rFC methods. The choice of reagent depends on the product being tested.

The PyroGene® rFC Assay showcased its ability to detect autochthonous endotoxin in a comparable fashion to LAL-based methods. 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). Validating PyroGene® rFC Assay in your lab is easy using Lonza's Validation Protocol.



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