A Q&A on USP <86>, Recombinant Innovation, and Future Trends

In recent years, the landscape of bacterial endotoxin testing **(BET)** has evolved beyond reliance on horseshoe crab-derived Limulus Amebocyte Lysate (LAL). Regulatory recognition, such as the introduction of USP <86>, and advances in recombinant and cartridge-based technologies have opened the door to more sustainable, efficient, and reliable testing methods.

In this Q&A, Courtney Wachtel, Product Manager from Charles River, provides insight into the implications of USP <86>, practical considerations for labs transitioning from LAL to recombinant methods, and the forward-looking potential of cartridge-based approaches.

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The Evolution of the Horseshoe Crab

Historically, horseshoe crabs were harvested extensively for fertilizer and livestock feed, and even killed by fishermen who saw them as predators of valuable shellfish. Over time, awareness of their ecological importance and the need for conservation has grown, shifting public perception toward preserving this unique species.

In 2011, the Atlantic States Marine Fisheries Commission (ASMFC) and LAL manufacturers developed "Best Management Practices for Handling Horseshoe Crabs for Biomedical Purposes." These guidelines set standards for careful collection and handling, and by 2023, these practices had been reinforced, updated, and integrated into operational requirements, fostering responsible stewardship of horseshoe crab populations.

ASMFC stock assessments, such as the 2019 Benchmark and 2024 update indicate that LAL manufacturing has a negligible impact on horseshoe crab populations. For instance, in 2024 the Delaware Bay population alone was estimated at around 56 million adults, and coast-wide LAL-related mortality represents a fraction of a percent of that total.

Q: How has the LAL testing process become more resource-efficient over time?

Courtney: Early LAL tests, particularly gel-clot assays, required substantial raw lysate. Over time, technology advanced to more efficient assays, like kinetic chromogenic methods that use significantly less lysate and, eventually, microfluidic assays that reduce lysate usage by up to 95%. Additionally, recombinant technologies now enable endotoxin testing without using horseshoe crab lysate altogether.

Q: What is recombinant BET, and why has it gained traction?

Courtney: Recombinant BET uses genetically engineered enzymes to detect endotoxins, reducing reliance on horseshoe crab-derived LAL. Initially limited to one vendor and facing regulatory uncertainties, recombinant methods have become more accessible and increasingly recognized as sustainable alternatives to LAL. Recent regulatory developments and market demand for environmentally responsible approaches have accelerated their adoption.

Q: Why were pharmaceutical companies slow to adopt recombinant methods, and what has changed?

Courtney: Companies initially hesitated because LAL was well-established, compendial, and backed by decades of data. Switching to recombinant methods required new equipment, training, and extensive validation without clear regulatory framework. As sustainability considerations, regulatory guidance, and market competition have improved, more companies are embracing recombinant tests.

Q: How have recent USP updates impacted the use of recombinant reagents in endotoxin testing, and has global regulatory harmonization been achieved?

Courtney: The inclusion of recombinant tests in official compendial chapters like USP <86> in 2024 and EP 2.6.32 in Europe has marked a turning point. These chapters provide guidance on validation, verification, and quality standards, making it easier for laboratories to integrate recombinant methods and move closer to full regulatory equivalency with LAL.

While USP <86> and EP 2.6.32 are significant steps, alignment is still limited. For example, EP 2.6.32 currently includes only Recombinant Factor C (rFC), and not the newer Recombinant Cascade Reagent (rCR). The industry and regulators are working to gather more data to support harmonization and full compendial recognition.

Q: How do these developments ultimately benefit pharmaceutical quality control and patient safety?

Courtney: By adopting recombinant tests that are sustainable, efficient, and increasingly recognized by

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regulators, pharmaceutical companies can ensure consistent product quality while reducing environmental impact. This modernization of endotoxin testing supports reliable, safe products for patients and aligns with global sustainability goals, benefiting the entire healthcare ecosystem.

Q: What is the outlook for LAL and recombinant methods moving forward?

Courtney: LAL will remain important for the foreseeable future, providing continuity for legacy methods and comparative studies. However, as recombinant technologies mature, gain regulatory clarity, and prove their sustainability and reliability, they are expected to become a more common, trusted tool in the quality control arsenal.

Q: With USP <86> now recognizing recombinant reagent-based endotoxin testing, what steps should pharmaceutical laboratories take to transition from traditional methods?

Courtney: Laboratories should review USP <86> and assess their current testing workflows. They can start small-scale feasibility studies comparing traditional LAL assays with recombinant methods under actual laboratory conditions. Engaging early with suppliers like Charles River to review validation packages and performing method verification in line with USP <1226> ensures a smooth transition. As more companies adopt recombinant methods, organizations will gain confidence that these approaches can meet regulatory requirements and quality standards without compromising reliability.

Q: How does USP <86> pave the way for broader adoption of recombinant cascade reagents, and how do these methods compare to rFC regarding performance and sustainability?

Courtney: USP <86> establishes a recognized framework for recombinant methods, which includes both Recombinant Factor C (rFC) and Recombinant Cascade Reagents (rCR). This recognition signals to manufacturers that investing in recombinant technologies is viable from both a compliance and sustainability standpoint. rCR methods maintain a similar sensitivity and specificity profile to rFC but are designed to more closely mimic the naturally occurring cascade reaction found in traditional LAL, potentially streamlining method suitability testing and regulatory acceptance. This could further reduce reliance on animal-derived resources while ensuring test accuracy and robustness.

Q: In light of USP <86>, what does the future hold for cartridge-based recombinant endotoxin testing solutions, and how might they improve efficiency and workflow in the QC laboratory?

Courtney: Cartridge-based recombinant endotoxin testing integrates reagents into a self-contained, standardized format that simplifies the testing process. As laboratories adopt recombinant methods recognized in USP <86>, cartridge technology offers a highly controlled environment for reactions, reducing analyst variability and potential contamination risks. For example, Charles River's cartridge technology is designed to work seamlessly with LAL and rCR reagents, enabling rapid, automated analysis that enhances throughput and reproducibility with the option to switch between animal-derived and animal-free reagents. Such platforms also minimize reagent waste, streamline training, and significantly shorten turnaround times compared to manual, reagent-intensive methods.

Q: How can data generated from cartridge-based recombinant methods support the eventual inclusion of these technologies in regulatory monographs?

Courtney: Regulators welcome industry-supplied data on performance across a wide range of sample types, as this evidence builds the case for broader acceptance. By demonstrating consistent accuracy, precision, and equivalence of cartridge-based recombinant methods under diverse conditions, pharmaceutical companies and service providers like Charles River can contribute to a robust data pool. Over time, this evidence can help influence monograph updates, making

recombinant methods fully interchangeable with LAL at a compendial level, thus encouraging faster industry-wide adoption and ongoing innovation.

Q: With recombinant methods continuing to evolve, how might next-generation cartridge technologies further enhance the sustainability and reliability of endotoxin testing?

Courtney: As recombinant reagents and cartridge platforms advance, we may see further reductions in reagent consumption, along with improved stability and shelf life of test components. This will not only reduce the environmental footprint but also improve the consistency of testing. Integrated data management and connectivity features can enable real-time traceability, ensuring compliance and facilitating data-driven decision-making in QC processes. Charles River and other industry leaders are already exploring enhancements that could further simplify testing, lower costs, and provide more actionable insights, ultimately driving better quality outcomes for patients.

If you are ready to transition to a more sustainable endotoxin testing solution, **click here**.

About Courtney Wachtel - Product Manager, Endotoxin Detection Assays and Services, Charles River Microbial Solutions

Courtney Wachtel is the Product Manager focusing on endotoxin detection assays and services for the Microbial Solutions division of Charles River Laboratories. In this role, she identifies customer needs and supports the commercialization objectives of key products. She holds a BS in Biology from the Georgia Institute of Technology, with certification in both Integrative and Biomedical biology, and has 10 years of experience in the pharmaceutical industry.

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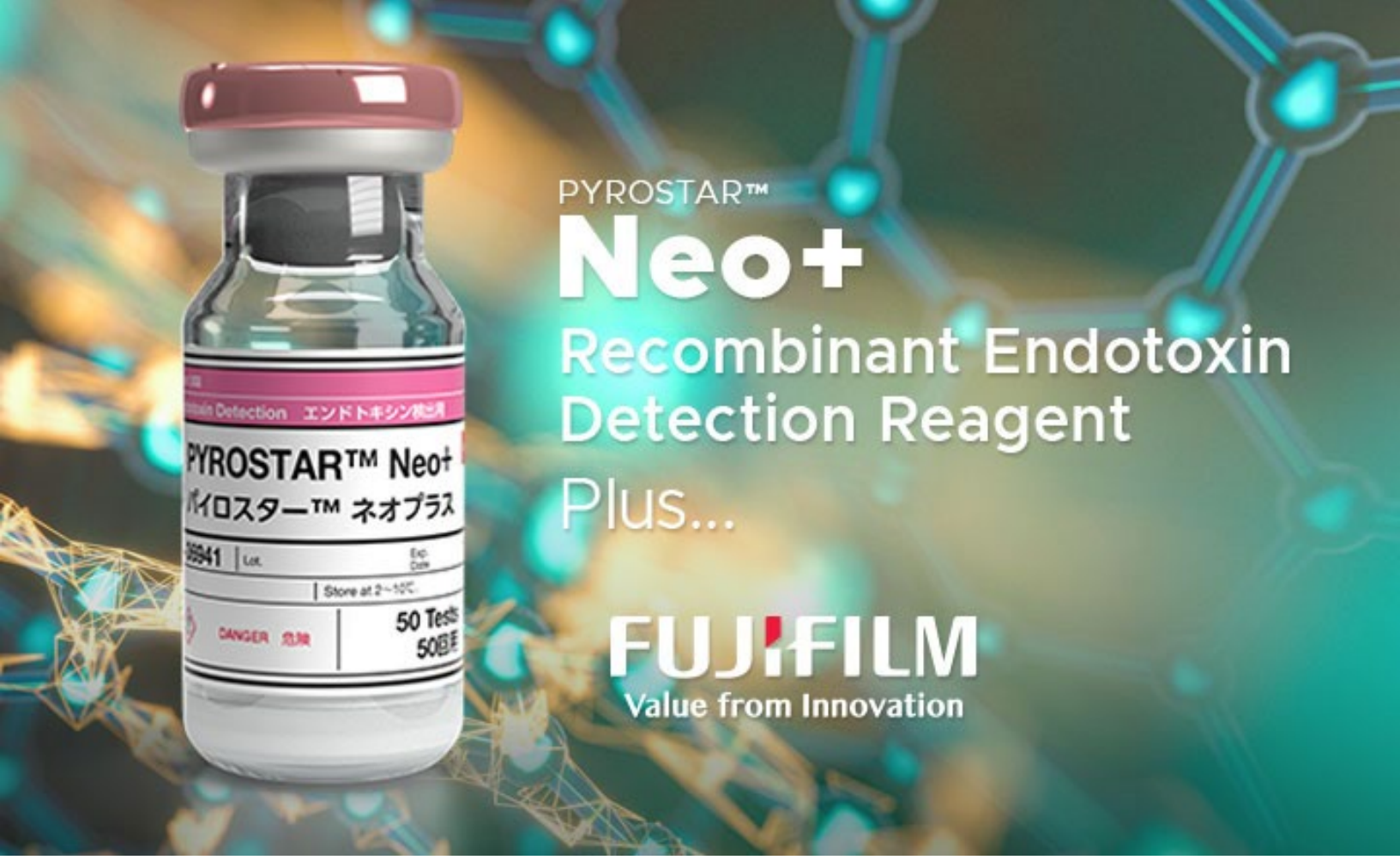

Sustainable and Compendial Endotoxin Testing with Recombinant PYROSTAR™ Neo+

FUJIFILM Irvine Scientific's commitment to sustainable and accurate endotoxin testing is reshaping the industry. By collaborating closely with laboratories, they're not just providing products but fostering a partnership that drives innovation and environmental responsibility.

In this rapidmicrobiology interview, Timothy Francis and Delaney Novak, Technical Specialists, LAL Division of FUJIFILM Irvine Scientific, share their knowledge and experience of helping labs implement more sustainable and accurate endotoxin testing solutions and talk us through the features and benefits of using FUJIFILM Wako's rCR (Recombinant Cascade Reagent) PYROSTAR™ Neo+.

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Q: How does the methodology for PYROSTAR™ Neo+ compare to the methodology of natural LAL?

Timothy and Delaney: Naturally derived LAL reagent is the basis for the test methods found in USP <85> for BET (Bacterial Endotoxin Testing). These are gel clot, turbidimetric, and chromogenic techniques. USP <86> for BET with recombinant reagents outlines two techniques—the fluorometric quantitative technique and the chromogenic absorbance technique. The fluorometric technique differs greatly from traditional LAL (Limulus Amebocyte Lysate) testing. However, the chromogenic technique is nearly identical to the chromogenic technique of USP <85> .

Since PYROSTAR™ Neo+ follows the chromogenic technique of <86>, its methodology is the same as the kinetic chromogenic technique of natural LAL. However, this cannot be said of every USP <86> compliant test, as the fluorometric techniques have no parallel technique in USP <85>.

One advantage of using rCR (Recombinant Cascade Reagent) PYROSTAR™ Neo+ is that is uses the same methodology, equipment, and accessories as natural LAL does. When using PYROSTAR™ Neo+, you will find that it is virtually identical to KCA (kinetic chromogenic assay) LAL methodology and does not require any special buffer for reagent dissolution nor fluorescence readouts. Additionally, PYROSTAR™ Neo+ can be used on an incubating absorbance plate reader and uses the same wavelengths of 405 and 650 nm that are used in a regular kinetic chromogenic assay.

Q: How does PYROSTAR™ Neo+ differ from other recombinant LAL products, such as rFC (recombinant Factor C)?

Timothy: rFC was the first recombinant BET reagent. By using synthesized “Factor C” (the protein in LAL that binds to endotoxin) along with a fluorescent reporter, an endpoint assay in which the endotoxin concentration can be quantified based on the fluorescent response was created. This reader and reagents used are distinct from natural LAL measurement techniques.

Neo+ is a recombinant cascade reagent (rCR), in which the complete 3-factor protein cascade of natural LAL is replicated, allowing for the same chromogenic substrate used in chromogenic LAL to be used to create Neo+’s signal response to endotoxin. This produces an endotoxin assay remarkably like natural LAL’s results and allowing for the advantages of kinetic measurements to be realized in the recombinant reagent’s results.

rFC was the pioneer method that allowed for a synthetic BET reagent. rCR reagents including PYROSTAR™ Neo+ are a more complete synthetic replication of natural LAL reagents and techniques.

Q: What preliminary testing or verification is needed to meet the requirements for USP <86>?

Timothy: The preliminary validation of accessories, personnel training, and IQ/OQ and calibration of instruments remains the same with USP <86> as those required of USP <85>.

Additionally, the assurance testing of the standard curve for each lot of Neo+ and the interference testing of each product remains unchanged from USP <85>.

For a product that is new to BET testing and does not currently have monographic requirements, no additional preliminary testing and verification is required.

For products with existing USP <85> monographic requirements, comparison testing with current natural LAL reagent and some additional testing as required by the regulatory body may be necessary.

Q: What expectations are there that monographs will include USP <86>?

Timothy and Delaney: Currently, monographs that list only USP <85> as the required methodology for Bacterial Endotoxin Testing can still use recombinant methodologies outlined in USP <86> as a compendial alternative method. As a result, a full validation as outlined in USP <1225> is not required, but as outlined in USP <1226>, the verification considerations found in USP <86> can be considered.

This means that users who would like to transition to recombinant reagents will need to perform verification and

product-specific testing, which is the same preliminary testing that is required for new users of natural LAL as well.

These considerations in USP <86> are nearly the same as those already found in <85> with the addition of a comparison study with <85> and potentially other considerations asked of by the regulatory bodies. A common example would be recovery of NOEs (Naturally Occurring Endotoxins) found at the client's site.

While there is not a specific timeline to update each monograph to include USP <86>, end users of specific monographs are encouraged to share data with the USP when using recombinant methodologies.

Q: What considerations would a client need to take if they have a product without a monograph and an endotoxin limit that was calculated according to the directions of USP <85> (with K/M)?

Delaney: Like USP <85>, USP <86> also includes guidelines on calculating the endotoxin limit for a drug product, as well as how to calculate the MVD (Maximum Valid Dilution), for a product based on the test sensitivity and endotoxin limit. Additionally, the guidelines for calculating an endotoxin limit have remained unchanged. Users can still calculate endotoxin limits for their products by using K/M, which is the threshold pyrogen dose, or "K," divided by the maximum bolus dose per hour, or "M." However, a client should perform preparatory tests, including product-specific testing as well as interference testing, when adopting a new methodology.

Q: How has the formulation of PYROSTAR™ Neo+ changed from its predecessor, PYROSTAR™ Neo?

Timothy: The recombinant proteins found in Neo+ remain the same as found in Neo. The only changes were made to the buffers and excipients in the formulation.

The performance of the reagent remains unchanged as outlined in the specifications and package insert.

However, this change in formulation allows for some improvements in performance. Heparin-based products and certain cellular products presented interference to Neo. This interference is eliminated with Neo+. Also, the stability of negative controls is increased with Neo +. Additionally, the new formulation of Neo+ allows the reagent to be used on both a tube reader and a microplate reader, whereas Neo was only validated for use on a microplate reader.

Q: Can this chapter be applied to medical devices?

Delaney: Currently, USP <161>, which outlines guidance for BET for medical devices, lists USP <85> as required testing for these devices. However, with USP <86> now being a compendial alternative method, users can now use recombinant LAL reagents for BET for medical devices, following <1226> verification requirements.

Q: Is this chapter harmonized?

Delaney: While USP <85> on BET is harmonized with Ph. Eur. Chapter 2.6.14 and JP General Test No. 4.01, USP <86> on BET with recombinant reagents is not harmonized with any other pharmacopeia currently.

Currently, the European Pharmacopoeia only lists rFC as a compendial method for BET in Chapter 2.6.32. This differs from USP <86>, which outlines both rFC and rCR as compendial methods for BET. Additionally, while recombinant methodologies are now considered to be compendial methods in the US and Europe, recombinant LAL methodologies are still only a "compendial guidance chapter" in Chinese, Japanese, and Korean pharmacopeias.

Q: What research has been published and is available to evaluate PYROSTAR™ Neo+?

Delaney: For PYROSTAR™ Neo+, we offer a primary validation package that covers aspects of linearity, range, quantitation/detection limit, accuracy, and precision following USP <1225> and ICH Q2(R2) guidance on adoption of an alternative method.

Additionally, there has been various research published on PYROSTAR™ Neo, which includes product-specific client data, including the following:

- Kikuchi, Y, et al. 2023. Collaborative Study of Bacterial Endotoxins Test Using Recombinant Factor C-Based Procedure for Detection of Lipopolysaccharides (Part 3).

- Bolden, J. 2023. An Ongoing Analysis of Recombinant and Naturally Derived BET Reagent Comparability. Indianapolis: Eli Lilly and Company.
- Kim S, et al. 2023. LPS Administration during Fertilization Affects Epigenetic Inheritance during Embryonic Development .
- Yoneda E, et al. 2024. Evaluation of Lipopolysaccharide and Interleukin-6 as Useful Screening Tool for Chronic Endometritis.

Q: How does FUJIFILM support validation for PYROSTAR™ Neo+?

Timothy and Delaney: At FUJIFILM, we supply a primary validation package for Neo+ that includes both a test plan and a test report so that users can replicate testing on site and compare results. We are also available to assist in creating product-specific test plans to accommodate <1226> verification requirements. Additionally, we can facilitate the transition to recombinant LAL for users who currently use a natural LAL methodology.

Those who currently use a kinetic chromogenic LAL assay will find that the transition to Neo+ does not require the purchase of any additional materials or equipment.

A user transitioning from the kinetic turbidimetric method may need to ensure their reader can perform a measurement at the correct wavelength, but all other materials should remain the same.

Users transitioning from quantitative endpoint methods may need an additional reader if their current reader cannot perform kinetic reads.

Finally, users transitioning from the gel clot method, as they would transitioning to any quantitative method, will need new equipment and materials. However, as we would with any potential client, we at FUJIFILM will be able to aid the client in transitioning and validating the new equipment and methods.

To make sure your endotoxin method is the right choice for your products, visit www.**wakopyrostar.com**

About Timothy Francis

Timothy Francis is the Senior Technical Specialist for the LAL Division of FUJIFILM Irvine Scientific. He holds a B.S. in Biochemistry and a M.S in Science Education. He comes into the Technical Specialist role with 5 years of experience teaching the natural sciences at a college level. He is proficient at taking the complex, technical aspects of a topic and breaking them down into clear, understandable pieces that all connect back to the big picture. He draws upon this experience to provide professional technical support and training for the PYROSTAR™ line and to help you with your technical needs.

About Delaney Novak

Delaney Novak is a Technical Specialist for the LAL Division of FUJIFILM Irvine Scientific. She holds a B.S. in Environmental Science alongside a minor in Biology. She enjoys working in a collaborative environment and is always open to addressing new challenges and answering complex questions. She applies these skills to further support any technical needs or concerns you may have in your bacterial endotoxin testing endeavors.

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Lonza's rFC:
Sensitivity That Sets the Standard

Lonza's groundbreaking rFC (Recombinant Factor C) assay is transforming endotoxin testing in the bioprocessing industry. This innovative alternative to traditional LAL assays offers a reliable and efficient method for detecting bacterial endotoxins, which are essential to help ensure product safety. According to Allen Burgenson, Global SME in Bioprocessing Testing at Lonza, implementing Lonza's rFC assay allows companies to enhance their product safety measures while optimizing their testing processes in the critical field of endotoxin detection.

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Lonza

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Q: How does the Lonza rFC assay compare to Lonza's LAL offering?

Allen: Lonza's PyroGene® recombinant Factor C assay was introduced in 2003 as a sustainable alternative to using Limulus Amebocyte Lysate, which is derived from the blood of the American Horseshoe Crab (Limulus polyphemus). The assay has the same sensitivity as our Kinetic QCL® LAL assay (0.005 EU/mL), and is less susceptible to interferences, including enhancement due to the presence of beta-glucans. Side-by-side comparisons show comparable results in most applications.

Q: How does the PyroGene® rFc Assay work? What applications is it most suitable for?

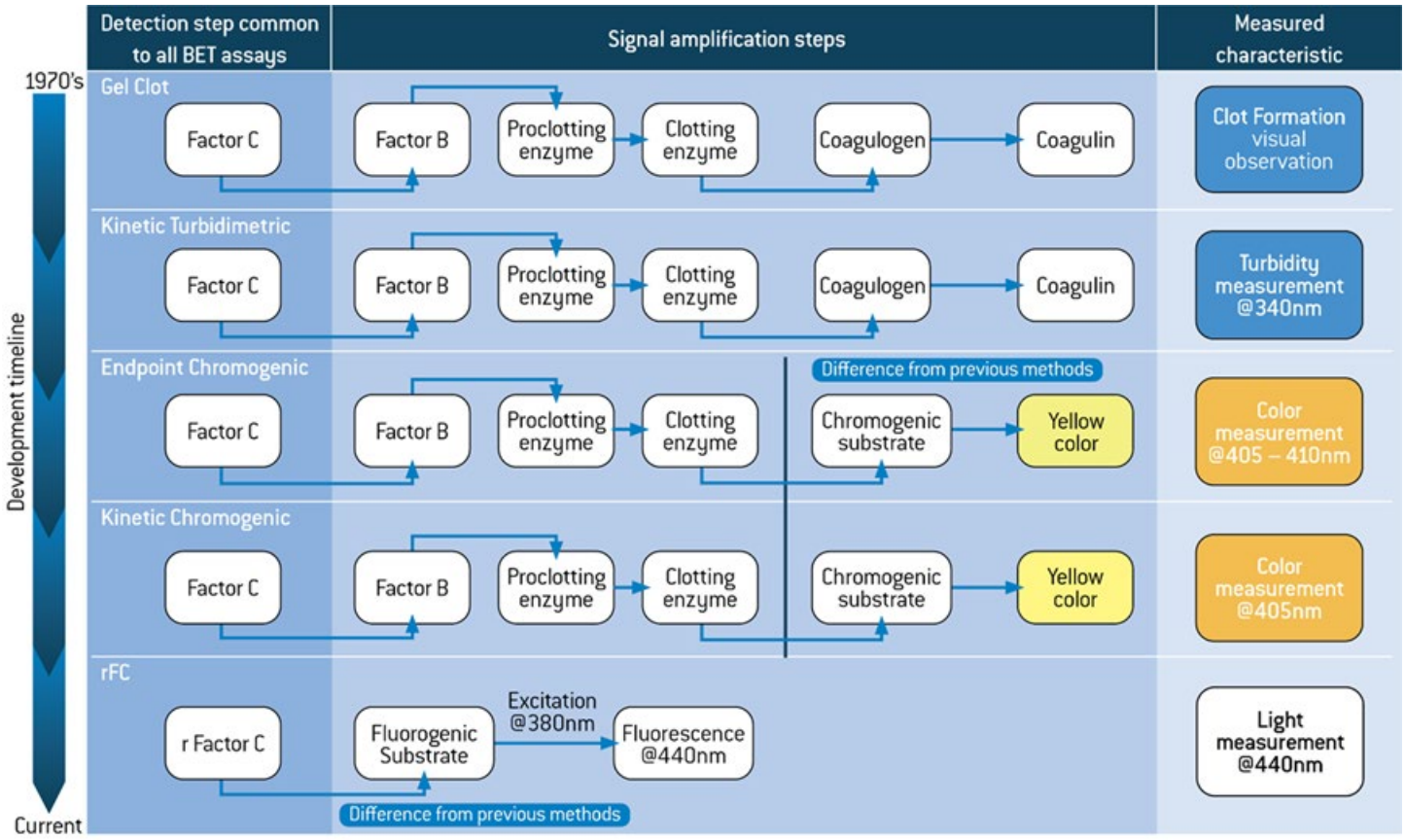
Allen: The PyroGene® rFC assay uses a recombinant form of Factor C that is found in every other endotoxin detection reagent (see Figure 1 below). It directly cleaves a fluorogenic substrate to yield light upon excitation, eliminating the need for the signal amplification steps found in classical assays which are subject to interferences.

The PyroGene® rFC assay works in almost every application that our classical LAL reagents are used in, with the exception of products that are fluorescent. Most current applications are for water, which accounts for approximately 75-80% of all pharmaceutical testing.

Q: What are the most common problems labs face with LAL-based testing?

Allen: All LAL-based assays are subject to "enhancement" or a reaction making the apparent endotoxin level appear higher than it actually is. Such interference must be overcome using a modified assay or a substance like β-glucan blockers that block the Factor G pathway contained in LAL reagents. The PyroGene® rFC assay doesn't follow the Factor G pathway.

Figure 1 - BET Assay Reaction Comparison



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Lonza

"The PyroGene® rFC assay is a liquid preparation and as such, needs no reconstitution step. You simply mix the volume of components you need to test your samples, greatly reducing wasted reagents. This is ideal in high-volume or automated laboratories."

Q: What makes Lonza an advanced solution when compared to other options in the market?

Allen: The PyroGene® rFC assay is a liquid preparation and as such, needs no reconstitution step. You simply mix the volume of components you need to test your samples, greatly reducing wasted reagents. This is ideal in high-volume or automated laboratories.

Q: Is the PyroGene® rFC assay suitable for automation?

Allen: Absolutely! The PyroGene® rFC assay has been used on the PyroTec® PRO robotic system in dozens of facilities around the world. Some are high-throughput facilities testing thousands of samples in a month, and the ease of preparing large volumes of reagent as needed is a significant benefit to them.

Q: How can Lonza support with documentation for audits?

Allen: Our dedicated teams can provide documentation to support customer audits and regulatory filings. Additionally, the method validation was published in the Jan/Feb 2010 issue of Pharmacopeial Forum, the official journal of the United States Pharmacopeia. A reprint of the article is also available upon request.

Q: What level of support can Lonza offer?

Allen: Lonza's Scientific Support team and Global SME group are ready to assist customers with a wide range of applications, including testing, validation support, and regulatory filings. We also offer hands-on assay training, either at our facility or yours.

Q: Can you give any effective strategies on how to transition to rFC methods?

Allen: Yes, Lonza can provide a step-by-step validation protocol for our PyroGene® rFC assay. In addition, our Scientific Support, Services, and Global SME teams can provide support for product validation, comparability, and more.

Are you ready to revolutionize your endotoxin testing process? Experience the Lonza difference for yourself. Visit Lonza.com/PyroGene

About Allen Burgenson - Global Subject Matter Expert - Testing Solutions at Lonza Walkersville, Inc.

Allen L. Burgenson has over 35 years of experience in industries regulated by the FDA, including Foods, Drugs, Biologics, Medical Devices, and Cosmetics. He has worked in R&D, QC, QA, Regulatory Affairs, and now Marketing as an SME for endotoxin detection. Allen is involved in several scientific organizations including, the immediate Past-Chair of the Horseshoe Crab Advisory Panel for the Atlantic States Marine Fisheries Commission (ASMFC) and the immediate Past-President of the Capital Area Chapter of the Parenteral Drug Association (PDA). He was Chair of the 2004 PDA Annual Meeting. He is a co-author of three PDA Technical Reports including, TR-50 and 51 regarding Mycoplasma detection and filtration, and TR-82 regarding Low Endotoxin Recovery (LER). Allen also contributed to USP Informational Chapter <1228.5> on Endotoxin Indicators. Allen has a BA in Microbiology from Rutgers University and an MS in Biotechnology Management from the University of Maryland.

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