Chapter <86>: Frequently Asked Questions

1

What is the animal-free alternative to Limulus amebocyte lysate (LAL)?

Alternatives to naturally sourced LAL are commercially available or currently in development. These recombinant reagents utilize one (rFC) or more (rCR) recombinant zymogen proteases cloned from the natural clotting cascade of horseshoe crabs to detect and quantify endotoxins activity.

2

What is the purpose of Chapter <86>?

Chapter <86> provides additional tests to the Bacterial Endotoxins Test <85> using recombinant Factor C or recombinant cascade reagents to detect or quantify endotoxins.

3

What kind of data was gathered and reviewed to inform Chapter <86>?

The USP Microbiology Expert Committee, which includes eight FDA representatives, gathered and reviewed scientific data obtained from literature review and submissions from stakeholders, as well as from USP-generated experimental data gathered during reference standard qualification.

4

Does this proposal replace LAL for endotoxin testing?

No, manufacturers that currently use LAL for endotoxin testing can continue to do so and Chapter <86> has no impact on them. The Bacterial Endotoxins Tests (BET) described in the new chapter are additional techniques to the current *Bacterial Endotoxins Test* described in Chapter <85>. The new chapter is intended to allow manufacturers to use non-animal derived reagents, in line with USP's commitment to reduce the use of animal-derived materials.

5

From a compendial perspective, how does Chapter <86> allow for the use of rFC and other cascade reagents?

This chapter provides methods for the use of rFC or rCR and steps for how to verify their use for a specific product. Under the provisions of the chapter, manufacturers of new biopharmaceuticals can choose to use rFC or rCR without the need to demonstrate comparability to the current method using LAL. Manufacturers of existing products that want to switch to animal-free reagents need to show this comparability. This is a normal approach and information on how to do this is readily and freely available. Please note that regulatory authorities may require supplemental data prior to acceptance, and users are encouraged to consult each regulatory authority. An example of supplemental data may include a comparative study of the material tested by techniques described in this chapter and those in <85>.

6

Will FDA require additional validation to use rFC or rCR?

The new Chapter <86> outlines steps to use endotoxin testing with rFC or rCR. It is a normal requirement for any method that it needs to be validated and shown that it is fit for use. Regulatory authorities may require supplemental data and users are encouraged to discuss with each regulatory authority.

Chapter <86>: FAQ, continued...

7

How does this proposal differ from other global pharmacopeias?

This proposal is similar to the European Pharmacopeia's and the Japanese Pharmacopeia's approach. USP is additionally proposing to add rCR and the associated method, which is not in the current EP chapter, as we considered it a suitable addition based their recent commercial availability by multiple manufacturers.

USP	*European Pharmacopeia*	*Japanese Pharmacopeia*
Unless specified in an individual monograph or General Notices, the tests in this chapter are considered alternative tests and users must meet the requirements in *General Notices* 6.30.	The replacement of an LAL-based method prescribed in a monograph by an rFC-based method is considered as the use of an alternative method as described in the Ph. Eur. General Notices.	<G4-4-180> describes procedures and consideration in measurement when using recombinant protein-reagents for endotoxin assay as alternative methods, in addition to lysate reagents and test methods in Bacterial Endotoxins Test.
A test for bacterial endotoxins using rFC or rCR can be used in the same way as LAL-based methods, after demonstration of its fitness for use for the specific substance or product. Regulatory authorities may require supplemental data and users are encouraged to discuss with each regulatory authority.	A test for bacterial endotoxins using rFC can be used in the same way as LAL-based methods, after demonstration of its fitness for use for the specific substance or product.	If these reagents for endotoxin assay are used as an alternative method, confirm that accuracy, precision, sensitivity, specificity, etc. are equal or better compared to Bacterial Endotoxins Test <4.01> using lysate reagents.
To use recombinant reagents, supplier's primary validation data can be used.	The rFC can be used in the same way as LAL-based methods, after demonstration of fitness for use for the specific substance or product.	The recombinant protein-reagents for endotoxin assay are not identical to "an amoebocyte lysate prepared from blood corpuscle extracts of horseshoe crab" specified in Bacterial Endotoxins Test <4.01>.
Includes methods for rFC and rCR.	Includes methods for rFC.	Includes methods for rFC and rCR.
Reference	Reference	Reference

Chapter <86>: FAQ, continued...

8 **Will Chapter <86> be harmonized?**

This is a topic for discussion between several pharmacopeias. The proposed Chapter contains many similarities with the European and Japanese pharmacopeia. If Chapter <86> becomes an official standard, it will be further discussed among pharmacopeias with the intent to obtain harmonization as much as possible.

9 **Will there be an opportunity for stakeholders to comment on the proposed chapter?**

The Chapter comment period will be open from Nov. 1, 2023, through Jan. 31, 2024. We welcome questions or comments through our pre-publication on USP's website in advance of the official comment process.

10 **When will Chapter <86> be included in the *USP–NF*?**

At the end of the comment period, all comments on the proposed monograph are collected and sent to the relevant Expert Bodies for review. The Expert Committee may revise the document based on feedback and send it to the Expert Committee for review. Our USP scientific liaisons review all the public comments, organize the information received and provide science-based recommendations to the Expert Committee. Depending on the comments received, the draft Chapter may be republished for another round of comments, or the chapter may be balloted by the Expert Committee for incorporation into the *United States Pharmacopeia–National Formulary* (*USP–NF*).

11 **Does USP's reference standard apply to bacterial endotoxin testing using rFC and rCR?**

There is no impact on USP's Reference Standard for Endotoxins. Tests described in the new Chapter <86> utilize the standard in the same manner as <85>.