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November 24, 2020

Leslie Furr
12601 Twinbrook Parkway
Rockville, MD 20852-1790, USA

Reference to Correspondence Number – C273155
Proposed <1085.1> Use of Recombinant Reagents in the Bacterial Endotoxins Test – Photometric and Fluorometric Methods Using Recombinantly Derived Reagents – USP PF 46(5)

Dear Ms. Furr:

PDA is pleased to have the opportunity to provide comments on the proposed USP Informational Chapter <1085.1>, released for public comment on September 1, 2020. We recognize that the purpose of the proposed chapter is to provide guidance on the qualification and validation of recombinant reagents as alternatives to naturally sourced reagents from horseshoe crab hemolymph for the purposes of quantitating endotoxin activity.

Our comments were prepared by an international group of expert volunteers with experience in drug product regulation, development, and manufacture specifically related to endotoxin testing, including the drafting of pharmacopeial guidance. The following pages present some concerns with the proposed chapter overall, as well as a number of technical comments. The specific technical comments are organized by the draft's section headings.

Of particular concern to the group was:

1) The inclusion of additional hurdles for the use of recombinant reagents for endotoxin detection compared to other USP guidance on the use of alternative methods
2) The inclusion of procedures for ensuring supplier quality as this is not specific for recombinant reagents and these considerations are not part of a description provided by USP for other, similar reagents.

PDA is a non-profit, international, professional association of more than 10,000 individual member scientists having an interest in the fields of pharmaceutical, biological, and device manufacturing and quality. These comments were prepared by a committee of experts with experience in the practice of pharmacy and pharmaceutical manufacturing including members representing our Board of Directors, our Science Advisory Board, and our Regulatory Affairs and Quality Advisory Board.

If there are any questions, please do not hesitate to contact me.

Sincerely,

Richard M. Johnson

Digitally signed by Richard M. Johnson
DN: cn=Richard M. Johnson, o=PDA, ou=President & CEO, email=johnson@pda.org, c=US
Date: 2020.11.30 07:37:17 -05'00'

Richard Johnson
President, PDA

CC: Glenn Wright, PDA; Joshua Eaton, PDA

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General Comments	Rationale
This proposed chapter seems to be attempting to dissuade the use of rFC	USP introduces exclusive hurdles for rFC compared to other USP guidance (e.g., alternative sterility tests)
The USP should engage all stakeholders to develop a General Test as originally planned.	There is extensive technical understanding of the underlying biotechnology science which is supported by current peer-reviewed literature produced by experts in the field. The drafting committee needs to consider and incorporate the most recent, relevant, peer-reviewed data (see PDA article doi:10.5731/pdajpst.2020.012187 for examples)
Further international dialogue on this topic should be a priority given the large volume of parenterals entering the market from vaccine needs and other products.	The rationale for why USP is diverging from other pharmacopeia needs to be resolved. A harmonized test expedites drug development and approval

Specific Comments to the Text

Section	Current Text	Proposed Change	Rationale
Briefing	……..requires demonstration of comparability based on criteria recommended in this chapter proposal and other *USP* chapters, principally *Validation of Alternative Microbiological Methods* ⟨1223⟩, *Validation of Compendial Procedures* ⟨1225⟩, and *Guidelines on the Endotoxins Test* ⟨1085⟩ as noted.	Remove references to <1223> throughout the document	Endotoxin is not a microbiology test (Reference the FDA guidance from 2012)
Briefing	"..preapproval…"	Change to 'approval'	FDA would review as part of a filing. Possibly reference a suggestion to discuss with FDA. The document shouldn't speak to what other health authorities would require. Seems to ignore EP 2.6.32.
Briefing	Photometric and Fluorometric Methods	Fluorometric Methods	To our knowledge the Photometric Method (Pyrosmart, Seikagaku) is not broadly available to the US market at this time. Compared to rFC the availability of end-user data is limited. Pharmacopeias do not recommend methods not available or fairly unknown. The Charles River photometric method is not yet available.
Background	(kinetic turbidimetric assay)	(turbidimetric assay)	Both endpoint and kinetic assay are in use

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Section	Current Text	Proposed Change	Rationale
Background	"Recombinant Cascade Reagents"	Confirm that this is what the reagent supplier calls them.	Harmonize verbiage
Background	A Species of horseshoe crab	From either one of two species	Stylistic
Background	Fig 2, (rFC cascade) Fig 3 (Recombinant Cascade Chromogenic Reaction)	Harmonize the two figure titles.	Stylistic change
Validation of alternative methods	3. Comparability: …. comparability of the recombinant reagents to naturally sourced lysate using endotoxins from autochthonous manufacturing sources is of particular importance.	Rewrite section and delete term "endotoxins from autochthonous manufacturing sources"	The term "endotoxins from autochthonous manufacturing sources" has not been used in the relevant literature and needs further explanation. There are published data which demonstrates comparability for rFC and LAL using environmental endotoxins (Bolden et al., 2020. PDA Journal of Pharmaceutical Science and Technology August 2020, pdajpst.2020.012187). It is not sufficiently justified to request additional comparability data using environmental endotoxins for each product-specific validation.
Validation of alternative methods	Prior to validation of an endotoxins test using recombinant reagents, a user requirement specification (URS) should be produced per Validation of Alternative Microbiological Methods ⟨1223⟩.	Remove reference to <1223>	Test for bacterial endotoxins is not regarded as a microbiological method and thus <1223> does not apply.
Validation of Alternative Methods (Comparability)	"demonstrate equivalency of results"	Remove this statement	Not in harmony with FDA 2012 Guidance which specified equivalent or better results, or alignment with <1225>.

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Section	Current Text	Proposed Change	Rationale
Validation of Alternative Methods	"Therefore, it is incumbent on the user of these reagents to assure that the manufacture…"	Remove most of this sentence	It imposes a greater requirement than other reagents used throughout the chapters in the USP. In a typical supplier audit, it would not be possible to assure all of the specified attributes. It is inappropriate for USP to request that a supplier audit should be conducted.
Validation of Alternative Methods	Unless otherwise indicated in the monograph	Remove	This is general knowledge and probably doesn't need to be stated here.
Preparatory Tests and General Notes	Apparatus: Reference ⟨85⟩. For fluorometric tests, qualify instruments per Analytical Instrument Qualification ⟨1058⟩ and calibrate the instrument according to the manufacturer's instructions.	Remove directions on how to qualify the instrument. <1085> is an informational chapter	<1085> is an informational chapter and, while the information in the chapter may be good information to have, it is not appropriate to include it in this USP standard.
Preparatory Tests and General Notes	Reagents and test solutions: Reference ⟨85⟩ except for recombinant reagents. For those, follow the manufacturer's instructions for storage, reconstitution, and use.	Remove	This is a general expectation and including it here doesn't seem to serve a purpose.
Comparability	Historically, prior to the acceptance of the LAL method as comparable to the rabbit pyrogen test it replaced, comprehensive studies were performed to assure that the LAL method could provide equivalent (or better) product quality decisions (*20*).	Remove this reference	References to historical requirements of moving from the Rabbit Pyrogen Test to LAL are not applicable with respect to the use of biotechnology to clone the natural protein and using the same Reference Standard Endotoxin calibrator for the same assay readout: i.e., the detection of endotoxin expressed in Endotoxin Units (EU).

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Section	Current Text	Proposed Change	Rationale
Comparability	Currently, although data are available on suitability (inhibition/enhancement) testing using recombinant reagents, comprehensive data demonstrating comparability of recombinant methods to LAL lysates in compendial articles containing *assayable levels* of endotoxins activity from autochthonous endotoxins are not available or have not been published in the public domain. Therefore, until such data become available it is up to each stakeholder who wishes to qualify a recombinant BET to undertake appropriate comparability trials.	Remove this reference	The USP-remark on lack of data on "autochthonous Endotoxins" is inaccurate, see PDA article doi:10.5731/pdajpst.2020.012187 Nearly all the cited literature establishes comparability or demonstrates that rFC is equivalent or superior to LAL. At least 1,087 unique samples containing environmental (real world) endotoxin were reported using rFC: most with head-to-head corresponding LAL data. 213 different relevant pharmaceutical products have been reported as using rFC (most with head-to-head corresponding LAL data) in 8 broad categories including: - large molecule/peptide drug product/drug substance - container closure components - small molecule drug product/API - buffers/pharma grade waters - vaccines - clinical samples - excipients/raw materials - plant extracts The European and Chinese Pharmacopoeias recognize the use of rFC for compendia purposes. Regulators will decide if data are sufficient, which is common regulatory approach for alternative methods.

Section	Current Text	Proposed Change	Rationale
Comparability	an appropriate analyte may be water taken from the upstream water for injection (WFI) purification stream after the carbon filters, for example, or in some cases deionized water may be used.	an appropriate analyte might be the product spiked with RSE or CSE	Unsterile water samples containing unknown contaminations including (autochthonous) endotoxins and beta-glucans are not appropriate for pharmacopeial proposals (standardization?), whereas products (or WFI for assay validation) spiked with RSE or CSE are. In general, the proposal to use contaminated batches (very rare) or batches contaminated with non-identified autochthonous contaminants seems exclusive for rFC, this is not requested for other relevant safety tests, like alternative sterility tests.
Comparability	Given that the recombinant reagents have no Factor G pathway, the use of a glucan blocker for the lysate reagent is highly recommended. This will reduce any effects of glucans on the lysate that may alter the comparability test result.	Reword to “This may reduce the effects of glucans on the lysate that may alter the comparability test result.”	Beta glucan blocking buffers do not always completely block all beta glucan (specificity). To say the use of blocking buffers in <1085.1> “…will reduce any effects of glucans…” is not true. (Roslansky and Novitsky, Sensitivity of Limulus amebocyte lysate (LAL) to LAL-reactive glucans, Journal of Clinical Microbiology, Nov. 1991, p. 2477-2483.) This seems self-evident; however it is not clear the way that this is written that one would need the beta glucan buffer for the <85> test and not for the rFC assay.

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Section	Current Text	Proposed Change	Rationale
Comparability	CSEs are not approved by any regional authority nor are they tested by USP laboratories. CSEs are secondary calibration analytes that may be derived from different strains of Escherichia coli and formulated differently among reagent suppliers. The use of one manufacturer's CSE with another manufacturer's reagent may result in a different potency determination, which could influence the comparability study outcome (see ⟨1085⟩). It is suggested that comparability studies employ the USP Endotoxin RS for calibration curves and positive product control (PPC) in order to eliminate any effects that an unmatched combination of reagent lot–CSE lot may have on the test result.	Clarify this statement	rFC suppliers provide matched endotoxin standards (CSE) paired to specific reagent batches and are calibrated to the RSE. It might be appropriate to use CSEs in a comparability study that are matched to a specific supplier (or RSE), but we agree it would be inappropriate to use a BMX CSE with a Lonza rFC per the stated example. Please clarify the statement as such.
Comparability	Relative Recovery calculation	Remove this whole statement and calculation reference	This is not correct. For example, if the acceptance criteria are both 50 to 200% recovery, if you spiked 5 EU in the product, and the assay was 10 EU, then it would be acceptable. If you then assayed by rFC and got 2.5 EU, this would be an acceptable result. But the calculation here would give 2.5/10 *100 = 25% recovery
Comparability	Historically, the source of endotoxins entering manufacturing processes has most often been aquatic Gram-negative bacteria colonizing water systems	Include additional, more recent references	There has been a lot of relevant history in the last 75+ years.
Points to Consider: Supplier Quality	Entire section	Remove	Procedure for ensuring supplier quality should not be included. It is not specific for recombinant reagents and it is not part of description for other similar reagents in USP. General GMP requirements should not be included in the chapter.