A SUPPLEMENT TO AMERICAN PHARMACEUTICAL REVIEW

American Pharmaceutical Review

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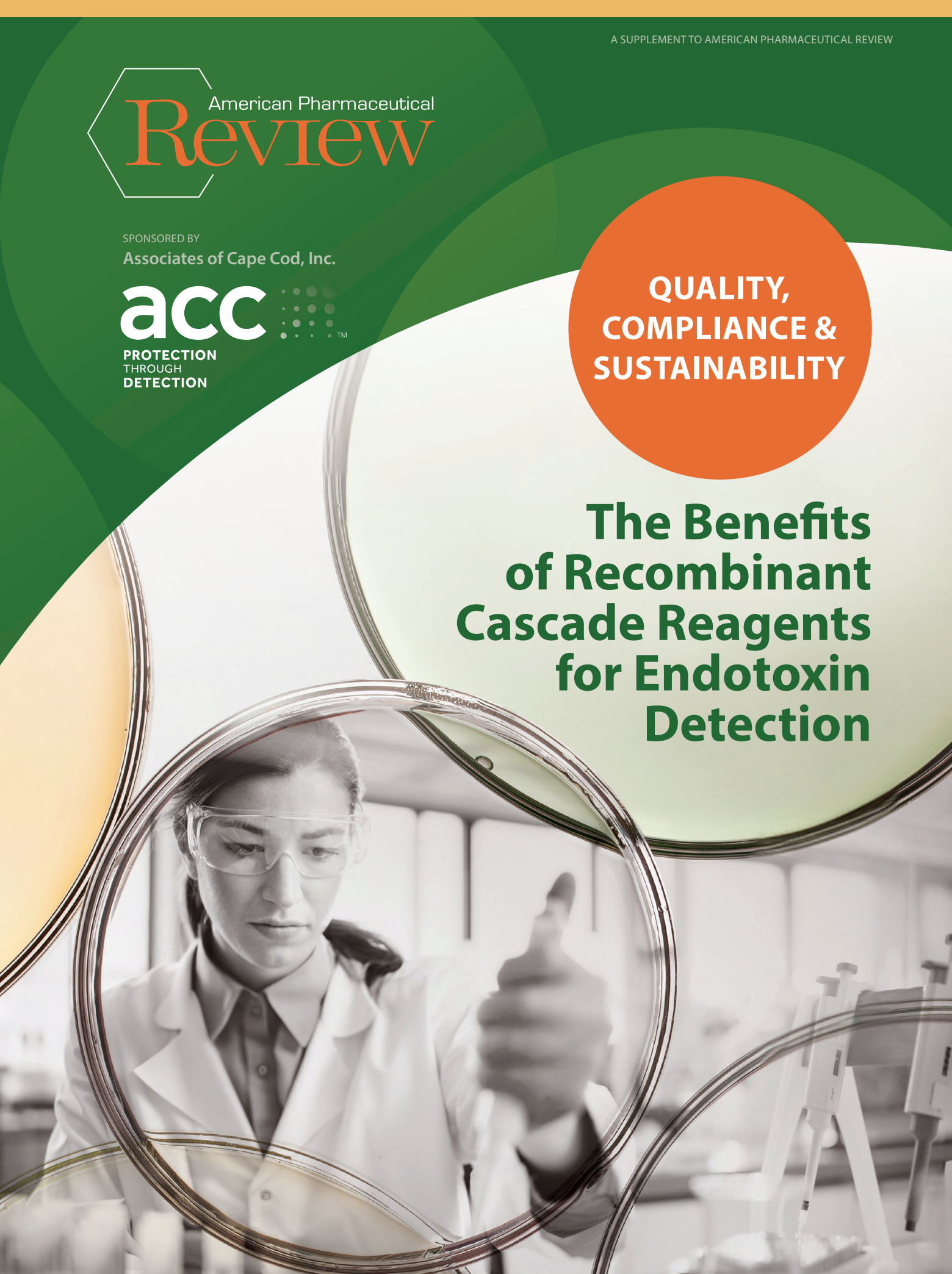
QUALITY, COMPLIANCE & SUSTAINABILITY

The Benefits of Recombinant Cascade Reagents for Endotoxin Detection



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Your journey from LAL to rCR begins here.

As the pioneer in endotoxin detection Associates of Cape Cod, Inc. (ACC) proudly recognizes and supports the scientific and regulatory validation of recombinant technologies, which offer sustainable alternatives that align with the industry's increasing focus on principled sourcing and environmental responsibility.

With that said, inclusion of recombinant reagents in USP <86> is the beginning of a broader transition. Real-world implementation will require a phased, multi-tiered approach that takes into account several complex variables.

As you navigate your own transformation journey — from qualitative to quantitative to recombinant — count on ACC for the highest-quality products and support.

PyroSmart®
NEXT GEN Recombinant Cascade Reagent (rCR)

More Than Recombinant. It's Recombinant Cascade.

Recombinant reagents are not created equal. **Recombinant cascade reagent, rCR,** mimics the entire Limulus amebocyte lysate (LAL) cascade, which means you can use the same instruments and preparation steps.

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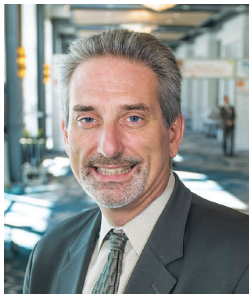
A Note from the Editor



Mike Auerbach
Editor in Chief
American Pharmaceutical Review

The pharmaceutical industry continues to evolve, with sustainability, ethical responsibility, and regulatory compliance driving innovation at every level - including quality control and safety testing. Nowhere is this more evident than in the field of endotoxin detection, where the transition from traditional Limulus Amebocyte Lysate (LAL) tests to recombinant cascade reagents (rCR) marks a pivotal moment for both science and environmental stewardship.

For decades, LAL tests - derived from the blood of horseshoe crabs - have been the cornerstone of bacterial endotoxin detection. However, growing concerns about the ecological impact of harvesting horseshoe crabs and the threat to these remarkable, ancient animals have propelled the search for sustainable alternatives. In response, the emergence of recombinant cascade reagents (rCR) represents not just a scientific breakthrough, but a compelling shift towards sustainability. Unlike traditional LAL, rCR products are produced using recombinant DNA technology, meaning they do not require animal-derived materials. This change drastically reduces pressure on horseshoe crab populations and aligns with the industry's commitment to biodiversity and animal welfare.

In addition to their environmental advantages, recombinant cascade reagents offer enhanced specificity and avoid cross-reactivity with 1,3-β-D-glucans - a limitation occasionally seen with traditional LAL tests. This improved performance has helped build confidence throughout the industry.

The last year has seen significant new developments. Regulatory bodies, including the European Pharmacopoeia and the US Food and Drug Administration, have issued updated guidance acknowledging the validity and equivalence of recombinant reagents for endotoxin testing. In fact, several jurisdictions have moved to formally recognize rCR-based tests, facilitating their adoption and streamlining approval pathways for manufacturers. These regulatory milestones underscore the growing consensus within the global scientific and regulatory communities that sustainable, animal-free alternatives can, and should, replace traditional methodologies wherever feasible.

Our publication takes an in-depth look at the expanding implementation of recombinant cascade reagents, from the scientific underpinnings to the practical considerations for manufacturers. We examine the tangible sustainability benefits, the evolving regulatory landscape, and the technical challenges and opportunities that accompany this transition.

The momentum behind rCR adoption is unmistakable—and as new regulatory frameworks and sustainability goals reshape our industry, recombinant technologies are set to become the new standard in endotoxin testing.

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May 12, 2025

ACC Position Statement on USP Chapter <86> Inclusion of Recombinant BET Reagents

The recent update to USP Chapter <86> to include recombinant bacterial endotoxins test (BET) reagents—specifically recombinant cascade reagents (rCR) such as ACC's own PyroSmart NextGen®, and first-generation recombinant Factor C (rFC) - marks a significant and welcome milestone in the evolution of endotoxin testing technologies.

As the pioneer in endotoxin detection with over 50 years of innovation, ACC proudly recognizes and supports the scientific and regulatory validation of recombinant technologies within the BET landscape. We believe recombinant reagents represent an important advancement, offering sustainable alternatives that align with the industry's increasing focus on principled sourcing and environmental responsibility.

ACC has been at the forefront of this shift, having introduced the world's first commercially available rCR reagent, PyroSmart NextGen®, four years ago. Since then, we have actively supported peer-reviewed studies and customer-driven comparability data to demonstrate that rCR reagents can match—and in many cases exceed—the performance and consistency of traditional LAL-based methods.

That said, it is important to emphasize that regulatory evolution does not immediately equate to industry-wide adoption. The inclusion of recombinant reagents in USP <86> is the beginning of a broader transition. Real-world implementation will require a phased, multi-tiered approach that takes into account the complexity of global regulatory expectations, the diversity of testing platforms, and the validation resources needed by manufacturers.

We caution against the misconception that the industry can or should move away from traditional LAL overnight. While innovation must be embraced, health and human safety must remain the primary driver. Traditional LAL reagents will continue to play a critical role in ensuring compliance and safeguarding public health as the industry gradually adapts to these new technologies.

ACC remains committed to supporting this transition through education, data transparency, and the continued development of robust, reliable solutions for endotoxin testing. We are proud to be a trusted partner to our customers and regulators as we navigate this next chapter - together.

MKT#25_292

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Specializing in chromogenic and turbidimetric reagent technologies, Associates of Cape Cod, Inc. (ACC) has been a global leader in endotoxin and (1→3)-ß-D-glucans detection products and services for over 50 years. ACC pioneered LAL testing methodology and was the first FDA licensed company to manufacture LAL reagents, and throughout the years has grown to be an internationally recognized leader in endotoxin detection.

In 2021, we were very excited to introduce another first when we launched the first commercially available sustainable BET Recombinant Cascade Reagent (rCR), PyroSmart NextGen®. PyroSmart NextGen® is completely horseshoe crab blood free and unlike first generation recombinant BET reagents (rFC), PyroSmart NextGen® uses the same LAL cascade as traditional LAL reagents, while eliminating the potential for (1→3)-ß-D-glucans. This eliminates the need to change test methods and purchase new specialized instrumentation as required by first generation rFC recombinant reagents. Simply put… same Instrument, same preparation steps, same method. Furthermore, with the proposed new standard, Chapter <86> from The USP Expert Committee to include rCR recombinant reagents, there's never been a better time to consider transitioning to PyroSmart NextGen®. **Keep your Method… Make an Impact!**

Our worldwide headquarters are located in Falmouth, Massachusetts. With a dedication to quality, ACC is certified to I.S. EN ISO 13485:2016 and ISO 13485:2016. We are FDA Inspected and operate DEA Licensed and CLIA-certified laboratories. Our endotoxin detection reagents, instruments and software are used within the Pharmaceutical, Medical-Device, Biotechnology, Compounding Pharmacy and Dialysis industries for quality control, product release and research. Our reagents are FDA licensed and can be used for testing in compliance with USP, EP and JP bacterial endotoxin test chapters, and our software is 21 CFR Part 11 Compliant.

ACC also operates a Contract Test Services (CTS) Laboratory which has specialized in testing for endotoxin and glucan contamination for over 40 years. Our CTS laboratory is GMP compliant, ISO registered and DEA licensed and is capable of handling all controlled drug substances except those included in Schedule 1. All testing services can be performed to FDA, USP, EP and/or JP regulatory guidelines. In addition to routine testing, our CTS Laboratory will customize endotoxin testing, troubleshoot difficult samples, develop and/or transfer LAL test methods, design and produce custom depyrogenation controls for oven validation and perform Low Endotoxin Recovery (LER) studies/protocols.

ACC also offers a clinical diagnostic product line – Fungitell® and operates a CLIA-certified laboratory specializing in (1→3)-ß-D-glucans analysis to support the diagnosis of Invasive Fungal Disease (IFD).

rCR Implementation Guide

In an era where secure supply chain and sustainability are paramount, the implementation of PyroSmart NextGen® (PSNG) - recombinant Cascade Reagent (rCR) for endotoxin testing is a necessary step forward. Designed for QC managers, QC directors, Regulatory Affairs professionals, and Sustainability Officers, this guide provides a roadmap for adopting rCR in your quality control systems.

Disclaimer: The information provided in this document is given for the purposes of education and discussion. It is not intended to be, and it should not be used as, a substitute for regulation and regulatory guidance. Decisions and actions should be based on the relevant regulations, guidance documents and pharmacopeial chapters, not on this document. This guide provides information about implementation of PSNG reagent only which is the only widely published recombinant cascade reagent commercially available. The analytical performance of other recombinant cascade reagents should not be assumed to be identical to PSNG.

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Additional information available at www.acciusa.com or via techservice@acciusa.com.

Why Implement PyroSmart NextGen® Now?

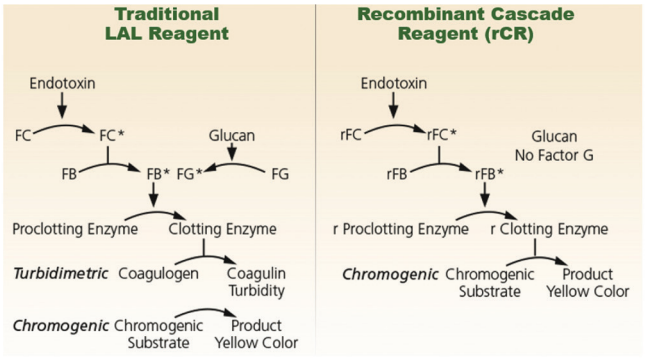
Implementing recombinant cascade reagent PyroSmart NextGen® (PSNG) offers several compelling benefits:

- **Regulatory acceptance:** the new US Pharmacopeia Chapter <86> Bacterial Endotoxin Test Using Recombinant Reagents (official as of 01 MAY 2025) includes recombinant cascade reagents (such as PSNG) as one of the options of recombinant reagents.[1]
- **Mitigation of supply chain risk:** globally, over 70 million BET tests are performed per annum, with 90% of all tests done using LAL reagents. Growing demand for medicines (thus growing need for BET assays) together with escalating restrictions to harvest horseshoe crabs create a supply chain risk. The use of PSNG mitigates this risk.
- **Non-animal derived reagent:** the use of PSNG reduces global dependency on wild marine arthropods as the raw material for a critical quality control test.
- **Sustainable production:** PSNG allows for aligning with global sustainability goals and the 3R ethical guidelines (Replacement, Reduction, Refinement).
- **Technical advancements:**
    1. Documented superior analytical performance: lot-to-lot reproducibility of PSNG (shown on Certificates of Compliance.[2]
    2. Rapid test with Time to Result shorter than LAL assays.[2]
    3. Documented specificity of PSNG to a range of endotoxins (including those with four fatty acid chains within Lipid A structures) and no cross-reactivity with 1,3-beta glucans.[3]
    4. Documented suitability of PSNG with a wide range of products, including lower degree of interference in many products.[3,4]
    5. Documented comparability/equivalency of PSNG to LAL in a variety of matrixes.[3-7]
- **Seamless Integration:** PSNG is compatible with existing photometric platforms, allowing for straightforward incorporation into existing BET procedures.
- **Standard of Quality:** PSNG is produced under rigorous cGMP conditions in FDA-licensed facility for manufacture of Limulus Amebocyte Lysate (LAL). PSNG FDA Drug Master File is available for review via US FDA (MF# 031277) – the only Master File for rCR to date.
- **Compatibility with automation systems for BET:** PSNG was proven to be adopted for high sample throughput productions.
- **Technical resources:** Access to Global Technical Services (available in local time and languages) regarding any specific implementation concerns and troubleshooting: techservice@acciusa.com. Available per request:

1. Reagent Transfer Protocol
2. Method Validation Package
3. Peer-reviewed publications
4. Contract Test Services

Principle of PyroSmart NextGen®

PSNG contains recombinant Factor C, Factor B and Pro-clotting enzyme co-lyophilized with chromogenic substrate into a reagent.[8] It is kitted together with a reconstitution buffer (in a number of kit configurations). As such, PyroSmart NextGen® is a reagent for kinetic chromogenic technique: a quantitative enzymatic assay based on the measure of change in absorbance at 405nm which is proportional to the dose of bacterial endotoxins present in the sample. The kinetic chromogenic technique is included in USP Chapter <85>.[9] Therefore, the stoichiometry and output of PSNG is well understood, published and accepted as a compendial technique. The dynamic range of PSNG is 50 to 0.001 EU/mL, depending on the type of existing absorbance reader.



rCR such as PSNG are included in USP <86> as one of two types of recombinant reagents. Per USP FAQ: <86> Bacterial Endotoxins Test using Recombinant Reagents, *"The Endotoxin Subcommittee to the USP Microbiology Expert Committee (MEC) developed Chapter <86> based on an extensive review of public and submitted confidential end-user data and validations, and the available peer-reviewed literature. Extensive data exists in the peer-reviewed literature for the BET using rFC and rCRs, and the MEC was also able to review non-public/confidential data during the development of the chapter. This information was considered in combination with the recognition that recombinant reagents are the biotechnology equivalent of natural Factor C, the biosensor responsible for endotoxin detection in horseshoe crabs. Additionally, the rCR enzymatic cascade begins with rFC. Combining all of this information and data, the MEC agreed that the inclusion of rCR was supported by the science and data reviewed. Advancing an rBET chapter is aligned with USP's commitment to expanding the use of animal-free methods and materials."*[10]

PSNG was cloned based on the genome of *Limulus polyphemus.*

Key Differentiators Between LAL and PSNG

Understanding the differences between Limulus Amebocyte Lysate (LAL) and PSNG recombinant cascade reagent is crucial when performing validation studies:

- **Source Material:** LAL is derived from horseshoe crabs, whereas PSNG is produced through recombinant DNA technology.
- **Specificity:** PSNG provides specificity to endotoxins only, no cross-reactivity with 1,3-beta glucans due to the absence of Factor G.
- **Composition:** PSNG formulation is based on decades of research and trials conducted by Seikagaku/ACC endotoxin experts. It reflects the most current knowledge of endotoxin biochemistry. Unlike LAL, PSNG formulation does not contain additional blood-derived proteins and peptides that regulate the blood clotting mechanism in horseshoe crabs.

Status of USP <86>

At present time, the methods described in USP <86> (official on 01 MAY 2025 in USP 2025 Issue 1) are considered to be alternative methods to USP <85> Bacterial Endotoxins Test. Per USP General Notices and Requirements, 6.30. Alternative and Harmonized Methods and Procedures: "An alternative method or procedure is defined as any method or procedure other than the compendial method or procedure for the article in question. The alternative method or procedure must be fully validated (see USP <1225> Validation of Compendial Procedures and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis."[11,12]

STEP 1: User Requirements

Successful implementation of PSNG rCR necessitates a clear understanding of user requirements in order to ensure an effective transition within quality control processes. This is achieved by producing a User Requirement Specification document. Meeting the user requirements will be demonstrated and/or tested as part of the validation protocol.

STEP 2: Equipment, Consumables and Resources Required

Successful implementation of PSNG rCR necessitates a clear understanding of specific user needs that ensure an effective transition within quality control processes:

- **Equipment:**

Equipment	Available Yes/No
Incubating absorbance reader: microplate reader or Pyros Kinetix Flex® tube reader	
Absorbance detection at 405nm wavelength	
Current software built with settings needed for PSNG	
A set of pipettes (P20, P100, P1000)	
Repeater or multichannel pipette	
Vortex mixer	

- **Consumables:** All must be certified free of interfering endotoxins and must be meet the vendor's requirements

Consumables	Available Yes/No
PSNG lot matched with CSE lot (Certificate of analysis available)	
Reaction vessels (i.e. Pyroplates 96well plate for microplate readers or 8x75mm glass reaction tubes for tube readers)	
Dilution tubes	
Pipette tips	
Repeater Combi-tip (for repeaters) or a reservoir (for multichannel pi-pette)	
LAL Reagent Water	

- **SOP Transfer:** Since the use of PSNG is like-to-like with kinetic photometric assays, existing LAL SOPs can be quickly adopted for use with PSNG.
- **Training and Education:** Training the analyst on the best practices of reagent handling and assay preparation, emphasizing the differences between the in-house LAL and PSNG reagent.

STEP 3: Risk Analysis

Before proceeding with implementation, conduct a product-specific risk assessment relevant to bacterial endotoxin:

- Evaluation of the regulatory barrier for each sample based on their regulatory status (whether monographed or non-monographed)*
- Evaluation of the likelihood of endotoxin contamination (based on historical data sets and manufacturing process)**
- Evaluation of severity of endotoxin contamination**
  - *Process microflora: corroborating data (i.e. bioburden data and associated identification of the bacteria; Total Organic Carbon data for purified waters) may be used to determine the occurrence of the most sever risk such as false negative results.*

The output of the risk analysis is grouping samples into three categories based on the regulatory threshold:

- **No regulatory threshold samples**
- **Low regulatory threshold samples**
- **High regulatory threshold samples**

PSNG may be implemented in a phase-wise process starting with the lowest regulatory threshold samples.

*Detailed information can be found in Appendix 1

**Detailed information can be found in Appendix 2

STEP 4: Implementation

PHASE I - No Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with no additional testing requirements. The end user shall follow the general expectations as defined in USP <86> under Preparatory Testing and USP <1085> under Method Suitability Testing in order to comply with all the scientific principles of the test:

1. Assurance of the criteria for the standard curve when using PSNG per USP <86>

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	|R| ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Notes:	1 lot of PSNG	

2. Method suitability testing when using PSNG per USP <1085>: must be demonstrated for each product individually (13)

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	|R| ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Product	Series of dilutions not exceeding MVD in duplicate	< Endotoxin limit
Positive Product Con-trols (PPC)	Series of dilutions spiked with CSE to the middle of standard series in duplicate	PPC recovery % = 50 – 200%
Notes:	1 lot of PSNG 1 lot of product	

3. Test for Interfering factors when using PSNG per USP <86>: must be demonstrated for each product individually

Minimal criteria	Setup	Required specifications
Standard series	3 concentrations in duplicate	|R| ≥ 0.980
Negative controls	LRW in duplicate	Onset time > onset time of the lowest standard
Product	A single dilution (< MVD) ex-pected to yield a valid PPC in duplicate	< Endotoxin limit
PPC	A single dilution spiked to the middle of the standard series in duplicate	PPC recovery % = 50 – 200%
Notes:	1 lot of PSNG 3 lots of product	

4. Internal review and approval (no regulatory filing)

PHASE II - Low Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with no additional testing requirements.

1. Confirm that primary method validation of PSNG was executed in accordance with USP <1225> Validation of Compendial Procedures to ensure the validation status of the method is suitable for its intended purpose. This can be done via:
   a. Request a letter of authorization from ACC to gain access to PSNG FDA MF# 031277
   b. Or obtain primary method validation package from ACC.[3]
2. Assurance of the criteria for the standard curve when using PSNG per USP <86> (same as for Phase I)
3. Method suitability testing per USP <1085>: must be demonstrated for each product individually (same as for Phase I)
4. Verify that the method is suitable for the product being tested in accordance with USP <1226> Verification of Compendial Procedures (14). Test for Interfering Factors per USP <86> must be performed for each product individually under actual conditions of use (same as for Phase I)
5. Equivalency testing to the in-house LAL method is not required within the context of USP <86>. However, a proactive review of the in-house approach with the relevant regulatory authority may help determine the regulator's current expectations relevant to the type of product tested (such as new drug product).
6. Internal review and approval
7. Regulatory filing:
   a. Annual reporting only for pharmaceutical waters
   b. Regulatory review and registration for new drug products

PHASE III - High Regulatory Threshold Samples

If already using one of the LAL kinetic photometric techniques (turbidimetric or chromogenic), the end user can seamlessly switch to PSNG with no additional testing requirements.

1. Confirm that primary method validation of PSNG was executed in accordance with USP <1225> Validation of Compendial Procedures to ensure the validation status of the method is suitable for its intended purpose. This can be done via:
   a. Request a letter of authorization from ACC to gain access to PSNG FDA MF# 031277
   b. Or obtain primary method validation package from ACC.[3]
2. Assurance of the criteria for the standard curve when using PSNG per USP <86> (same as for Phase I)
3. Method suitability testing per USP <1085>: must be demonstrated for each product individually (same as for Phase I)
4. Verify that the method is suitable for the product being tested in accordance with USP <1226> Verification of Compendial Procedures. Test for Interfering Factors per USP <86> must be performed for each product individually under actual conditions of use (same as for Phase I)
5. Equivalency testing to the in-house LAL method is not required within the context of USP <86>. However, a proactive review of the in-house approach with the relevant regulatory authority may help determine the regulator's current expectations relevant to the type of product tested.
6. Internal review and approval
7. Regulatory filing: Submit the results (after confirming the process with the appropriate review division) – possibly as reduced reporting category Supplement – Changes being affected or Special Report)

Note: If currently using one of the LAL photometric methods for testing specific samples, the method suitability with PSNG is likely to be the same for that sample.

Note 2: If currently using the gel clot test only, prior to Preparatory testing, the end user must install and validate one of the platforms for kinetic chromogenic measure (i.e. absorbance microplate reader powered by appropriate software).

Conclusions

The new USP chapter <86> significantly simplifies the implementation and review process for all sample types and pharmaceutical waters in particular. It demonstrates USP's commitment to expand the use of animal-free methods and materials. There are many signals that the transition from LAL to recombinant reagents for BET is no longer a choice, it is an expectation.

Implementing PyroSmart NextGen® rCR for BET is a strategic move towards greater sustainability, reliability, and efficiency in quality control of biopharmaceutical manufacturing.

By following this guide, your organization can seamlessly transition to PSNG, maintaining high standards of accuracy and compliance while meeting your sustainability goals.

Ready to make the switch? Contact our team today to learn more about how PSNG can enhance your endotoxin testing processes.

Appendix 1 – Sample Types

Globally, over 70 million BET tests are performed per annum, with 90% of all tests done using LAL reagents. Out of the 70 million BET tests, about 75% of all tests are performed on water samples for pharmaceutical purposes. Therefore, transitioning water testing from LAL to recombinant reagents can yield a dramatic reduction in the use of LAL and significantly improve your organization's Sustainability scorecard and Environmental, Social and Governance (ESG) scores.

Water for Pharmaceutical Purposes

There are different water samples tested daily for endotoxin and used within biopharmaceutical manufacturing: monographed and non-monographed samples. Monographed samples have their specific monographs listed in the US Pharmacopeia – National Formulary (USP-NF) and some of them include the test for Bacterial Endotoxin under the list of specific tests required to be performed along with an endotoxin specification.

Table 1. Estimation of typical samples tested in biopharmaceutical manufacturing

Samples	Percentage of sample types
Waters for pharmaceutical purposes	75%
Pharmaceutical ingredients	10%
Buffers and solutions	2%
In-process materials	3%
Finished products	10%

Table 2. List of water samples per USP <1231> Water for Pharmaceutical Purposes - monographed vs. non-monographed[14]

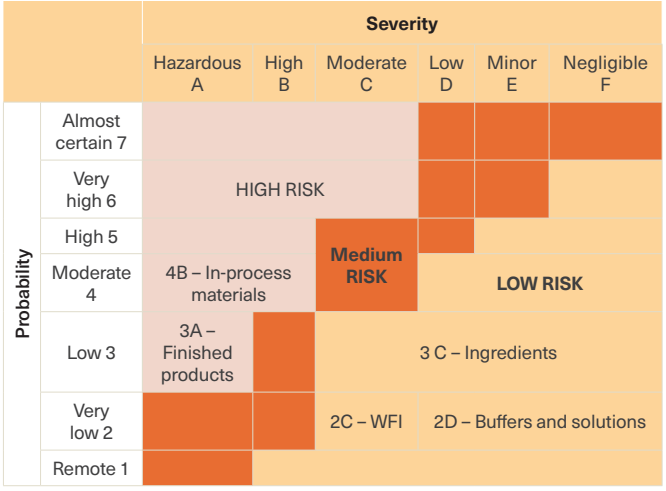
Samples	USP Monograph?	Reference to USP <85>?
Purified Water	Yes	No
Water for Injection (WFI)	Yes	Yes, < 0.25 EU/mL
Water for hemodialysis	Yes	Yes, < 1 EU/mL
Pure Steam	Yes	Yes, < 0.25 EU/mL
Sterile Purified Water	Yes	No
Sterile Water for Injection	Yes	Yes, < 0.25 EU/mL
Bacteriostatic Water for Injection	Yes	Yes, < 0.25 EU/mL
Sterile water for Irrigation	Yes	Yes, < 0.25 EU/mL
Sterile Water for Inhalation	Yes	Yes, < 0.5 EU/mL
Drinking water	No	N/A
Distilled water	No	N/A
Deionized water	No	N/A
Filtered water	No	N/A
High-purity water	No	N/A
Deaerated water	No	N/A

Appendix 2 – Risk Assessment Examples

Table 3. Estimation of probability of endotoxin ingress per sample type based on validated manufacturing process and historical data sets

Samples	Probability of endotoxin ingress
Water for Injection (WFI)	Very low
Pharmaceutical ingredients	Low
Buffers and solutions	Low
In-process materials	Moderate
Finished products	Low

Table 4. An example of Risk analysis of endotoxin ingress in pharmaceutical manufacturing per sample type



The output of the risk analysis is grouping samples into three categories of sample types based on the level of regulatory requirements needed towards implementation of PSNG as shown in Table 5.

Table 5. Final categories of sample types

Regulatory threshold	Sample types	Comments	Implementation requirements
NO	**Non-monographed water samples:** • Purified water • Sterile purified water • Drinking water • Distilled water • Deionized water • Filtered water • High purity water • Deaerated water	• Low risk	Follow USP <86>
LOW	**Monographed samples:** • WFI, SWFI • Pure steam • Pharmaceutical ingredients, buffers and solutions	• Low risk • Annual reporting only	Follow USP <86> Reference FDA MF# 031277 for PSNG *Equivalency studies should not be needed unless requested by the relevant regulatory authority*
	New non-monographed parenteral products	• High risk • New regulatory review and filing • New registration	
HIGH	**In-process materials** **Legacy monographed parenteral products**	• High risk • Re-validation • Regulatory review and approval for legacy products • Re-registration per country	Follow USP <86> Reference FDA MF# 031277 for PSNG *Equivalency studies should not be needed unless requested by the relevant regulatory authority*

References

1. Current USP <86> Bacterial Endotoxins Test using Recombinant Reagents
2. Associates of Cape Cod, Inc. - Certificates of Compliance
3. Stevens I et. al. Title: Advanced Recombinant Cascade Reagent PyroSmart NextGen® for Bacterial Endotoxins Test as Described in the Pharmacopeias, BPB, Vol.5, No. 5 105-114 (2022).
4. Shapovalova O V et. al. New direction in the determination of bacterial endotoxins: Analysis using recombinant Factor C. Pharmaceutical chemistry journal, 56, 1133-1139 (2022).
5. Kikuchi Y et. al. Collaborative study on the Bacterial Endotoxins test using Recombinant Factor Cbased Procedure for Detection of Lipopolysaccharides (Part 3). Pharmaceutical and Medical Device Regulatory Science, 54 (4), 341 – 351 (2023).
6. Kelley M et. al. Title: Evaluation of Recombinant Cascade Reagent PyroSmart NextGen® and Limulus Amebocyte Lysate Equivalency in a Plate and Tube Reader for Bacterial Endotoxins Testing, BPB Reports, Vol.6, No. 1 11-15 (2023).
7. Kelley M et. al. Title: A Demonstration of the Validation Process for Alternative Endotoxin Testing Methods Using PyroSmart NextGen® Recombinant Cascade Reagent, BPB Reports, Vol.6, No. 2 68-75 (2023).
8. PyroSmart NextGen® Package Insert
9. Current USP <85> Bacterial Endotoxins Test
10. https://www.uspnf.com/notices/86-alternative-faq
11. Current USP General Notices and Requirements
12. Current USP <1225> Validation of Compendial Procedures
13. Current USP <1085> Guidelines for Bacterial Endotoxins
14. Current USP <1226> Verification of Compendial Procedures
15. Current USP <1231> Water for Pharmaceutical Purposes

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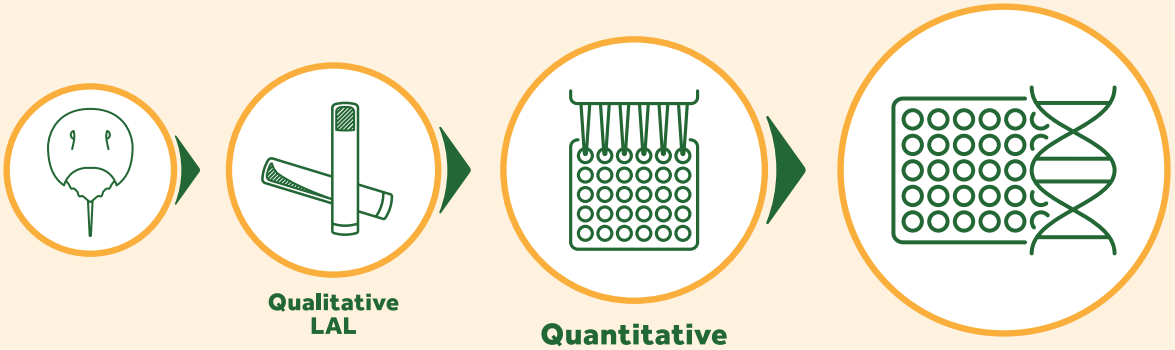
NEXT GEN Recombinant Cascade Reagent (rCR)

Your journey from LAL to rCR begins here.



More Than Recombinant. It's Recombinant Cascade.

- No Animal Content, No Horseshoe Crab Blood
- Same Cascade, Same Instrument, Same Preparation
- No Cross Reactivity with (1→3)-β-D-Glucan
- Better Lot-to-Lot Reproducibility vs. LAL

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Attainable Sustainability

ACC's Horseshoe Crab Sustainability Project

ACC's one-of-a-kind sustainability project meets a milestone of over one million juvenile crabs released!

In 2018 after working with local regulators to receive a class 1 type 4 aquaculture permit, ACC introduced our Horseshoe Crab Sustainability Project. This unique program was aimed at complementing our 50-year history of horseshoe crab conservation and ensuring a stable supply of horseshoe crabs now, and for future generations.

The program was so successful that, in 2019, we were able to secure grants to help organizations release horseshoe crabs in Asia. In 2021, we achieved a major milestone and released our 1,000,000th crab in the waters of Massachusetts. In 2022, we made another milestone when we were issued a US patent on the system! (US Patent #11425894). To date, over 1.4 million juveniles have been released in Massachusetts!!

Our team collects horseshoe crab eggs, facilitates fertilization through IVF, nurtures hatchlings as they mature into juveniles, and strategically releases them back into their natural environment. This program only uses eggs collected from bait crabs that are sacrificed for the eel, conch, and whelk fisheries, extending their genetic legacy for generations to come.

Please visit acciusa.com for more information and future updates!

Horseshoe Crabs & the Biomedical Industry — Know the Truth

What makes a horseshoe crab's blood so special?

Horseshoe crab blood carries factors that react to antigens found on and in gram-neg- ative bacteria walls by forming a clot. The clot isolates the bacteria and protects the crab from infection. The blood also begins a healing process similar to human healing: a wound forms a clot, a scab, and eventually heals.

What makes limulus amebocyte lysate, or LAL, so important?

The LAL test is the most sensitive, accurate, and cost-effective test on the market today to detect contaminating endotoxins. It was first approved by the FDA in the 1970s and is now considered the gold standard because it can, in a relatively simple test, detect endotoxin in the parts per billion. Prior to LAL, hundreds of thousands of rabbits were used to test

for endotoxins. Animals were injected with samples of the product being manufactured and monitored to see if they developed a fever which may indicate the presence of gram-negative bacteria. LAL-based assays are more humane, more accurate, and more cost-effective than rabbit-based tests. Plus, they can give results in a test tube, in about an hour. There are very few people you are likely to meet in your lifetime who have not benefited from a bacterial endotoxin test.

What types of things are tested with the blood?

If endotoxin enters your bloodstream, it can make you sick and possibly even kill you. For this reason, the US FDA has mandated that all injectable or indwelling materials must be tested for endotoxin contamination before being released for sale. This is to protect the public from products that are not sufficiently free of materials that can make a patient ill from exposure to gram-negative cell wall material. The test ACC manufactures is used for medical devices, such as knee replace- ments, stents, heart valves, and intravenous solutions; it's also used for drugs, vaccines, insulin, and chemotherapy drugs. In essence, anything injected or implanted into the human body must be free of endotoxin.

LAL is also used to make a diagnostic assay, Fungitell®, that can detect the presence of invasive fungal infections. This is a fast and accurate assay used in hospitals for critically ill patients and in patients who are at risk. In 2024 alone, over 900,000 people were tested using this assay.

I have read somewhere that crab blood is worth $15,000 a quart. Is this true?

Absolutely not — this is a myth sensation- alized by some media. Manufacturing LAL, which is made from the white blood cells of horseshoe crabs, is a complex process that is regulated by the FDA and must be done under extremely clean conditions. A typical LAL test costs less than $20. In terms of the impact it has had on human health and safety, it is safe to say it has saved many lives and is therefore priceless.

Where do the crabs you bleed come from?

Most of the crabs that come to our facility are from Massachusetts waters, Vineyard Sound, Nantucket Sound, and Buzzards Bay. Fishers catch them using different techniques, but they must follow strict reg- ulations on size, number of crabs harvested, and abide by strict quotas.

How does the process of bleeding crabs work?

Every crab that enters our facility is checked for health, has it's sex determined then the vendor and origin recorded. This is all reported to regulators monthly . The process itself is very similar to when people donate blood. The crabs are placed in a very clean laboratory, where we disinfect a portion of the shell and carefully insert a sterile needle. The crabs have a sinus in the dorsal aspect of their body just under the shell that holds excess blood; we collect from that region. The crabs are held in a very specific manner, limiting the blood that can be harvested from the dorsal sinus. The majority of the blood, which remains in the gill area, is untouched. The process takes only a few minutes and the crabs are held in darkened areas and kept moist before being returned to the supplier. Studies have shown that the crabs tolerate this process very well, and the overwhelming majority of animals survive.

What threats face the horseshoe crabs today, are they endangered?

Crabs in the United States are regulated and monitored carefully. They are not endangered and, in many areas, have growing populations. In other parts of the world, they are victims of pollution and humankind's development of coastal areas and are not so closely monitored.

Like any sea creature, horseshoe crabs are dependent on a suitable environment in which to live and reproduce. Water quality is an important factor, as is having suitable beaches in which to lay their eggs. Fertilizers, septic systems, and other forms of pollution can greatly reduce the quality of water on which the crabs depend. Sea walls, rip-rap, and jetties can manipulate the natural move- ment of sand on beaches and affect spawning habitat. Beach nourishment, the practice of bringing in truckloads of sand to beaches to replenish what's lost or make them look nice, can bury millions of eggs before they hatch. Crabs are also used as bait for conch and eels, which is another source of man-made mortality. Fortunately fisheries managers use the best science available to monitor the population and utilize structured decision making to determine quotas etc. It is safe to say there are tens if not hundreds of millions of crabs in the US.

What does ACC do to support conservation?

ACC has always promoted and practiced a catch-and-release fishery where the overwhelming majority of crabs survive the process of blood extraction. We work closely with fishermen and regulators to minimize the impact we may have on crab populations. ACC was instrumental in creating a minimum size limit for crabs to ensure only mature crabs are collected, and in helping to keep a biomedical-only fishery in Pleasant Bay, Massachusetts, where all the crabs collected are released. We have supported conservation efforts that include the use of bait bags, decreased catch limits, and prohibition of fishing for crabs around peak spawning periods. We also partic- ipate in the Massachusetts "rent-a-crab program," where crabs destined for use as bait are brought to our facility first. This program helps to limit the overall impact on crabs. ACC takes part in the Atlantic States Marine Fisheries Commission (ASMFC) Horseshoe Crab Advisory Panel, where we helped develop the Best Management Practices (BMPs) for the industry. We also collect data for the regulators from every crab that enters our facility, which is invaluable to understanding population dynamics. Most recently, ACC has implemented a one-of-a-kind sustainability project where we can hatch and grow juvenile crabs in the lab and then release them to the wild. You can learn more about this exciting new program on the ACC website.

What information should more people know about horseshoe crabs?

Horseshoe crabs and their ancestors have lived on Earth for approximately 400 million years and have survived several mass extinctions. They are not harmful and do not sting or bite.

When you see a horseshoe crab shell washed up on the beach, it is likely a molt, and not a dead crab. Crabs can only grow by shedding their shells and growing larger ones. Old shells are discarded, and many beachcomb- ers worry crabs are dying when they are really just growing up. Even as recent as the 1950s, crabs were destroyed by the tens of thou- sands by people on Cape Cod and elsewhere fearing they were harmful to shellfish beds or for use as fertilizer and pig food. In fact, they are useful for shell fishermen by helping to till and keep sediment aerated. They are an important part of the international ecosystem.

What can I do?

Water quality and human development are major threats to all fragile ecosystems such as the embayments where horseshoe crabs reproduce and grow. Do your part in limiting the impact humans have on water quality and beach erosion. If you ever see a crab upside down on the beach, gently roll it over so it can return to the water. And remember, the next time you or a loved one receives an injection, IV, or implant, be sure to thank a horseshoe crab!

Bacterial Endotoxins Testing Using Non-Animal Derived Reagents and Innovative Microfluidic Technology on Real World Samples

Jay Bolden and Kelly Smith, *Eli Lilly*
Meg Provenzano, Brian Short, and Hayden Skalski, *Veolia Water Technologies & Solutions-Sievers Instruments*

Background

Annex 1 encourages pharmaceutical companies to adopt new and innovative technologies in order to streamline their manufacturing processes. As well, companies are continually looking to create more sustainable laboratories. More recently, the USP announced the upcoming publication of a new chapter, USP <86> related to the use of recombinant reagents for endotoxin testing.

For these reasons, Veolia Water Technologies & Solutions-Sievers Instruments, in partnership with Eli Lilly, conducted a comparison study using a novel Bacterial Endotoxins Testing (BET) Platform to compare results between traditional Limulus Amebocyte Lysate (LAL) and newer recombinant cascade reagents (rCR). The Platform utilizes microfluidics and centripetal force; allows for assay set up in 85% of the time it takes to set up a traditional 96-well microplate; uses up to 90% less LAL or rCR; and is automated. It also increases efficiency and assures precise and accurate results, allowing manufacturers to meet Annex 1 and sustainability goals while remaining in full compliance with regulations to assure patient safety.

This poster presentation outlines the results of the endotoxin tests with data obtained from real-world samples, providing a practical comparison between the two reagent types.

Comparison Study

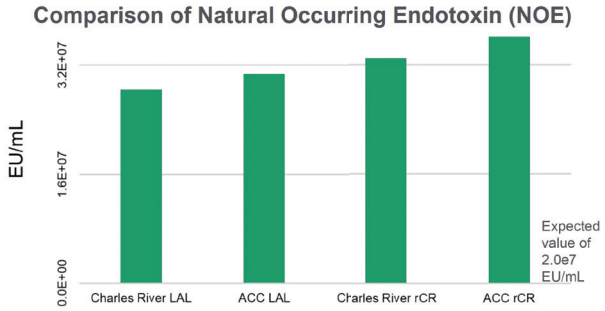
Compared recombinant cascade reagents (rCR) against traditional Limulus Amebocyte Lysate (LAL) reagents from two different vendors with a wide range of pharmaceutical samples and components from Eli Lilly

- Samples were diluted to non-interfering test dilutions to evaluate the PPC recoveries and sample suitability
- Testing was conducted using the Sievers Eclipse Bacterial Endotoxins Testing Platform
- The purpose of the study was twofold:
- To test naturally occurring endotoxins (NOE) as well as purified water with RSE to show endotoxins were recoverable with both LAL and rCR
- To challenge the Sievers Eclipse with both reagents to prove suitability for real-world samples utilizing 90% less lysate as well as recombinant reagents for sustainability

Samples

The following samples were used in the comparison study between Limulus Amebocyte Lysate (LAL) and recombinant cascade reagents (rCR):

- Two monoclonal antibodies
- Insulin
- Peptide
- NOE (see chart)
- Histidine and Sodium Acetate
- LRW
- Purified Waters
- Purified Waters with RSE spikes
- Polysorbate 80
- Counterfeit products
- Components (stopper, cartridge)
- Yeastolate



Conclusion

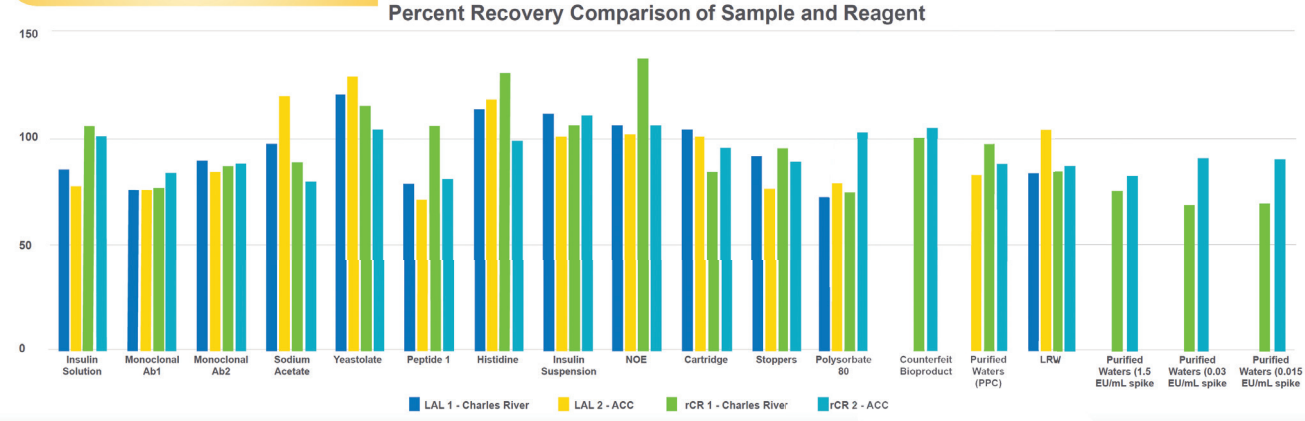
This study showed equivalent performance between LAL and rCR using the Sievers Eclipse for the detection of bacterial endotoxins in real-world samples, as well as naturally occurring endotoxins. Based on the evidence, it can be determined that the Sievers Eclipse Bacterial Endotoxins Testing Platform is able to successfully utilize recombinant cascade reagents, provided that the sample's compatibility has been verified.

Automation via the Eclipse's centripetal microfluidic platform offers the simplest form of BET automation available, providing significant time savings and reducing opportunities for error. With the availability of this innovative technology, BET assays can be automated while remaining fully compliant with compendia.

Benefits include:

- USP <85> and future <86> compliant
- Proven to work with both LAL and rCR reagents
- Up to 90% less reagent needed; aids in sustainability initiatives
- 27 total pipetting steps for 21 samples for increased efficiency
- Innovative technology aligned with Annex 1 guidelines

Results



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Resources:

Download this poster

Drivers of Implementation of Recombinant Technologies for Endotoxin Testing

As documented by the most recent US Pharmacopeia announcement,[1] the adoption of recombinant technologies for endotoxin testing is gaining momentum in the pharmaceutical industry. This article explores the fundamentals of using recombinant Cascade Reagents (rCR), their analytical performance and equivalency to LAL reagents, current developments within the regulatory framework (specifically updates provided by the USP and the factors accelerating their implementation.)

Principles of Bacterial Endotoxin Testing

The bacterial endotoxin test (BET) is an enzymatic assay crucial for detecting minute quantities of bacterial endotoxins down to 0.005 EU/mL or even 0.001 EU/mL, if applicable. BET is used daily as a quality control test ensuring the safety of biopharmaceuticals and medical devices. Despite its importance, BET predominantly relies on reagents prepared from marine arthropods, in the US, the horseshoe crabs Limulus polyphemus.

Traditional and Recombinant Endotoxin Testing Methods

These days, endotoxin testing employs two primary methods: the traditional Limulus Amebocyte Lysate (LAL) reagents and recombinant reagents which include two options: recombinant Cascade Reagents and recombinant Factor C reagents.

Traditional LAL Reagents

Traditional LAL reagents utilize the blood clotting mechanism of horseshoe crabs. The hemolymph of these crabs contains granular blood cells (amebocytes) filled with coagulation factors, including Factor C, Factor B, pro-clotting enzyme, and coagulogen. In the presence of endotoxins, Factor C triggers a clotting reaction which leads to a formation of blood clot (gel clot reagent) or a formation of turbidity (turbidimetric reagent) or a development of yellow color (chromogenic reagent).

Production of LAL Reagents

The production of LAL reagents involves several stages:

1. **Collection:** The hemolymph is collected from horseshoe crabs
2. **Centrifugation:** The hemolymph is mixed and centrifuged to collect amebocytes as a supernatant.
3. **Lysis:** The amebocytes are subjected to lysis.
4. **Formulation/Fill:** The raw lysate is formulated with buffers/excipients and a chromogenic substrate before being filled into vials and lyophilized.
5. **QC testing of the LAL reagent:** against USP Reference Standard Endotoxin and reference lysate.

The hemolymph serving as raw material for LAL can be subjected only to a limited incoming material inspection, thus is considered non-GMP. The production is conducted under current Good Manufacturing Practice (cGMP) conditions in ACC's FDA-licensed facility for LAL manufacture, ISO 13485:2016 certified.

Drivers for Recombinant Technology Adoption

Several factors advocate for the accelerated implementation of recombinant technologies in endotoxin testing.

Technical Improvements and Advancements

Typically, innovations aim to produce accurate, reproducible, and consistent results while enhancing laboratory productivity and efficiency. Recombinant Cascade Reagents (rCRs), such as PyroSmart NextGen®, have shown just that: improvement in accuracy, reproducibility and specificity to endotoxins compared to traditional LAL reagents. When paired with automated liquid handle systems, laboratory efficiency and high throughput are also considerably enhanced.

Supply Chain Risks

Over 70 million BET tests are performed annually, a trend that continues to grow with the ever-increasing and ageing global population. 90% of all BET tests are still conducted using LAL reagents. Growing restrictions to harvest horseshoe crabs as a result of local and federal laws, together with the growing demand, create a supply chain risk. Implementing rCR reduces our dependence on natural resources, and thus decreases the supply chain risk. Pharmaceutical Supply Chain Initiative (PSCI), representing 74 major pharmaceutical companies, has publicly called for transitioning from TAL reagents (derived from endangered Asian horseshoe crab Tachypleus sp.) to LAL or recombinant technologies to address conservation and welfare concerns.

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From the Pioneers in Endotoxin and Beta-D-Glucan Testing

Fifty years ago, ACC revolutionized the endotoxin detection industry by pioneering LAL testing methodology. We continued to innovate with the first and only FDA-cleared and CE marked rapid screening test for invasive fungal infections (IFI), Fungitell®.

Today, we are recognized as an international leader in endotoxin and glucan detection. Our legacy and commitment to quality stands, as we continue to provide **Protection Through Detection**™.

Our latest offering, **PyroSmart NextGen®**, is a recombinant cascade reagent (rCR) that replicates the LAL enzyme cascade without the use of animal content.

Our global headquarters are located in East Falmouth, Massachusetts, USA. In Europe, our office in Knowsley, UK, established 30 years ago, is supported by our distribution warehouse in Nijverdal, NL.

ACC is certified to I.S. EN ISO 13485:2016 and ISO 13485:2016. Our reagents are FDA licensed and can be used for testing in compliance with USP, EP and JP bacterial endotoxin test chapters, and our software is 21 CFR Part 11 Compliant.

ACC also operates a GMP-compliant, ISO-registered and DEA-licensed Contract Test Services (CTS) Laboratory. In addition to routine testing, our CTS Laboratory will customize endotoxin testing, troubleshoot difficult samples, develop and/or transfer LAL test methods, design and produce custom depyrogenation controls for oven validation and perform Low Endotoxin Recovery studies/protocols.

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Social and Corporate Responsibility

A growing number of investors have expressed their concern about corporate impacts on ecosystems and global dependencies on nature by joining the investor-led initiative Nature Action 100, which has more than 200 participants representing over US$ 28 trillion in assets under management or advice. The biotechnology/ pharmaceutical sector is one of eight systemically important sectors that the initiative has identified for initial investor engagement. Some pharmaceutical companies, such as Eli Lilly, have already transitioned to using recombinant technology, setting a precedent for the industry.

Conservation of Horseshoe Crabs

The Atlantic States Marine Fisheries Commission (ASMFC) monitors horseshoe crab populations and has been reporting positive developments in their numbers in the US. For example, over 16 million mature female horseshoe crabs and 50 million mature male horseshoe crabs have been recorded in the Delaware Bay region alone. The horseshoe crab, an integral keystone species of the Delaware Bay ecosystem, is much depended upon by many other species.

Recombinant Cascade Reagents: PyroSmart NextGen®

PyroSmart NextGen® is a reagent containing recombinant Factor C, Factor B and Pro-clotting enzyme co-lyophilized with chromogenic substrate. As such, PyroSmart NextGen® is a reagent for kinetic chromogenic technique. The kinetic chromogenic technique is described in the harmonized USP Chapter <85> Bacterial Endotoxins Test which specifies that the technique should be conducted using an LAL/ TAL reagent.[2] Therefore, while the kinetic chromogenic technique is compendial, the reagent used for the technique is considered an alternative reagent to LAL, when using it to test a compendial article per USP monograph.

Development and Validation

The development of PyroSmart NextGen® began in 2010, with its first iteration launched in Japan in 2015. The new generation reagent, launched globally in 2021, has been evaluated in numerous comparability studies.[3-7] To date, a total of nine peer-reviewed scientific publications, documenting the development, first generation PyroSmart®, two publications on method validation output of PyroSmart NextGen® and three comparability studies (https://www.acciusa.com/tools-and-resources/educational-content/acc-rcr-reference-list) were published.

Production of PyroSmart NextGen®

The production of LAL reagents involves several stages:

1. **Expression/Purification of recombinant proteins in a bioreactor:** the genes coding the factors were cloned based on *Limulus polyphemus genome*.
2. **QC testing of the recombinant proteins:** subjected to the same requirements as for production of recombinant therapeutic proteins

3. **Formulation/Fill:** The recombinant proteins are formulated with buffers/excipients and a chromogenic substrate.
4. **Fill/Lyophilization:** 1 vial provides enough of reagent for ½ of 96well microplate.
5. **QC testing of the final reagent:** subjected to the same QC testing as LAL reagents but with more stringent specifications.

Start to finish, PyroSmart NextGen® production is conducted under cGMP conditions in ACC FDA-licensed facility for LAL manufacturing and ISO 13485:2016 certified. FDA Master File for PyroSmart NextGen® is in preparation.

Precision and Accuracy

Precision is often expressed as repeatability, intermediate precision, and reproducibility. PyroSmart NextGen® has shown precision within acceptable values. For example, in Stevens et al, 2022, Correlation of Variation % based on endotoxin concentration was obtained within 20-35%.[3] A separate study by Kelley et al, 2023 showed that PyroSmart NextGen® had tighter precision than concurrently performed Pyrochrome when testing sodium citrate injection for endotoxin.[5]

Endotoxin Specificity

PyroSmart NextGen® was shown in multiple studies to detect only endotoxins, including endotoxins varying by the structure of the Lipid A part of the lipopolysaccharide.[3] Unlike all LAL reagents, PyroSmart NextGen® does not produce a response to 1,3-β-glucans which either mimic the reactivity of endotoxin (in its absence, causing false positive results) or have a synergistic affect with the present endotoxin (causing enhanced results). In Stevens et al, 2022, two series of Reference Standard Endotoxin (RSE) concentrations were prepared; one spiked with 200 pg/mL glucan (which is considered to be a significant concentration highly likely to cause a false positive response with the LAL reagents). PyroSmart NextGen showed no difference between the RSE and RSE + glucan standard curves, confirming its specificity to endotoxins.[3]

High degree of Linearity and Accuracy

High degree of linearity was confirmed in multiple publications and studies. Of note is Stevens et al. 2022 which included 24 onset times assays over three days, with each concentration analyzed in eight replicates, where the correlation coefficients ranged between 0.996 and 0.999, demonstrating a high degree of linearity and robustness.[3] The improved degree in linearity of standard curves generated by PyroSmart NextGen® leads to a high degree of accuracy: in absorbance microplate readers was determined as 71 to 140% for a wide range curve of 50 – 0.005 EU/mL (across different analysts and facilities, in 24 assays using eight replicates per concentration over three days).

Suitability for a range of sample matrixes

The evidence of suitability of PyroSmart NextGen® with a wide range of drug products has already been documented and continues to grow with the implementation of this reagent in the field. For instance, Stevens et al, 2022 tested 27 different finished products (injectables), showing equivalent suitability and improved suitability with some products.[3]

Comparison with LAL Reagents

Lot to lot reproducibility

PyroSmart NextGen® demonstrates significantly less variability compared to traditional LAL reagents. In a comparison of randomly selected eight consecutive lots of LAL kinetic chromogenic reagents, the obtained onset times of individual standard concentrations varied lot to lot by 10% and the potencies ranged from 11 to 19 EU/ng. PyroSmart NextGen®, however, showed the variability between onset times of individual contractions lot to lot as low as 1% up to 4%, indicating greater reproducibility of its enzymatic rate.

Performance and Equivalence

PyroSmart NextGen® has been demonstrated to be equivalent or superior in performance compared to traditional LAL reagents in multiple studies.[4-7] The reagent is a kinetic chromogenic assay, identical to traditional LAL chromogenic reagents but produced recombinantly. It offers high lot-to-lot reproducibility and precise results, with minimal sensitivity to 1,3-β-glucans, making it an easy to implement QC test.

Phased Approach for Implementation of PyroSmart NextGen®

The USP Microbiology Expert Committee has approved the inclusion of Chapter *<86> Bacterial Endotoxins Test using Recombinant Reagents* to the US Pharmacopeia – National Formulary (USP-NF).[8] The final text will be published for early adoption on 01 November 2025 and will become official on 01 MAY 2025.

ACC follows a phased approach for implementing PyroSmart NextGen:

- **Phase 1:** Convert testing of low-risk sample types from LAL to PyroSmart NextGen® to immediately to mitigate the supply chain risk: pharmaceutical grade water,[9] water for injection, pharmaceutical ingredients, buffers and solutions, cell culture media. Water testing for endotoxin constitutes between 70 – 80% of global samples daily. This presents a low regulatory threshold and may be subject to annual reporting only.
- **Phase 2:** Early implementation of USP <86> for expanding the testing to final products tested per USP monographs

following the requirements of USP <86> to verify suitability for the intended purpose under actual conditions of use (per USP <1226>) and to document comparability to LAL, where required.[8,10]

Conclusion

Adopting recombinant technologies for endotoxin testing is driven by technical advancements, supply chain risk mitigation, social and corporate responsibility, and conservation efforts. Recombinant Cascade Reagents, such as PyroSmart NextGen® offer a sustainable and reliable alternative to traditional LAL reagents: same compendial kinetic chromogenic technique, but with documented improvements. PyroSmart NextGen® is fully manufactured under cGMP conditions in FDA-licensed facility for LAL manufacturing, ISO 13485 certified, thus meets and exceeds quality requirements for the production of LAL. This high level of quality provides ensurances for accurate and reproducible results while reducing the reliance on natural resources. The pharmaceutical industry must accelerate implementation of innovations in BET to reduce the global dependency on natural resources (such as the horseshoe crabs) and to advance the specificity and reproducibility of BET assays.

References

1. *Expert Committee approves endotoxin testing using non-animal derived reagents (usp.org)*
2. *USP <85> Bacterial Endotoxins Test*
3. *Stevens I et. al. Title: Advanced Recombinant Cascade Reagent PyroSmart NextGen® for Bacterial Endotoxins Test as Described in the Pharmacopeias, BPB, Vol.5, No. 5 105-114 (2022).*
4. *Kelley M et. al. Title: Evaluation of Recombinant Cascade Reagent PyroSmart NextGen® and Limulus Amebocyte Lysate Equivalency in a Plate and Tube Reader for Bacterial Endotoxins Testing, BPB Reports, Vol.6, No. 1 11-15 (2023).*
5. *Kelley M et. al. Title: A Demonstration of the Validation Process for Alternative Endotoxin Testing Methods Using PyroSmart NextGen® Recombinant Cascade Reagent, BPB Reports, Vol.6, No. 2 68-75 (2023).*
6. *Kikuchi Y et. al. Collaborative study on the Bacterial Endotoxins test using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides (Part 3). Pharmaceutical and Medical Device Regulatory Science, 54 (4), 341 – 351 (2023).*
7. *Shapovalova O V et. al. New direction in the determination of bacterial endotoxins: Analysis using recombinant Factor C. Pharmaceutical chemistry journal, 56, 1133-1139 (2022).*
8. *USP <86> Bacterial Endotoxins Test using Recombinant Reagents - Draft - https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/usp-nf-notices/86-bacterial-endotoxins-tests-using-recombinant-reagents.pdf*
9. *USP <1231> Water for Pharmaceutical Purposes*

Additional information available at www.acciusa.com or via techservice@acciusa.com or technicalservices@acciuk.co.uk.

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FDA Master File Available

*ACC is proud to announce that we now have an active FDA Master File for **PyroSmart NextGen®**.*

First-Gen. **Second-Gen. NEXT-GEN.**

Your Journey From LAL to rCR Begins Here

ACC is proud to announce that we now have an active FDA Master File (MF 031277) with US Food and Drug Administration (FDA) CBER for our recombinant cascade reagent for Bacterial Endotoxins Test (BET), **PyroSmart NextGen®**.

This significant milestone offers qualifying customers added confidence and streamlined regulatory support when transitioning to recombinant technologies. With the FDA Master File, you can reference our data directly in your regulatory submissions, reducing the burden of validation and accelerating your path to approval. This aligns with the expectations as defined in USP Chapter <86> Bacterial Endotoxin Test using Recombinant Reagents.

Why it matters:

- Simplified regulatory submissions via FDA Master File reference
- Confidence in a fully validated recombinant Cascade Reagent (rCR)
- Seamless transition from traditional LAL to sustainable, next-gen endotoxin testing
- Backed by over 50 years of innovation in endotoxin detection

Make the switch with confidence—**ACC is with you every step of the way.**

Learn more at **acciusa.com/BETransformed**.

PyroSmart®
NEXTGEN Recombinant Cascade Reagent (rCR)

QC Microbiology Training

BEST QC Microbiology Training

Bioburden, Endotoxin, Sterility Testing — Innovative educational programs designed specifically for you.

BEST is a three-day innovative educational program designed specifically for you and your laboratory staff.

This program, brought to you by ACC and MilliporeSigma, will focus on both in-process and product release quality control and will provide an overview of relevant methods in each area. Basic technical laboratory skills, however, are assumed as a prerequisite for participation. The course will consist of class presentations and demonstrations of laboratory applications. This program is only available in the US.

DAY ONE

Bioburden Testing

Dependable Tests Based on Membrane Filtration

Bioburden testing is critical for monitoring water quality and raw materials and for ensuring that manufacturing processes remain in microbiological control. During the first day of the training, you will learn about:

- Advantages and limitations of membrane filtration
- How to choose the right membrane for your application
- The regulations governing bioburden testing
- How to develop a sampling plan for bioburden testing
- How to qualify and validate a method
- How to set alert and action limits
- How to interpret bioburden test results
- How to troubleshoot membrane filtration issues
- Rapid methods for bioburden testing
- Hands-on session using a manifold and Milliflex Plus Pump

DAY TWO

Endotoxin Testing

BET Methodology & Background

The Bacterial Endotoxin Test (BET) is sed for the detection and quantitation of endotoxins from gram-negative bacteria. Reagents are primarily used to test for endotoxins in injectable pharmaceuticals, biological products, and medical devices. They are also used in renal dialysis centers and a wide range of other applications. During the second day of training, you will learn:

- What are endotoxin and BET reagents
- The regulations governing bacterial endotoxin testing
- The methodology for bacterial endotoxin testing
- How to qualify a chosen BET method
- How to validate samples and how to test them routinely
- How to analyze and interpret data
- How to address sample interference
- Hands-on session
- Learn about sustainable recombinant reagents

DAY THREE

Sterility Testing

A Complete Solution for Reliable Results

Sterility testing is considered the most essential QC Microbiological test for releasing sterile final product. This test is heavily regulated and harmonized across most of the globe. During the third day of training, you will learn about:

- The history of sterility testing
- The global harmonized regulations overview
- Environmental monitoring requirements for sterility testing
- Deep dive into USP <71>
- Advantages and limitations of direct inoculation sterility testing
- Advantages and limitations of open funnel sterility testing
- Advantages and limitations of closed system sterility testing
- Overview of sterility testing media and rinse fluids
- Most common sterility questions
- Hands-on session—Steritest Equinox

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Program Schedule & Fees

This program is only available in the US. For program dates and fees, please contact your local ACC representative or check our website at acciusa.com. To receive additional information or to register for a program, contact the US office below.

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