



Hyglos GmbH - Am Neuland 1/3 - 82347 Bernried am Starnberger See - Germany

April 1, 2020

FOOD AND DRUG ADMINISTRATION

Division of Dockets & Management
5630 Fishers Lane, rm. 1061
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STEPHEN HAHN

Commissioner
Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

ALEX AZAR II

Secretary
Department of Health and Human Services
200 Independence Avenue SW

CITIZEN PETITION TO RECOGNIZE RECOMBINANT FACTOR C

The undersigned (Petitioner) hereby submits this petition 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>

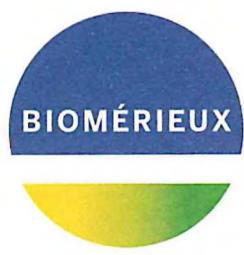
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Action Requested

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

The Petitioner hereby requests the Commissioner of the Food & Drug Administration (FDA or the Agency) 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 to amend the biologics regulations using Executive Orders 13771 and 13777. The FDA could review existing regulations to identify opportunities for replacement or modification that will result in meaningful burden reduction, while allowing the Agency to achieve its public health mission. For example, if the FDA updated its *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers* to include a statement that the Agency will accept rFC as equivalent to LAL for pharmaceutical test purposes and require only the same verifications that required in USP <85>, this action would remove the need for the rFC addition to the compendia for all practical purposes.²

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 proclamation 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 is calling for the advancement of vaccines and pharmaceutical agents in light of the COVID-19 pandemic.³

There is important precedent that the Petitioner calls out to the Agency in support of this petition. In 1976, the U.S. experienced an outbreak of swine flu (influenza A virus subtype H1N1) which led to a mass immunization program under President Gerald Ford. The President urged pharmaceutical companies to start manufacturing massive quantities of vaccines to inoculate every man, woman, and child before the flu season began.⁴ Ten days after the immunization program started, however, people were sickened by bacterial contamination within the vaccine.

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"The Public Health Legacy of the 1976 Swine Flu Outbreak," Rebecca Kreston, *Discover*. September 30, 2013. <https://www.discovermagazine.com/health/the-public-health-legacy-of-the-1976-swine-flu-outbreak>



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The federal government responded by conducting further pyrogenic tests on rabbits, resulting in the euthanization of over 400,000 rabbits. However, a more affordable endotoxin test utilizing the blood of horseshoe crabs was in development but had not yet been embraced by the pharmaceutical industry. At the FDA's urging, pharmaceutical companies began running large-scale comparisons of the rabbit and horseshoe crab pyrogenic tests and ultimately opted to support the faster and cheaper horseshoe crab option.⁵ Today, the Petitioner asserts that, with the FDA's leadership amid the COVID-19 pandemic, there is an immediate opportunity to innovate and rapidly move away from the use of rabbits and horseshoe crabs to the use of rFC.

Finally, the European Pharmacopeia has recently adopted its own similar standard regarding rFC, and the Japanese and Chinese Pharmacopeias are close to adopting this type of standard as well.⁶ The Petitioner requests the FDA's strong support in updating its Guidance and BLA regulations in encouraging 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

⁵Crab Wars: A Tale of Horseshoe Crabs, Bioterrorism, and Human Health, William Sargent (2002).

⁶https://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



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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 (see Table 2).⁷ 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 selling point for rFC).

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 humans (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.).

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.⁸ 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:

⁷ 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.

⁸“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.



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- 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.⁹
- 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.¹⁰
- 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* 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, please find attached 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 article¹¹(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*.

⁹<https://www.lonza.com/news/2018-11-08-14-00>

¹⁰Submitted to FDA/CBER in 2008 (BBMF-13800).

¹¹<https://journals.plos.org/plosbiology/article/comments?id=10.1371/journal.pbio.2006607>

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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 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.*”¹²

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.¹³

...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 70,000 (with a range of 23,000–140,000; [Atlantic States Marine Fisheries Commission, 2016](#)).

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 the LAL manufacturer opposing animal welfare advances and being accused of animal welfare abuse, including by The Humane Society of the United States and Harvard University’s

¹²Before the Collapse, Ugo Bardi, Springer Nature, 2020.

¹³The 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>



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Animal Law and Policy Clinic.^{14,15,16} These accusations suggest that the manufacturer is adhering 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. 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.¹⁷

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.¹⁸ 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).

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.^{19,20} 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.

Conclusion

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/>

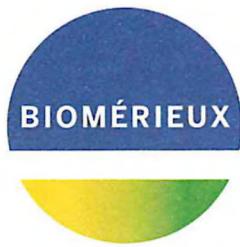
¹⁶ <http://crueltyfreeinvesting.org/charles-river-laboratories/>

¹⁷ 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.

¹⁸ “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/>

¹⁹ https://www.ecfr.gov/cgi-bin/text-idx?SID=b71ac4c111db08a3637cf868d07227cf&mc=true&tpl=/ecfrbrowse/Title21/21cfr25_main_02.tpl

²⁰ ASMFC 2019 Horseshoe Crab Stock Assessment: https://www.asmfc.org/uploads/file/5cd5d6f1HSCAssessment_PeerReviewReport_May2019.pdf



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In summary, the Petitioner requests the Agency consider the following actions to recognize rFC as a method equivalent to existing compendial methods of bacterial endotoxin testing including LAL and TAL:

- Amend the biologics regulations using Executive Orders 13771 and 13777. Existing regulations present opportunities for replacement or modification that will result in meaningful burden reduction.
- Update the *Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers* 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>.
- Utilize the Emergency Use Authorization to approve rFC as a valuable tool in the fast-tracking of diagnostics and vaccines for the current COVID-19 pandemic.
- Demonstrate strong support in updating Guidance and BLA regulations 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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