USP Evolving Position on Bacterial Endotoxins Tests

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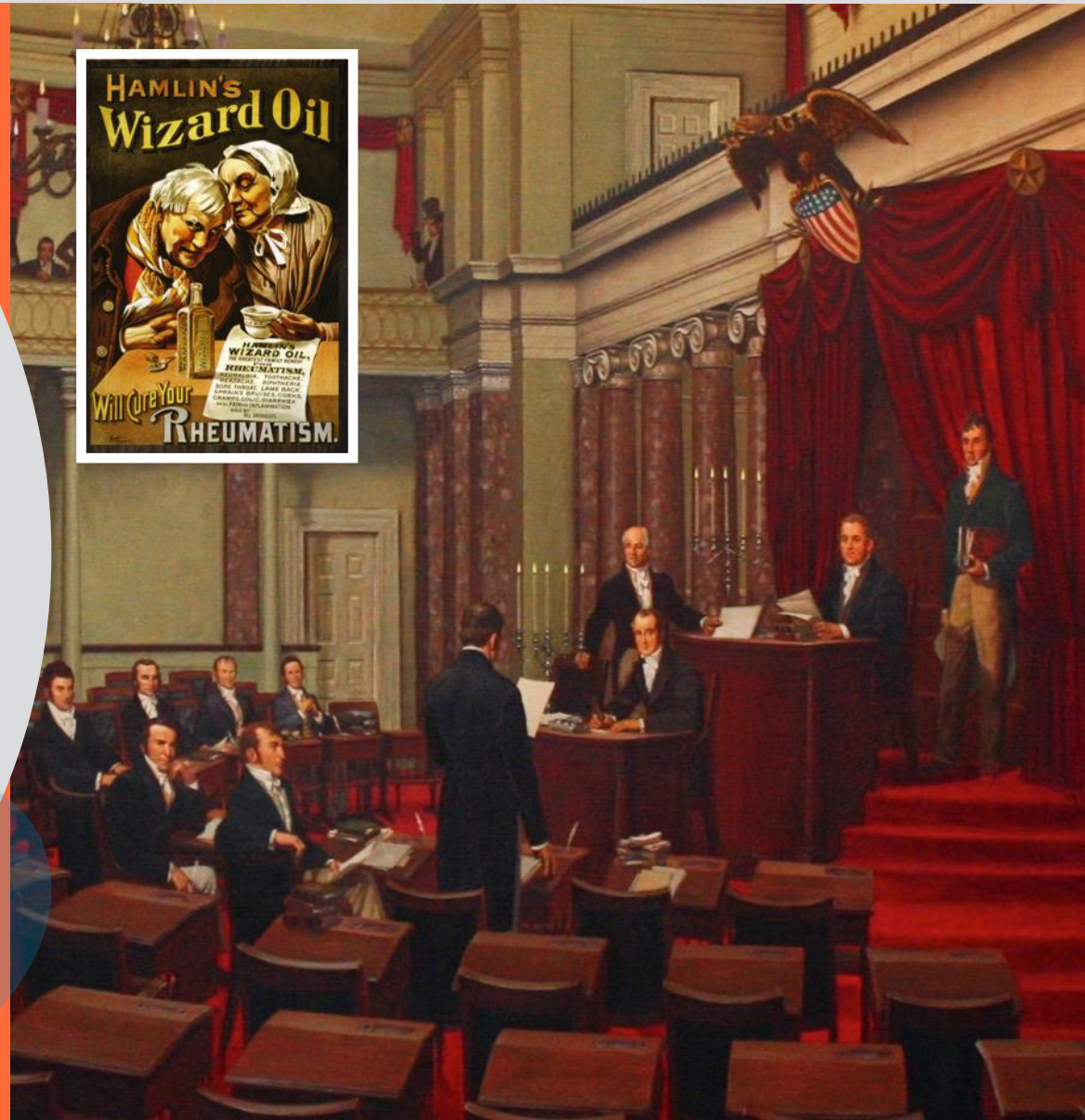
August 2024

1 Introduction to USP

USP's Enduring Mission

To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.

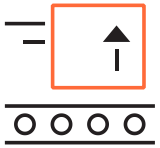


More than 7,000 USP standards support quality across the supply chain

Manufacturing

- Standards
  - Drug and API monographs
  - Compounding monographs
  - General chapters
  - Characterized reference materials
- Manufacturer capability building
  - Advanced Manufacturing, incl. Pharmaceutical Continuous Manufacturing
  - USAID-funded PQM+ program
  - API and excipient verification

Distribution



- Standards
  - Good Distribution Practices
  - Packaging and distribution

Administration

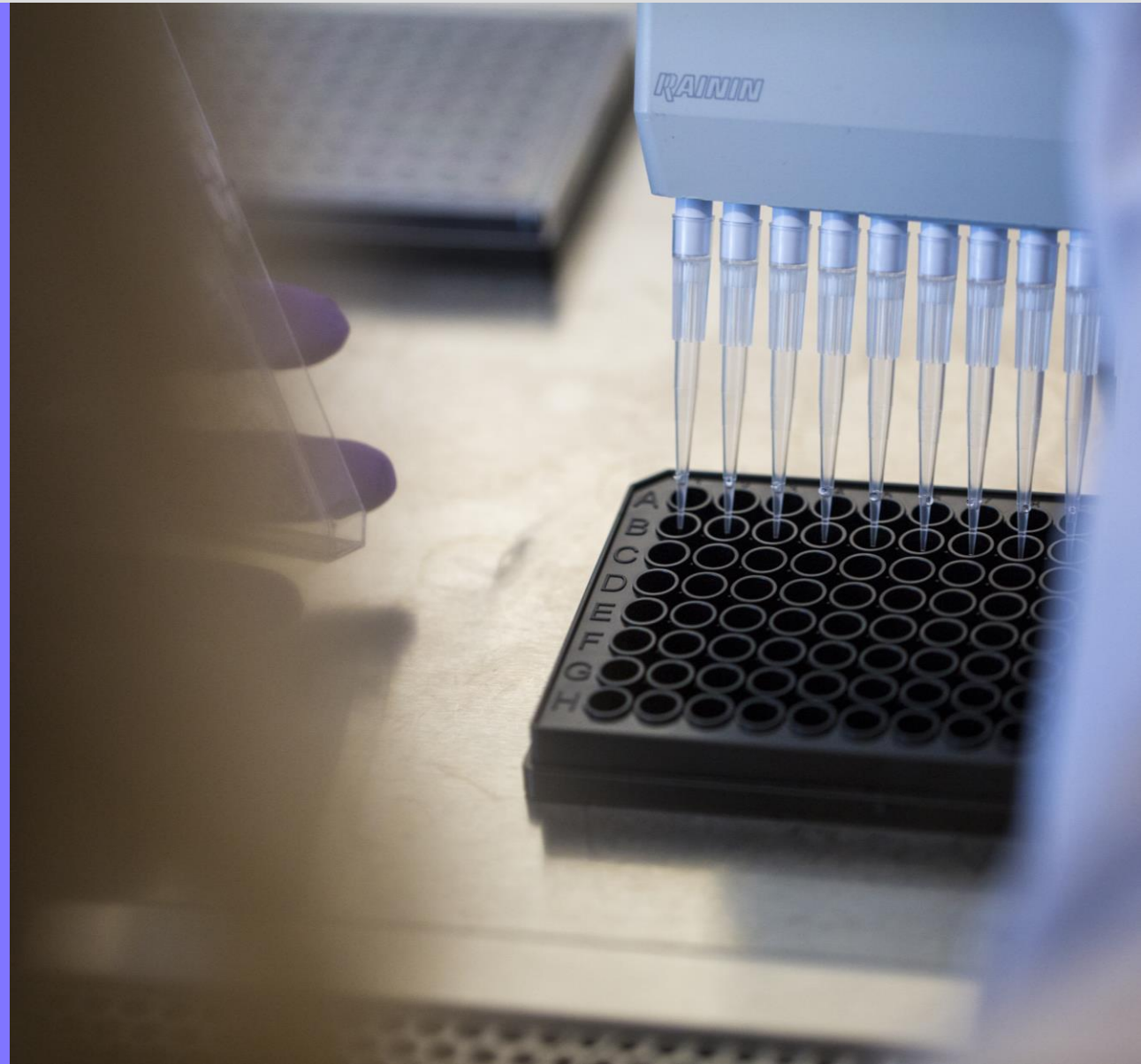
- Standards
  - Labeling
  - Nomenclature
- COVID-19 Vaccine Handling Toolkit

- USP does not enforce its standards
- USP has longstanding role in determination of whether drugs (including biologics) are deemed “adulterated” or “misbranded”
- Enforcement of USP standards is the responsibility of FDA and states that have adopted or adapted USP standards

Endotoxins and Pyrogens Testing

History

- Pyrogenicity has been associated with infections since the 6th Century BC
- Siebert established the rabbit as the preferred model for pyrogens detection in 1923
- The Rabbit Pyrogens Test was introduced in the 12th revision of USP (1942)
- Nearly 50 years have passed since LAL testing was accepted
- Alternative tests have been developed



Comparison of Pyrogen Tests

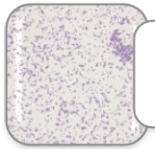
- **Rabbit Pyrogen Test (RPT) <151>**
  - Measurement of body temperature response after injection into rabbits
- **Limulus Amebocyte Lysate (LAL) <85>**
  - Hemolymph clotting upon contact with endotoxins
- **Recombinant Reagents <86> (rFC/rCR)**
  - Alternatives to LAL
    - Recombinant factor C (rFC)
    - Recombinant cascade reagent (rCR)
- **Monocyte Activation Test (MAT)**
  - Measurement of immunologic reaction of blood cells (release of cytokines) upon contact with pyrogens

Pyrogen	RPT	LAL	rFC/rCR	MAT
Endotoxin	+	+	+	+
Non-endotoxin	+	-	-	+
Human-specific	-	-	-	+
Yeasts & molds	+	-	-	+
Virus	+/-	-	-	+

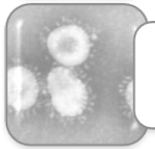
What are Endotoxins?

Pyrogens

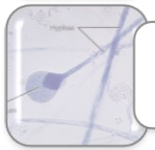
Non endotoxin pyrogens



Components of Gram-positive bacteria

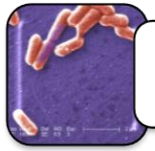


Viruses



Yeasts and molds

Endotoxins



Components of Gram-negative bacteria

Bacterial Endotoxins Test <85>

Current endotoxin testing chapter <85> uses a reagent isolated from the blood of horseshoe crabs (*Limulus* species)

Specific enzyme members of a cascade system are isolated from the blood and are identified as the limulus amebocyte lysate (LAL).

When endotoxin comes into contact with LAL, it initiates a series of enzymatic reactions that result in the activation of a pathway to the production of at least three serine protease zymogens (factor C, factor B, and pro-clotting enzyme)

Endotoxin Reagents

LAL vs Recombinant Reagents

LAL	Recombinant Factor C (rFC)	Recombinant Cascade reagents (rCR)
Natural extract from Horseshoe crab • Factor B • Factor C • Factor G • Proclotting enzyme • Coagulogen • Several other components ► Factor G activates beta glucans and gives false-positive results ► Turbidimetric and chromogenic detection methods	**Synthetic** • Factor C ► Only Fluorescence detection due to substrate ► Different detection method compared to LAL	**Synthetic** • Factor B • Factor C • Proclotting enzyme ► Chromogenic substrate similar to LAL ► Same analytical methodology as Chromogenic LAL

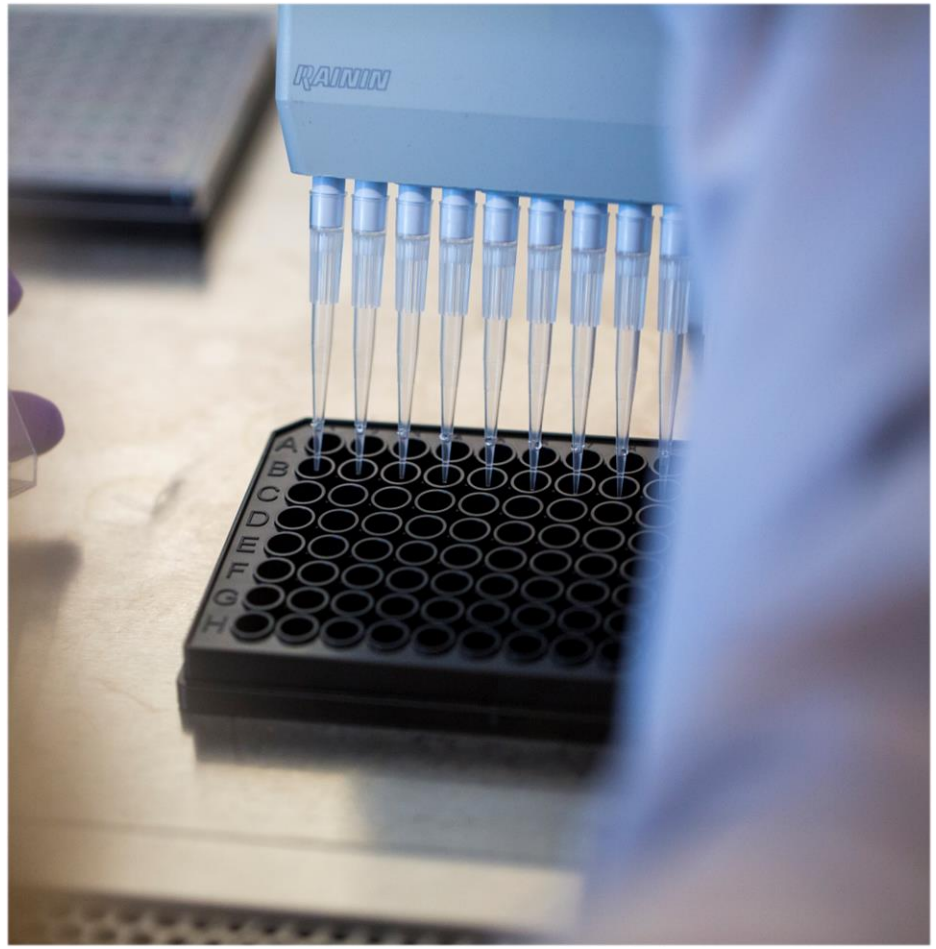
Endotoxin Reagents

Current issues

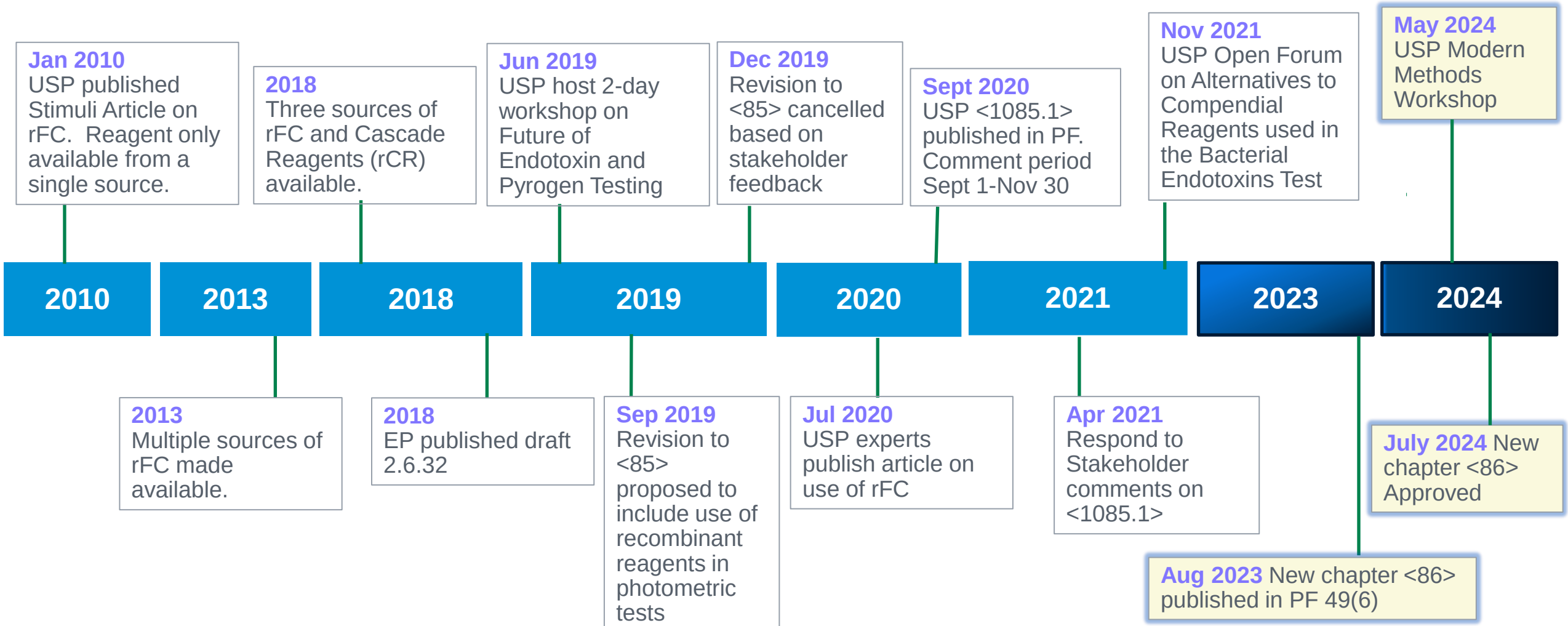
- Sustainability of horseshoe crab as the sole raw material source for BET
- Recombinant zymogen proteases as possible alternatives to the natural lysate reagents
  - Single supplier up to 2013
  - Currently multiple suppliers of rFC/rCR

Benefits

- Reduction in the use of animals as a reagent source
- Less variability for recombinant reagents compared to LAL
- Possible consistent and stable reagent supply not subject to environmental pressures



Endotoxin Reagents Timeline



Endotoxins

USP Proposals to add recombinant reagents

2019

Original proposal to add rFC to <85>

- Published data did not support comparability (only suitability)
- Approach was abandoned in favor of an informational chapter

2020

<1085.1>

PF 46(5)

- Included all recombinant reagents (one recombinant cascade reagent was near commercialization, and another was in late-stage development)

2021

<1085.1>

future publication

- Modified to include ALL ASSAYS that are replacements for the BET
- Never published

2023

<86> drafted by new SC

PF 49(6)

- Include recombinant reagents
  - rFC
  - rCR

<86> Bacterial Endotoxins Test Using Recombinant Reagents

Note: The chapter is a draft proposal and final content may change based on stakeholder feedback

- The MEC accepted proposed chapter <86> for publication in *Pharmacopeial Forum 49(6)*
- Includes both end-point fluorescence using rFC and kinetic chromogenic using rCR
- Until cited in a monograph or the general notices this chapter will be viewed by USP as an alternative method
  - Users are directed to evaluate the necessary experiments to demonstrate that the method is suitable for use for their product.
- Users should evaluate the primary validation package for the reagent used (provided by the vendor) and should prepare the suitability tests that need to be conducted to confirm the method validity for the specific product to which it will be applied.
- Reagent providers are encouraged to make available the non-specific validation packages (potentially as a DMF filed with FDA that can be referenced by the users)

Implementation

- At the present time <86> is considered to be an alternative method until cited in a specific chapter or monograph
- Use of recombinant reagents is believed to be the future gold standard for endotoxin testing
- Water testing is a significant portion of endotoxin testing for the industry
  - “Simplest” matrix– fewer interferences and large sample volumes

Summary

<86> drafted by new SC

- Three requirements:
  - Validation (supplier provides data);
  - Verification (suitability for the intended use);
  - Equivalency (when used as alternative).
- FDA engagement
- Future alignment with global pharmacopeias

Endotoxins and Pyrogens SC: Current and future status

General Chapters	Update	
<86> Bacterial Endotoxins Test Using Recombinant Reagents	PF 49(6)	• New general chapter <86> published in PF 49(6)

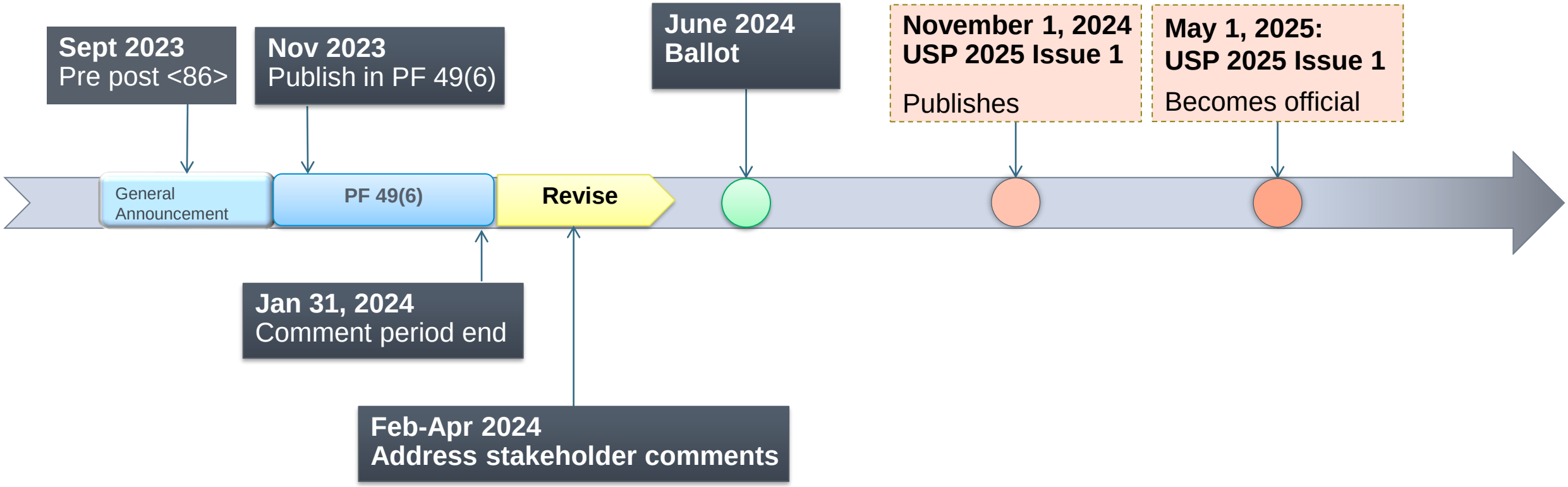
Action	Date
USP <86> General Announcement	22-Aug-2023
PF 49(6) Posting Date	01-Nov-2023
Comment Due Date	31-Jan-2024
Official Ballot (USP-NF 2025 Issue 1)	21-Jun to 01-Jul-2024
▶ Chapter Published – Early Adoption	Nov 2024
USP 2025 Issue 1 – Official Date	May 2025

USP Schedule

View the latest announcement

Recombinant Reagents for Endotoxins Test

Publication Timeline for <86>



General Comments Overview

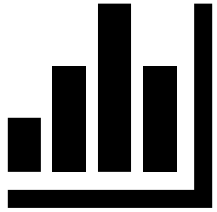
- The comments on the new USP chapter largely focus on the need for clarification and harmonization of standards, encouraging the adoption of recombinant reagents over traditional methods involving horseshoe crabs, and concerns regarding implementation and integration into current practices. There are repeated calls for the USP to expedite the adoption process and ensure global consistency. Additionally, there are suggestions and requests for specific amendments to the chapter and to provide more guidance, where needed.

Opportunities for Stakeholder Participation

REACH OUT WITH YOUR

NEEDS

COMMENTS



DATA

EXPERTISE

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Thank You

The standard of trust