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Re: Docket No. FDA-2020-P-1405

AMENDED CITIZEN PETITION TO RECOGNIZE RECOMBINANT FACTOR C

The undersigned (Petitioner) hereby submits this Amendment (Amendment One) to the petition previously filed on April 23, 2020 under Title 21, Volume 1, of the Code of Federal Regulations (21 CFR 10.30) regarding Section 211.165 (Testing and release for distribution) of the Federal Food Drug and Cosmetic Act.[1]

[1] https://www.fda.gov/regulatory-information/laws-enforced-fda/federal-food-drug-and-cosmetic-act-fdc-act; https://www.ecfr.gov/cgi-bin/text-

Action Requested

What rule, order, or other administrative action does the petitioner want FDA to issue, amend or revoke?

The Petitioner hereby amends its previous request to the Commissioner of the Food & Drug Administration (FDA or the Agency) to focus only on one element of the request: to recognize recombinant Factor C (rFC) as a method equivalent to existing compendial methods of bacterial endotoxin testing [Limulus amoebocyte lysate (LAL) and Tachypleus tridentatus lysate (TAL)] and update the FDA's *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers* (June 2012) (the 2012 Guidance).

Petitioner requests the FDA to include a statement that the Agency will accept rFC as equivalent to LAL for pharmaceutical test purposes (as per European Pharmacopeia (EP) Chapter 2.6.32) and require only the same verification as that required in U.S. Pharmacopeia (USP) <85>. This action would remove the need for the rFC addition to the compendia for all practical purposes[2] and would also agree with recent FDA comments on USP <1085.1> draft chapter.

If finalized, this action will provide regulated industry with the flexibility, as appropriate, to employ advances in science and technology as they become available without diminishing public health protections. As necessary, the FDA will describe the appropriate tests for particular products in manufacturers' Biologics License Applications (BLAs). Just as the initial rabbit pyrogen test required in the Code of Federal Regulations was superseded by an interpretation that post pyrogen testing would allow for the singular use of LAL in lieu of rabbit pyrogen testing, a similar statement would enable the user to choose the most appropriate endotoxin test for the specific product after initial testing with LAL.

Moreover, in light of the ongoing coronavirus (COVID-19) pandemic, there is a viable possibility that synthetic products like rFC could have beneficial applications concerning COVID-19. The FDA's Emergency Use Authorization (EUA) is fast-tracking the development of new diagnostics and the U.S. government continues to call for the advancement of vaccines and pharmaceutical agents in light of the ongoing COVID-19 pandemic.[3]

Given that the European Pharmacopeia has recently adopted its own similar standard regarding rFC (per EP 2.6.32) and the Japanese and Chinese Pharmacopeias are close to adopting this type of standard as well,[4] the Petitioner therefore requests the FDA's strong support now in updating the

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[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers

[3] https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

[4] https://www.edqm.eu/sites/default/files/medias/fichiers/PressRelease/european_pharmacopoeia_press_release_outcome_of_the_165th_session_of_the_european_pharmacopoeia_commission_december_2019.pdf

2012 Guidance to encourage the USP to demonstrate its global leadership and to harmonize with other leading pharmacopeias.

Statement of Grounds

The factual and legal grounds for the petition, including all supporting material, as well as information known to the petitioner that may be unfavorable to the petitioner's position.

The Petitioner asserts that rFC has been shown to be equal to or more effective in specific instances than using horseshoe crab blood to detect bacterial endotoxins in vaccines, intravenous drugs, and medical equipment for human use. In June 2012, the FDA acknowledged the use of rFC in its *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers.* The Petitioner is requesting the FDA's action to support rFC in the USP standard-setting process for the following reasons described below.

Bacterial endotoxins cause fever in human beings, so ensuring the availability of a reliable and stable biomedical test in the form of rFC represents an innovative and viable (non-animal) substitute approach to bacterial endotoxin testing. rFC does not rely on draining the blood of ecologically vulnerable horseshoe crabs and therefore successfully presents a state-of-the-art synthetic substitute to using horseshoe crab blood for bacterial endotoxin testing purposes.

The availability of rFC as a synthetic substitute for LAL will allow for a continuation of the historical paradigm of increasing process control to prevent end product failure or end product under sampling ("testing into compliance"), which is a well-known caveat associated with sterility testing, an end-product test only, and has unfavorable statistical ability to detect contaminants. As LAL costs increase due to various impacts stated here, it may serve to put a lid on needed in process control performance.

Substantiation Claims

The Japanese reference study by the Japanese Pharmaceutical and Medical Device Regulatory Science department (Kikuchi et al.) showed, independently from reagent manufacturers, that, in purified waters such as those produced and used in the pharmaceutical industry, the recovery of endotoxin is equal or better than LAL when using tests performed by rFC.[5] The Kikuchi study also substantiated significant differences when natural, contaminated waters are tested. This is due to the presence of an interfering pathway in LAL that detects non-endotoxin substances (β-glucans). This is a common "false positive" problem encountered by industry that is overcome by the use of rFC (a major advantage for rFC).

[5] Collaborative study on the bacterial endotoxins test using recombinant factor C-based procedure for detection of lipopolysaccharides, Kikuchi et al., Vol. 48, No. 4, pg. 252-260, 2017.

One LAL commercial producer based in the U.S., in an effort to thwart rFC inroads, has provided "natural water" comparison results to USP from water they requested from users that is "pre-filtered water," which is not water tested for endotoxins by drug manufacturers. Drug manufacturers test purified water where the contaminants are of biofilm origin, not from bleed through from all-natural sources. From a scientific perspective, this is clearly an attempt to use the false pathway of LAL to prove non-equivalence to rFC. This misinformation has been accompanied by false, full page advertisements claiming that rFC cannot detect endotoxins of the very bacterial types already demonstrated to be equally recovered in the Kikuchi study.

The "recombinant revolution" has proceeded unhindered ever since Eli Lilly and Company first produced recombinant human insulin in 1982. Hundreds of lifesaving therapies have been developed based upon the "central dogma of biology" (Watson and Crick) that describes the two-step process, transcription and translation, by which the information in genes flows into proteins: DNA → RNA → protein. This paradigm has allowed for the production of recombinant enzymes, cytokines, antibodies, growth factors, etc. The recombinant production of naturally occurring proteins is now accomplished without harvesting them from human (growth hormone) or animals (insulin) and with other associated important benefits including: quality (lack of contaminants including viral, bacterial, etc.), "at will" production, sustainable production, and potentially more local production to aid in pharmaceutical oversight (auditing, etc.). See Table 2 below for current peer-reviewed studies showing the equivalence or superiority of recombinant reagent assays.

Table 2. Peer-reviewed studies showing the equivalence or superiority of recombinant reagent assays compared to LAL.

1. Comparison of Limulus amebocyte lysate test methods for endotoxin measurement in protein solutions, J. Pharm. Biomed. Analysis–Chen, L. and Mozier, N. (2013)
2. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides, PMDA– Kikuchi, Y., et al., (Part 1) (2017)
3. Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides, PMDA–Kikuchi, Y., et al., (Part 2) (2017)
4. Results of a harmonized endotoxin recovery study protocol evaluation by 14 BioPhorumOperations Group (BPOG) member companies, Biologicals–Bolden J., et al. (2017)
5. Application of recombinant Factor C reagent for the detection of bacterial endotoxins in pharmaceutical products, PDA-JPST–Bolden, J. and Smith, K. (2017)
6. Study on the Applicability of Recombinant Factor C Method for Detection of Bacterial Endotoxin, China Pharmaceuticals–Pei, Y., et al. (2019)
7. Application of a Recombinant Three-Factor Chromogenic Reagent, PyroSmart, for Bacterial Endotoxins Test Filed in the Pharmacopeias, Biol. Pharm. Bull–Muroi, M., et al. (2019)
8. Comparison of LAL and rFC Assays-Participation in a Proficiency Test Program between 2014 and 2019, Microorganisms–Piehler, M., et al. (2020)

9. Comparison of LAL and recombinant Factor C endotoxin testing assays in human vaccines with complex matrices, PDA-JPST– Marius, M., et al. (2020)
10. Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices, Biologicals–Marius, M., et al. (2020)
11. Evaluation of recombinant factor C assay for the detection of divergent lipopolysaccharide structural species and comparison with Limulus amebocyte lysate-based assays and a human monocyte activity assay, J. Med. Microbiol.–Abate, W., et al. (2017)

Several large pharmaceutical quality control labs have adopted rFC testing. These companies have demonstrated equivalence of LAL and rFC for pharmaceutical test purposes as per USP <1225>. They have tested and released tens of thousands of results on both purified water and raw materials. Most recently, the FDA approved a finished drug product tested only by rFC. rFC manufacturers have also performed validation comparisons, including one submitted to USP in 2010 and published in the Pharmacopeial Forum by USP.[6] Following these world-wide efforts, USP requested comments on rFC's inclusion as a compendial method in Chapter <85> last year, but now has apparently halted its consideration. This stands at odds with the increasing traction that regulatory acceptance has already gained, as noted by the following activities:

- On January 1, 2021 the European Pharmacopeia's new general chapter adopting rFC as compendial took legal effect.
- In December 2019, as noted above, the 165th European Pharmacopeia Commission announced that it expects to include Chapter 2.6.32 (Test for bacterial endotoxins using recombinant Factor C) as one of the 4 general chapters to be adopted in its next version, effective January 1, 2021.
- In January 2019, rFC was listed and described as a new compendia method for bacterial endotoxin testing in the Chinese Pharmacopeia, following the European Pharmacopeia, Japanese Pharmacopeia, and USP (drafts). The 4th version of the Chinese Pharmacopeia will be effective in 2020.
- In December 2018, the European Pharmacopeia released a draft of their new compendial Chapter 2.6.32 dedicated to the rFC method.
- In September 2018, the FDA approved the first drug released using a recombinant method for endotoxin testing instead of traditional LAL-based methods, for a monoclonal antibody drug treatment for the prevention of migraines in adults.[7]
- In July 2016, Chapter 5.1.10 officially became effective. 510(K) submissions have been approved by the FDA using PyroGene™ rFC assay as a final release test. U.S. manufacturer Lonza has also submitted a comprehensive FDA Master File.[8]

[6] "Stimuli to the Revision Process: A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin" Bruce Loverock, et. al., Pharmacopeial Forum, 36 b, 2010.
[7] https://www.lonza.com/news/2018-11-08-14-00
[8] Submitted to FDA/CBER in 2008 (BBMF-13800).

- In July 2015, rFC became officially recognized by the European Pharmacopoeia as an alternative endotoxin detection methodology to the LAL and Rabbit Pyrogen Tests in the new draft of Chapter 5.1.10.
- In June 2012, the FDA issued the document *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers* (the 2012 Guidance, as referenced above) which allows for the use of rFC-based assays as alternatives to LAL-based assays.

In support of the action requested herein by the Petitioner, Petitioner previously submitted find letters and articles of support from:

- Members of the New Jersey delegation to the U.S. Congress (sent to USP)
- Revive & Restore - letter and PLOS journal article[9] (sent to USP)
- The International Union for the Conservation of Nature, Horseshoe Crab Specialist Group (sent to USP and European Pharmacopeia)
- The Royal Society for the Prevention of Cruelty to Animals (sent to European Pharmacopeia)
- Atlantic States Marine Fisheries Commission (sent to USP)
- Piehler, Maike & Roeder, Ruth & Blessing, Sina & Reich, Johannes. (2020). Comparison of LAL and rFC Assays—Participation in a Proficiency Test Program between 2014 and 2019. *Microorganisms*. 8. 418.
- Marius, Vacher, & Bonnevay. (2020). Comparison of LAL and recombinant Factor C endotoxin testing assays in human vaccines with complex matrices. *PDA Journal of Pharmaceutical Science and Technology*.
- Pharmaceutical companies, PDA and FDA responses to the USP <1085> draft Chapter on recombinant factor C are accessible in the following USP link: https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/usp-nf-notices/1085-1-pf-comments-redacted.pdf These comments support the use of rFC and point to the apparent intention of USP to make the alternative validation more difficult for users.

Following the Petitioner's April 23, 2020 submission, with 14,590 public comments have since been submitted to Docket No. FDA-2020-P-1405, with the vast majority in support of rFC. In particular, the Petitioner commends the Agency to review the submission by the Physician's Committee for Responsible Medicine (PCRM), which provides suggested language to revise the 2012 Guidance that the Petitioner supports.

Environmental Claims

The general conservation situation of the extant American horseshoe crab, *Limulus polyphemus*, has been thoroughly scientifically substantiated. However, the plight of the animal is routinely

[9] https://journals.plos.org/plosbiology/article/comments?id=f10.1371/journal.pbio.2006607

minimized for commercial purposes, given the vast profit margins gained from government regulated monopolies that license the access to horseshoe crab harvest from public shores along the Atlantic coast. These are not privately-owned harvest areas. The science of conservation and, alternatively, collapse and subsequent extinction has revealed certain characteristics that support the fact that "*growth is slow, but ruin is rapid*."[10]

The following two paragraphs, excerpted from the study "The Role of Horseshoe Crabs in the Biomedical Industry and Recent Trends Impacting Species Sustainability," present the significant environmental harm and repercussions of continued horseshoe crab harvests.[11]
[12]

> …regulations have been adopted to enhance the traceability and record keeping of horseshoe crab harvest, which has historically been difficult to track. However, these regulations do not restrict or limit LAL harvest in any significant manner. Still, sometimes-lethal biomedical bleeding process and associated behavioral changes pose a risk to horseshoe crab viability after bleeding and once returned to the waters. As a result, regulators and environmentalists are concerned that current trends and overfishing of this marine arthropod will significantly impact the surrounding ecosystem…

Atlantic States Marine Fisheries Commission reports on horseshoe crab harvest mortality date back to 2004. From 2004 to 2012, the number of crabs delivered to biomedical bleeding facilities increased from 343,126 to 611,827, or by about 78%; while total mortality correspondingly increased by 75% (Atlantic States Marine Fisheries Commission, 2013). The percentage of horseshoe crabs that died prior to being bled more than doubled from 2008 to 2012 (Atlantic States Marine Fisheries Commission, 2013), which may be attributed to deleterious harvest and transportation practices. The maximum harvest mortality limit of 57,500 set by the ASMFC (based on the 15% mortality allowance) has been exceeded at times by more than 20,000 horseshoe crabs every year since 2007 (Atlantic States Marine Fisheries Commission, 1998, 2013). More recently, ASMFC data has estimated the mortality of horseshoe crabs harvested for the biomedical industry to be 100,000 Atlantic States Marine Fisheries Commission, 2019 Review of the Interstate Fishery Management Plan Horseshoe Crab 2019 Fishing Year Approved October 21, 2020[13]).

The largest U.S. manufacturer of LAL provides laboratory animals for research purposes which is sometimes a necessary part of new drug development. However, there is a growing public record of LAL manufacturers opposing animal welfare advances and being accused of animal welfare

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[10] Before the Collapse, Ugo Bardi, Springer Nature, 2020.
[11] The Role of Horseshoe Crabs in the Biomedical Industry and Recent Trends Impacting Species Sustainability, Jordan Krisfalusi-Gannon, Front. Mar. Sci., 05 June 2018, https://doi.org/10.3389/fmars.2018.00185

[13] http://www.asmfc.org/uploads/file/5f99c5a32019HorseshoeCrabFMP_review.pdf

abuse, including by The Humane Society of the United States and Harvard University's Animal law and Policy Clinic.[14,15,16,17] These accusations suggest that the manufacturers adhere to outdated animal welfare practices.

Horseshoe crabs are estimated to be over 400 million years old, with only one of 4 species in the world located in the U.S. Outside the U.S., the crabs are endangered with some bordering on extinction. In the U.S., horseshoe crabs live and breed along the Atlantic coast, primarily in the Delaware Bay area between New Jersey and Delaware.

In the U.S. each year, approximately 500,000 horseshoe crabs are collected for biomedical use and drained of a significant percentage of their blood. After bleeding, the crabs are returned to the sea, where their mortality rate within the first two weeks is calculated to be between 15-30 percent (or approximately 50,000). Moreover, bleeding is alleged to affect the female horseshoe crabs' ability to reproduce.[18]

In addition to biomedical companies, horseshoe crabs are important to the sustainability of vulnerable shorebird populations, such as the red knot, which eats the eggs of the horseshoe crab to fatten up before long migratory journeys to Canada and South America.[19] Bird societies and other environmental groups have formed a coalition in support of horseshoe crab conservation and are also supportive of the use of rFC, per the attached letter sent in February 2020, to the Atlantic States Marine Fisheries Commission (ASMFC).

Questions have recently emerged in the public sector as to the need for continued harvesting of horseshoe crabs given the availability and scalability of recombinant reagent production.[20]

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[14] "Humane Society of the United States undercover investigation shows plight of dogs in a laboratory being dosed with pesticides and drugs." Press Release. March 12, 2019. https://www.humanesociety.org/news/humane-society-united-states-undercover-investigation-shows-plight-dogs-laboratory-being-dosed

[15] "Animal welfare groups sue government over treatment of research primates." *Boston Globe.* November 6, 2019. https://clinics.law.harvard.edu/blog/2019/11/animal-welfare-groups-sue-government-over-treatment-of-research-primates/

[16] "Ghastly Slaughter of Research Monkeys Prompts Calls for Oversight." Fox News. March 17, 2010. http://www.foxnews.com/scitech/2010/03/17/ghastly-slaughter-research-monkeys/

[17] http://crueltyfreeinvesting.org/charles-river-laboratories/

[18] Sub-lethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, *Limulus Polyphemus*, Rebecca L. Anderson et al, Biol. Bull. 2013 Dec; 225(3): 137–151.

[19] "With bird populations at stake, naturalists renew calls to halt horseshoe crab harvest in Delaware Bay." Jon Hurdle, NPR, June 21, 2019. https://stateimpact.npr.org/pennsylvania/2019/06/21/with-bird-populations-at-stake-naturalists-renew-calls-to-halt-horseshoe-crab-harvest-in-delaware-bay/

[20] https://www.thestate.com/news/local/environment/article248306895.html

Environmental Impact

According to 21 CFR Part 25.25 (Environmental Impact Statements), the Petitioner does not submit an EIS, but points the FDA to the horseshoe crab inventory statistics collected by the ASMFC.[21,22] These statistics should be taken into consideration as a public source of information, but the Petitioner submits that the information may be under-reported due to the current collection methodology used by the ASMFC.

Supply Chain

On February 24, 2021, President Biden issued an executive order to upgrade the readiness of America's supply chain, including in the Pharmaceutical industry. This executive order presents an opportunity to increase the safety of essential test reagent supply by adopting the recommendations proposed herein. The Executive Order may be accessed here: https://www.whitehouse.gov/briefing-room/presidential-actions/2021/02/24/executive-order-on-americas-supply-chains/

The order stipulates:

> "(iv) The Secretary of Health and Human Services, in consultation with the heads of appropriate agencies, shall submit a report identifying risks in the supply chain for pharmaceuticals and active pharmaceutical ingredients and policy recommendations to address these risks. The report shall complement the ongoing work to secure the supply chains of critical items needed to combat the COVID-19 pandemic, including personal protective equipment, conducted pursuant to Executive Order 14001 of January 21, 2021 (A Sustainable Public Health Supply Chain). The report shall include the items described in section 4(c) of this order.

Conclusion

In summary, the Petitioner amends its prior request to ask the Agency only consider the following actions to recognize rFC as a method equivalent to existing compendial methods of bacterial endotoxin testing including LAL and TAL at this time:

- Update the *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers* (June 2012) to include a statement that the Agency will accept rFC as equivalent to LAL for pharmaceutical test purposes and require only the same verification as that required in USP Chapter <85>.

[21] https://www.ecfr.gov/cgi-bin/text idx?SID=b71ac4c111db08a3637cf868d07227cf&mc=true&tpl=/ecfrbrowse/Title21/21cfr25_main_02.tpl
[22] ASMFC 2019 Horseshoe Crab Stock Assessment: https://www.asmfc.org/uploads/file/5cd5d6f1HSCAssessment_PeerReviewReport_May2019.pdf

- Demonstrate strong support in updating the 2012 Guidance to encourage the USP to demonstrate global leadership and harmonize with other leading pharmacopeias.

Certification

The undersigned certifies, that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petition which are unfavorable to the petition.

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