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Advancing Endotoxin Detection with Recombinant Cascade Reagents

American Pharmaceutical Review™

A SUPPLEMENT TO AMERICAN PHARMACEUTICAL REVIEW

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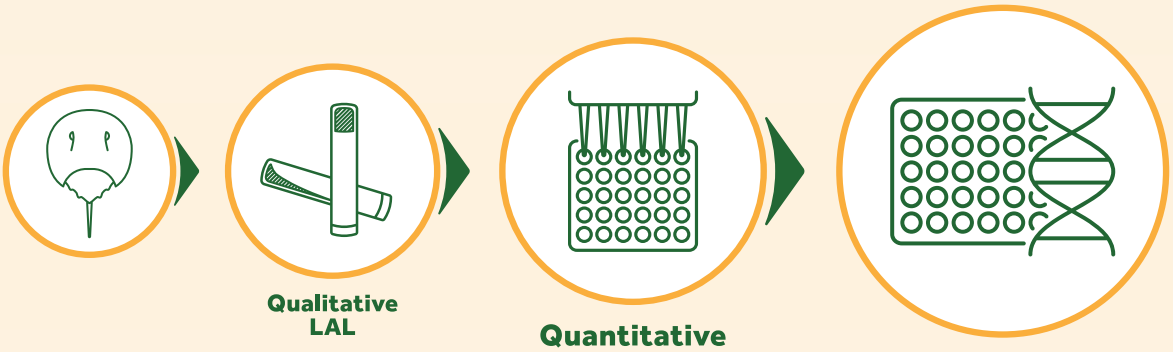

More Than Recombinant. It's Recombinant Cascade.

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- No Cross Reactivity with (1→3)-β-D-Glucan
- Better Lot-to-Lot Reproducibility vs. LAL

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A Note from the Editor

Mike Auerbach
Editor in Chief
American Pharmaceutical Review

The pharmaceutical industry's shift from traditional Limulus Amebocyte Lysate (LAL) testing to recombinant Factor C (rFC) and recombinant cascade reagents (rCR) has reached a turning point. For decades, LAL — derived from horseshoe crab blood — was the standard for bacterial endotoxin detection. Concerns over the ecological toll on these ancient animals drove the search for animal-free alternatives, which now also offer better specificity, avoiding false positives from 1,3-β-D-glucans.

The past year brought landmark regulatory progress. USP Chapter <86>, granting recombinant reagents full compendial status, became official in May 2025, followed by a revised Chapter <1085> (effective February 2026) broadening terminology beyond "lysates". In March 2026, the FDA issued updated guidance aligning with USP <86> and removing LAL-specific language . In Europe, the Pharmacopoeia Commission integrated rFC as "Method G" into chapter 2.6.14 in January 2026, putting it on equal footing with LAL.

Conservation gains followed suit: Atlantic States Marine Fisheries' (ASMFC) 2025 stock assessment showed coastwide horseshoe crab status improving to "good," with a continued zero-female-harvest policy for 2026–2027 . New York enacted a Horseshoe Crab Protection Act in December 2025, phasing out commercial harvest entirely by 2029.

With regulators on both continents now aligned and crab populations trending positive, recombinant technologies are fast becoming the new standard in endotoxin testing.

This supplement, produced with Associates of Cape Cod (ACC), unpacks that transition from every angle. ACC opens with a position statement on the evolving landscape of recombinant BET reagents, alongside a company profile detailing its role in advancing animal-free testing. From there, we offer a practical guide to implementing ACC's PyroSmart NextGen recombinant cascade reagent for BET testing under USP requirements in biopharmaceutical manufacturing, followed by a head-to-head comparison of two leading LAL systems to help manufacturers benchmark performance during transition. We close by examining what it takes to attain sustainability at scale — the operational, supply-chain, and conservation gains achievable as the industry moves decisively toward recombinant technology.

Contents

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124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536-4445

t 888.395.2221 / 508.540.3444
e custservice@acciusa.com

July 13, 2026

ACC's Position Statement on the Evolving Landscape of Recombinant BET Reagents

Patient safety and environmental stewardship are both non-negotiable. At Associates of Cape Cod, Inc. (ACC), we are committed to protecting both while leading a science-based transition toward the next generation of bacterial endotoxin testing (BET) technologies.

ACC has always been committed to:

- Protecting patients today
- Responsibly scaling the technologies of tomorrow
- Ensuring scientific continuity
- Avoiding unnecessary disruption to pharmaceutical quality systems

ACC has been at the forefront of developing and implementing the latest in recombinant BET technologies, which are increasingly being recognized by regulatory authorities and adopted throughout the pharmaceutical industry. We believe innovation is essential, but we also recognize that meaningful change must be grounded in sound science, practical implementation, and regulatory confidence.

The transition to recombinant technologies requires more than simply adopting a new reagent. It demands thoughtful validation strategies, a clear understanding of regulatory expectations, and confidence that product quality and patient safety will never be compromised. ACC is committed to helping organizations navigate this transition by providing scientific expertise, implementation guidance, technical support, and educational resources that enable informed, evidence-based decisions.

Protecting patients has always been the foundation of ACC's mission. We will continue to advocate for technologies and compendial advancements that demonstrate improved patient safety while maintaining the scientific rigor expected by regulators and manufacturers worldwide.

This commitment is reflected in our strong support of PyroSmart NextGen®, ACC's recombinant cascade reagent (rCR) technology, which has demonstrated outstanding performance, and, in many applications, enhanced endotoxin detection capabilities compared with traditional LAL methods.

At the same time, ACC recognizes that traditional Limulus Amebocyte Lysate (LAL) reagents continue to serve an essential role within the pharmaceutical industry. The transition to recombinant technologies cannot—and should not—occur overnight. A responsible evolution requires continued access to proven LAL methods while companies complete validation activities, satisfy regulatory requirements, and implement new technologies at a pace that maintains product quality and protects patients.

ACC remains committed to advancing recombinant BET through scientific innovation, transparent data, collaborative education, and practical implementation support. As the industry continues its evolution, we are proud to serve as a trusted partner to pharmaceutical manufacturers and regulators around the world—helping ensure that progress is achieved responsibly, sustainably, and always with patient safety as the highest priority.

MKT#26_346

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Associates of Cape Cod, Inc. (ACC)

124 Bernard E. Saint Jean Drive
E. Falmouth, MA 02536
Tel: 888-395-2221
Email: Custserrvice@acciusa.com

www.acciusa.com

For more than 50 years, Associates of Cape Cod, Inc. (ACC) has been a global leader in bacterial endotoxin testing (BET) and (1→3)-β-D-glucan detection products and services. Specializing in recombinant, chromogenic, and turbidimetric BET reagent technologies, ACC has built its reputation on scientific innovation, product quality, and an unwavering commitment to patient safety.

As the pioneer of Limulus Amebocyte Lysate (LAL) testing methodology and the first FDA-licensed manufacturer of LAL reagents, ACC has played a defining role in advancing endotoxin testing worldwide. Today, the company continues that legacy by leading the industry's transition toward sustainable recombinant technologies.

In 2021, ACC introduced another industry first with the commercial launch of PyroSmart NextGen®, the world's first commercially available Recombinant Cascade Reagent (rCR) for bacterial endotoxin testing. Completely free of horseshoe crab blood, PyroSmart NextGen® utilizes the same three-factor enzymatic cascade as traditional LAL while eliminating reactivity to (1→3)-β-D-glucans. Unlike first-generation recombinant Factor C (rFC) reagents, PyroSmart NextGen® allows laboratories to maintain their existing validated workflows without requiring new instrumentation or significant method changes—same instrument, same preparation steps, same method.

As regulatory acceptance of recombinant BET technologies continues to expand, including the introduction of USP Chapter <86> Bacterial Endotoxins Test Using Recombinant Reagents, there has never been a better time to begin the transition to recombinant testing. ACC supports customers throughout this journey by providing scientific expertise, implementation guidance, technical support, educational resources, and validation assistance. Your Journey from LAL to rCR Begins Here!

Headquartered in Falmouth, Massachusetts, ACC is dedicated to maintaining the highest standards of quality and regulatory compliance. The company is certified to EN ISO 13485:2016 and ISO 13485:2016, is FDA inspected, and operates CLIA-certified laboratories. ACC's endotoxin detection reagents, instrumentation, and software are trusted by pharmaceutical, biotechnology, medical device, compounding pharmacy, and dialysis organizations worldwide for quality control, product release, and research applications. ACC's FDA-licensed reagents support testing in accordance with USP, European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP) bacterial endotoxin testing requirements, while its software solutions are fully compliant with 21 CFR Part 11.

ACC also operates one of the industry's most experienced Contract Testing Services (CTS) laboratories, specializing in endotoxin and glucan testing for more than 40 years. The GMP-compliant and ISO-registered laboratory provides comprehensive analytical services, including routine endotoxin testing, method development and validation, method transfers, troubleshooting of difficult samples, custom depyrogenation controls for oven validation, and Low Endotoxin Recovery (LER) study design and execution. The laboratory is authorized to handle all controlled substances except those classified as Schedule I and performs testing in accordance with FDA, USP, EP, and JP regulatory guidelines.

Beyond endotoxin testing, ACC offers its Fungitell® clinical diagnostics product line and operates a CLIA-certified clinical laboratory specializing in (1→3)-β-D-glucan testing to aid in the early diagnosis of Invasive Fungal Disease (IFD). Together, ACC's pharmaceutical quality and clinical diagnostic solutions reflect the company's enduring commitment to advancing science, protecting patients, and supporting laboratories around the world.

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Guide for Implementation of Recombinant Cascade Reagent (rCR) PyroSmart NextGen® for Bacterial Endotoxins Testing (BET) When Testing Per USP In Biopharmaceutical Manufacturing.

PyroSmart®
NEXT GEN Recombinant Cascade Reagent (rCR)

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.

MKT#26-344

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Why Implement PyroSmart NextGen® Now?

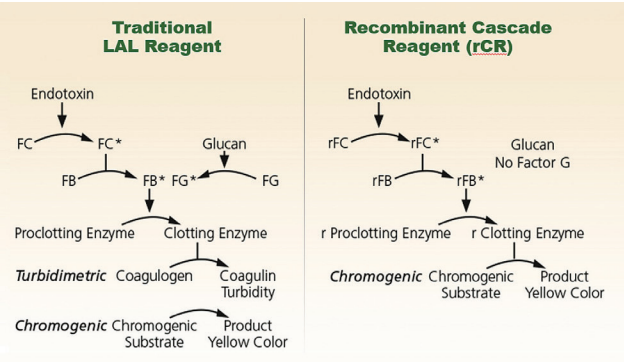
Implementing recombinant cascade reagent PyroSmart NextGen® (PSNG) offers several compelling benefits:

- Regulatory acceptance:
    1. *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]
    2. FDA Guidance for Industry 2026 Level 2 document "Pyrogen and Endotoxins Testing: Questions and Answers"[2] (released on 18 MAR 2026) as part of FDA's 2025 Roadmap to reducing animal safety testing in preclinical studies[3] provides flexibility for manufacturers to transition from the LAL reagents to recombinant reagents. On 20 APR 2026, the FDA released a follow up report summarizing the progress in implementing the roadmap and clarifying the next steps.[4] "Available data shows that the recombinant reagents deliver equivalent performance without the harvesting and use of marine animals. The transition to use of recombinant reagents for bacterial endotoxin testing also demonstrates how alternatives can address multiple concerns simultaneously—animal welfare, supply chain resilience, and environmental conservation."
    3. Bayer announced that the FDA approved AMBELVIST® (gadoquatrane), a new, intravenous macrocyclic gadolinium (Gd)-based contrast agent (mGBCA) indicated for contrast-enhanced magnetic resonance imaging (MRI) to detect and visualize lesions with abnormal vascularity in the central nervous system (CNS) and non-CNS body regions in adult and pediatric patients, including term neonates (15 JUN 2026).[5] The release testing of Ambelvist is validated and registered with PSNG rCR and LAL. AMBELVIST is a next-generation mGBCA with a novel tetrameric structure and high relaxivity (a measure of signal enhancement). This is the first FDA approval for a product to be released using rCR.
- Mitigation of supply chain risk: globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). Growing demand for medicines (thus growing need for BET assays) together with escalating restrictions to harvest horseshoe crabs for biomedical purposes create a supply chain risk.[6] 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.[7]
    2. Rapid test with Time to Result shorter than LAL assays.[7]
    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.[8]
    4. Documented suitability of PSNG with a wide range of products, including lower degree of interference in many products.[8,9]
    5. Documented comparability/equivalency of PSNG to LAL in a variety of matrixes.[6-13]
- 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.[14] It is kitted together with a reconstitution buffer (different configurations available). 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*>.[15] 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."*.[16]

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>

This current status and upcoming changes of USP <86> are described below:

At the time of writing this article (JUL 2026)

- USP <*86*> is a non-applicable chapter (official on 01 MAY 2025 in USP 2025 Issue 1)
- The methods described in USP <*86*> 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.".[17,18]

Based on the revisions to water monographs proposed in Pharmacopeial Forum 52,[1] it is projected that as of 25 SEP 2026 with the publication of USP-NF 2027, Issue 2:

- USP <*86*> will become an applicable chapter
- The methods described in USP <*86*> will become compendial for the testing of pharmaceutical waters per 7 USP monographs (Water for hemodialysis, Water for injection, Bacteriostatic water for injection, Sterile water for inhalation, Sterile water for injection, Sterile water for irrigation and Pure steam, official on 01 APR 2027).

After 25 SEP 2026, the methods described in USP <*86*> will remain alternative for samples/products tested per other monographs than the 7 water monographs.

USP Microbiological expert committee is actively working on ways of implementing methods described in USP <*86*> as compendial methods for all products.

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 microplate reader (e.g. Agilent Epoch 2)	
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)	
96well microplates (non-treated, non-coated) e.g. ACC Pyroplates*	
Dilution tubes	
Pipette tips	
Repeater Combi-tip (for repeaters) or a reservoir (for multichannel pipette)	
LAL Reagent Water	
*Not all 96well microplates are equal. The validation conditions for PSNG specify the use of non-coated, non-treated microplates together with a low onset OD of 0.03 (= mOD of 30).	

- 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 specific 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.

STEP 4: Implementation

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[19] 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

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 Controls (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	

**Detailed information can be found in Appendix 1 **Detailed information can be found in Appendix 2*

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) expected 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)

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 review of the PSNG Primary method validation package (request via *techservice@acciusa.com*).
2. Assurance of the criteria for the standard curve when using PSNG per USP <*86*> (same as for no regulatory threshold samples).
3. Method suitability testing per USP <*1085*>: must be demonstrated for each product individually (same as for no regulatory threshold samples).
4. Verify that the method is suitable for the product being tested in accordance with USP <*1226*> *Verification of Compendial Procedures*.[20] Test for Interfering Factors per USP <86> must be performed for each product individually under actual conditions of use (same as for no regulatory threshold samples).
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. Filing:
   a. Annual reporting only for pharmaceutical waters
   b. Regulatory review and registration for new drug products

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 review of the PSNG Primary method validation package (request via *techservice@acciusa.com*).
2. Assurance of the criteria for the standard curve when using PSNG per USP <*86*> (same as for no regulatory threshold samples).
3. Method suitability testing per USP <*1085*>: must be demonstrated for each product individually (same as for no regulatory threshold samples).
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 no regulatory threshold samples).
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. FDA's approvals of multiple products using recombinant reagents (including using rCR)

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 (*techservice@acciusa.com*).

Successful implementation of PSNG rCR necessitates a clear understanding of user requirements in order to ensure an effective transition within quality control processes.

Appendix 1 – Sample Types

Globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). 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.

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%

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.

The output of the risk analysis is grouping samples into three categories of sample types based on the level of regulatory

Table 2. List of water samples per USP **<1231> Water for Pharmaceutical Purposes** - monographed vs. non-monographed[21]

Samples	USP Monograph?	Reference to USP <85>?	Future Reference to USP <86>?
Purified Water	Yes	No	No
Water for Injection (WFI)	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Water for hemodialysis	Yes	Yes, < 1 EU/mL	Yes, < 1 EU/mL
Pure Steam	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile Purified Water	Yes	No	No
Sterile Water for Injection	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Bacteriostatic Water for Injection	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile water for Irrigation	Yes	Yes, < 0.25 EU/mL	Yes, < 0.25 EU/mL
Sterile Water for Inhalation	Yes	Yes, < 0.5 EU/mL	Yes, < 0.5 EU/mL
Drinking water	No	N/A	N/A
Distilled water	No	N/A	N/A
Deionized water	No	N/A	N/A
Filtered water	No	N/A	N/A
High-purity water	No	N/A	N/A
Deaerated water	No	N/A	N/A

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

		Severity					
		Hazardous A	High B	Moderate C	Low D	Minor E	Negligible F
Probability	Almost certain 7						
	Very high 6	HIGH RISK					
	High 5			Medium RISK			
	Moderate 4	4B – In-process materials			LOW RISK		
	Low 3	3A – Finished products		3 C – Ingredients			
	Very low 2			2C – WFI	2D – Buffers and solutions		
	Remote 1						

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

Globally, over 70 million BET tests are performed per annum, with 85% of all tests done using LAL reagents (as of 2026). Out of the 70 million BET tests, about 75% of all tests are performed on water samples for pharmaceutical purposes.

requirements needed towards implementation of PSNG as shown in Table 5.

References

1. *Current USP <86> Bacterial Endotoxins Test using Recombinant Reagents*
2. *Pyrogen and Endotoxins Testing: Questions and Answers | FDA, accessed on 07 JUL 2026.*
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20. *Current USP <1226> Verification of Compendial Procedures*
21. *Current USP <1231> Water for Pharmaceutical Purposes*

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

Comparability of Two LAL Systems

Pyros Kinetix® Flex tube reader/Pyrotell® -T vs Agilent Epoch 2 plate reader/Pyrochrome®

This technical white paper summarizes a comparability study conducted to assess whether the Epoch 2 plate reader used with ACC Pyrochrome® LAL chromogenic reagent yields performance comparable to, or better than, the Pyros Kinetix® Flex tube reader used with Pyrotell®-T for routine endotoxin testing under validated test parameters. The study was designed to evaluate system suitability, standard curve performance, replicate precision, and variability across multiple days, reagent lots, and analysts. The findings can be used to support customer decisions related to reader transition, method bridging, and onsite implementation.

Background

Instrument updates and replacements are a common part of the lifecycle of analytical methods used in pharmaceutical quality control. In addition, with the growing need for a modern, high-throughput systems for endotoxin testing, the plate reader platforms are better suited for end-to-end automation than tube readers. Agilent/BioTek Epoch 2 absorbance platereader is one of the platforms that can be used for as a replacement for tube readers – it is validated for use with multiple reagents (both LAL and recombinant cascade reagent) and is compatible with ACC's proprietary Pyros® eXpress software.

Both systems (tube and plate readers) are validated as part of their IOPQ and the reagents are both compendial reagents for endotoxin testing, it is expected that final sample results (adjusted for dilution) will be within 50 – 200% of each other (per USP <85>). This was documented in a publication[1] where over 100 unique water samples were tested on tube and plate reader. The primary objective of the publication was to determine whether equivalency can be observed between LAL and PyroSmart NextGen® recombinant cascade reagent on a statistically significant number of samples.

This study focuses solely on endotoxin standard performance compared directly for both systems to help determine what to expect from Epoch 2 as a replacement system and how to support the transition to the new system without introducing variability in performance.

Study Objective

The objective of this study was to compare performance between the PKFlex tube reader with Pyrotell-T reagent and the Epoch 2 plate reader with Pyrochrome reagent under routine-use conditions representative of large customer testing environments. The comparison was intended to support transition planning from PKFlex to Epoch 2 by assessing whether Epoch 2 could deliver equivalent or improved assay performance across critical analytical parameters.

Study Design

The study included two reader/reagent configurations tested in parallel using the same USP Reference Standard Endotoxin (RSE) preparations. Testing was performed over three days with two reagent lots each and two analysts. For each instrument, two standard curves were generated per run, resulting in total of 6 curves per system. This design enabled evaluation of within-run performance, across-day consistency, lot-to-lot effects, and analyst-to-analyst variability.

- Configurations compared: PKFlex/Pyrotell-T versus Epoch 2/Pyrochrome
- 6 standard curves per system
- 7 replicates per each concentration
- Two reagent lots
- Three study days
- Two analysts
- Shared RSE preparations and common supplies where applicable

Table 1. Test plan

	PKFlex/Pyrotell-T			Epoch 2/Pyrochrome		
Analyst	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
A	Batch 1	Batch 2		Batch 1	Batch 2	
B			Batch 1			Batch 1

Materials and Methods

Table 2. Materials and Equipment

Item	PN/catalog number	Lot number/CIN
PK Flex	PKF96-X	PKF96-9F-2678
Epoch 2	EPOCHNS	24101482
Pyroplates	CA961-10	19025090
Reaction tubes	TK100-10	224-25 *195-25 – test on 28 May 26
15mL centrifuge tubes	PN00517	14922018
250ul tips	PPT25	30404435
1000ul tips	PPT10	17004541
Pipette	P200	001347
Pipette	P100	001344
Repeater	N/A	001971
Vortex	N/A	389422
RSE	N/A	R172RO
Pyrochrome batch 1	CG1500-25	CKG0062
Pyrochrome batch 2	C1500-5	CK0049
Pyrotell-T batch 1	T0051-5	525-09-045-T
PYROTELL-T batch 2	T0051-5	523-01-001-T
Glucashield	GB051-5	1207101
Glucashield	GB051-5	1207120
LRW	WP10001	AL30822104

Pyrotell-T is an LAL reagent for a kinetic turbidimetric assay.[2]
Pyrochrome is an LAL reagent for a kinetic chromogenic assay.[3]
Both reagents are FDA-licensed and are used per USP <85> Bacterial Endotoxins Testing[4]

Acceptance Criteria

Comparability was assessed using routine onset assay conditions appropriate to each configuration. Key performance indicators included negative control separation, standard curve linearity, replicate precision (CV%) based on onset time, and variability of slope and Y-intercept. Acceptance criteria were derived from reagent validity requirements and internal expectations for photometric reagent performance.

With the growing need for a modern, high-throughput systems for endotoxin testing, the plate reader platforms are better suited for end-to-end automation than tube readers.

- Negative controls must have onset times greater than the lowest standard concentration.
- Standard curves must have an absolute correlation coefficient of at least 0.980.
- Replicate CV% (onset time) should be less than 15% (7 replicates).
- Slope CV% target: less than 10%.
- Y-intercept CV% target: less than 3%.

Results

See Table 3 and Table 4.

Discussion

Key Findings

The study showed that Epoch 2/Pyrochrome met all acceptance criteria and delivered highly consistent performance across all runs. By comparison, PKFlex/Pyrotell-T also performed acceptably overall but showed a small number of exceptions primarily at the lowest concentration of 0.001 EU/mL and in one slope variability assessment. These findings indicate that Epoch 2 offers a robust technical bridge for customers transitioning from PKFlex.

System Suitability

Negative controls met expectations for all Epoch2 curves and for 5 out of 6 PKFlex curves. Standard curve linearity passed

Table 3. Summary of results

Reader	Day	SC#	Onset times in seconds (mean of 7 replicates)					CV% (onset time)			
			Neg.	0.001	0.01	0.1	1	0.001	0.01	0.1	1
PKFlex	1	1	>7873	5386	2674	1588	1093	3.97	5.87	0.76	0.71
		2	7470	4668	2654	1577	1063	16.42	3.27	0.91	0.36
	2	1	>9821	6056	3330	2002	1351	10.75	2.51	1.22	1.09
		2	>9203	4995	3329	2033	1381	15.78	0.91	1.27	0.73
	3	1	>3858	3585	2469	1588	1095	2.88	1.81	2.39	1.16
		2	3830	4235	2605	1624	1111	3.88	5.49	1.79	2.15
Epoch 2	1	1	>5133	4139	2820	1671	924	2.30	3.01	2.29	1.70
		2	4845	4137	2894	1724	950	4.73	1.24	1.34	1.50
	2	1	>6621	4476	3005	1826	1083	4.29	1.58	1.35	2.43
		2	6421	4667	3026	1880	1105	7.26	2.85	4.10	3.85
	3	1	>4217	3882	2716	1700	1012	2.82	13.70	2.77	5.37
			>4447	4085	2865	1740	1018	1.42	1.63	1.74	2.92

Table 4. Comparison of Reference Values (obtained from Certificate of Analysis) and Study Results

Reagent	Reader Type	Mean Onset time for 0.001 EU/mL	Means of Standard curve descriptors			CV%		Specifications for CV%	
			Slope	Y-int.	R	Slope	Y-int.	Slope	Y-int.
Pyrotell-T 523-01-001-T	CofA (PKFlex)	5340s	-0.182	2.997	-0.996	1.3	0.7	<10	<3
	525-09-045-T	4821s	-0.203	3.053	-0.997	6.9	1.2		
Pyrotell-T 523-01-001-T	CofA (PKFlex)	5388s	-0.181	3.020	-0.995	5.3	1.1		
	Pyrotell-T	5526s	-0.202	3.120	-0.998	21	0.9		
Pyrochrome CKG0062	CofA (ELx808)	3747s	-0.200	3.010	-0.996	2.7	0.3		
	Epoch 2	4231s	-0.207	3.020	-0.997	2.6	0.5		
Pyrochrome CK0049	CofA (ELx808)	4588s	-0.210	3.040	-1.000	1.5	0.2		
	CKG0062	4572s	-0.210	3.050	-0.999	1.4	0.0		

across all datasets (see Table 4). These results demonstrate that both systems generated analytically suitable curves under the study conditions.

Precision and Variability

Replicate precision was acceptable overall for both systems; however, PKFlex showed isolated failures at 0.001 EU/mL, where two of 6 curves exceeded the replicate CV% criterion. There were no failures on Epoch 2 (see Table 3).

For inter-assay variability, slope and Y-intercept were evaluated as the standard curve descriptors dictating the back-calculated values (see Table 4). Epoch 2 met slope and Y-intercept CV% expectations for both Pyrochrome lots. PKFlex met Y-intercept variability targets for both Pyrotell-T lots, but one lot exceeded the slope CV requirement, indicating greater variability in that configuration.

Comparison against Reference Values

Both systems were also evaluated against historical performance of the reagent lots as obtained during release testing (see Table 4). While both PKFlex and Epoch 2 systems mirror the performance as obtained during release testing, there is a high degree of alignment between the plate reader reagent lot specific performance (although release testing was performed on BioTek/Agilent ELx808 plate reader, the predecessor of Epoch 2): mean of onset times, slope, Y-intercept, and correlation coefficient were all closely aligned.

This shows that Epoch 2/Pyrochrome performance is robust and repeatable and thus this system can serve as a reliable replacement configuration for customer applications.

Executive Summary

A side-by-side comparability study was performed using PKFlex/Pyrotell-T and Epoch 2/Pyrochrome configurations under routine-use conditions. 6 standard curves were generated per system across three study days using two reagent lots and two analysts. Results demonstrated that both systems met acceptance criteria for linearity, while Epoch 2 showed more consistent low-end precision and lower degree of curve-parameter variability to the Pyros Kinetix Flex system:

- Negative controls passed for all Epoch 2 curves and for 5 of 6 PKFlex curves.
- Correlation coefficient criteria met across all datasets.
- CV% (onset time):
  - PKFlex: isolated failures in replicate precision at 0.001 EU/mL
  - Epoch 2: met for all datasets
- Slope and Y-intercept CV% criteria:
  - PKFlex: met for one Pyrotell-T lot but failed slope CV% criterion for the other Pyrotell-T lot
  - Epoch: met for all datasets

Epoch 2/Pyrochrome met all criteria for all datasets and also this study data mirrored very closely data obtained for these reagent lots as obtained during release testing.

Overall, the study supports Epoch 2/Pyrochrome as a suitable replacement configuration for routine use, subject to customer-specific onsite validation requirements. For customers planning a transition from PKFlex, these results demonstrate a strong technical basis for bridging and implementation.

Customer Implications

For customers considering a transition from PKFlex to Epoch 2, these results provide practical evidence that the new reader/reagent configuration can support routine endotoxin testing with strong analytical performance. The data suggests that customers may expect equivalent or improved consistency, particularly at the low end of the standard curve where variability is often most visible. As with any method transition, onsite qualification or validation should be completed according to local quality requirements and intended use, including any automated platforms.

Conclusion

This comparability study demonstrates that Epoch 2 used with Pyrochrome provides a technically sound replacement for PKFlex with Pyrotell-T under the study conditions evaluated.

Both systems met core analytical requirements, but Epoch 2 delivered stronger consistency in critical performance areas. In addition, Epoch 2 and Pyros express software also support the use of sustainably sourced PyroSmart NextGen recombinant cascade reagent under the same conditions as Pyrochrome. This is considered a safe choice for the future of endotoxin testing.

The findings support customer adoption of Epoch 2 as part of a controlled transition strategy for routine endotoxin testing.

About Technology

Limulus amebocyte lysate (LAL) assays are widely used to detect bacterial endotoxins, which are components of the outer membrane of Gram-negative bacteria. In a kinetic turbidimetric assay, endotoxin activates the LAL cascade and the sample becomes progressively cloudy over time; in a kinetic chromogenic assay, the same biological reaction releases a yellow color signal that is measured over time. Both formats are compendial approaches used for endotoxin testing, but they differ in how the reaction is detected by the instrument.

Epoch 2 reader can be utilized together with other reagents such as recombinant kinetic chromogenic reagent PyroSmart NextGen® to perform endotoxin testing per USP <86>.[5]

As of 25 September 2026, the status of Chapter <86> is expected to change from non-applicable to applicable and for water testing per one of the 7 water monographs from alternative to compendial.

For more information, visit *www.acciusa.com* or contact Technical Services at *techservice@acciusa.com*.

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2. *Current USP <85> Bacterial Endotoxins Test*
3. *Pyrotell-T Instructions for use: https://acciusa.com/assets/documents/package-inserts/pyrotell-t-en.pdf*
4. *Pyrochrome Instructions for use: https://acciusa.com/assets/documents/package-inserts/pyrochrome-en.pdf*
5. *Current USP <86> Bacterial Endotoxins Test using Recombinant Reagents*

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-negative 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 replacements, 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 sensationalized 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 regulations 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 movement 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 participate 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.

There are very few people you are likely to meet in your lifetime who have not benefited from a bacterial endotoxin test.

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 beachcombers worry crabs are dying when they are really just growing up. Even as recent as the 1950s, crabs were destroyed by the tens of thousands 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!

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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PROTECTION THROUGH DETECTION

Your Goals. Your Endotoxin Experts.

A Partnership In Change

Where do you start? Bacterial endotoxin testing is an essential tool for quality control of raw materials, in-process sample screening, and in-process water system maintenance. It's also a test required by regulatory bodies to release your finished products.

Whether you're bringing testing in house, looking to switch from gel-clot to kinetic, validating a sustainable recombinant reagent, or converting to ACC as your supplier, you can count on ACC every step of the way. With 50 years of experience in the BET industry, we have your answers.

ACC Is With You at Every Step

Our experts will learn about your company and tailor a process to help you meet your goals.

1 QUALIFY ACC AS SUPPLIER

Ensure compliance with strict quality and safety standards, reducing the risk of product recalls, regulatory penalties, and supply chain disruptions.

2 METHOD DEVELOPMENT OF SAMPLES

Optimize test sensitivity, reduce interference from complex drug formulations, and validate the most effective BET detection approach.

3 VALIDATE SYSTEMS/ EQUIPMENT

Minimize the risk of false positives or negatives, enhances data integrity, and ensure consistent performance over time.

4 VALIDATE SAMPLES

Ensures accurate and reliable results by confirming that the test method is suitable for the specific drug formulation.

5 WRITE PROCEDURES

Well-documented procedures help minimize errors, improve reproducibility, and ensure that all personnel follow the same validated methods.

6 TRAIN STAFF

Well-trained personnel minimize errors, enhance test consistency, and maintain data integrity.

7 IMPLEMENT NEW PROCESSES IN QMS

Continuous improvement through QMS updates strengthens quality control, enhances staff accountability, and supports reliable, reproducible test results, ultimately ensuring product safety and regulatory readiness.

8 YOUR GOAL!

SUCCESSFUL VALIDATED CHANGE