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CITIZEN PETITION

The presence of bacterial endotoxins in parenteral drugs, biological products, and medical devices raises serious patient safety issues. It is well known, for instance, that these contaminants can induce endotoxemia, a dangerous medical condition that causes patients to develop high fever and quickly can progress to sepsis, septic shock, and even death. As the recent outbreak of bacterial infections by contaminated powered infant formula shows, the safety risks posed by inadequate manufacturing process input and final release product testing are real and can lead to tragic and far-reaching consequences.[1]

For the past 50 years, the leading assays used to detect possible endotoxic contamination in biological products, pharmaceuticals, and medical devices—and thereby prevent potentially fatal endotoxemia from inflicting the patients who later use those products—depend on the activity of Limulus Amebocyte Lysate ("LAL"), which is prepared from the blood of horseshoe crabs and contains three distinct proteins: Factor C, Factor B, and Pro-clotting Enzyme. Once exposed to endotoxins, these proteins undergo a remarkable series of chemical reactions commonly referred to as the "LAL cascade," which, when coupled with additional substrates, causes either the formation of a gel clot or produces a visible change in color that establishes the presence (or confirms the absence) of endotoxins in the sampled materials.

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[1] *See FDA Provides New Updates on Activities to Mitigate Infant Formula Supply Challenges, Abbott Nutrition Agrees to Take Corrective Actions at Facility to Produce Safe Infant Formula* (May 16, 2022), *available at* (last accessed July 19, 2022).

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Because LAL is derived from blood and its intended use is to protect patient health by preventing exposure to endotoxin contamination, FDA has always required manufacturers of LAL-based end-product endotoxin tests to seek and obtain the Agency's premarket approval of a Biologics License Application ("BLA") under the Public Health Service Act ("PHSA"), Pub. L. No. 78-410, 58 Stat. 682, 702 (1944) (codified as amended at 42 U.S.C. § 262). As FDA explained when it first concluded that LAL tests are "biological products" subject to the PHSA's premarket licensure requirement:

> The Commissioner of Food and Drugs, who is charged with administering section 351 of the [PHSA] and the provisions of 21 CFR Part 273, finds that such a product ***is a biological product applicable to the prevention or treatment of disease in man***, in that [its] utilization [is] for the detection of bacterial endotoxins to prevent unsafe drugs from being administered.

FDA, *Status of Biological Substances Used for Detecting Bacterial Endotoxins*, 38 Fed. Reg. 1404, 1404 (Jan. 12, 1973) (emphasis added); *see also* FDA, *Licensing of Limulus Amebocyte Lysate—Use as an Alternative for Rabbit Pyrogen Test*, 42 Fed. Reg. 57,749, 57,749-50 (Nov. 4, 1977) (reiterating that LAL "is a biological product subject to license under section 351 of the [PHSA]").

Petitioner Charles River Laboratories Inc. ("Charles River") has marketed naturally sourced LAL as a bacterial endotoxin test for testing of manufacturing process inputs and release of biological products, pharmaceuticals, and medical devices under BLA No. 1197. Since this BLA was first approved in 1989, Charles River has sought and obtained approval for additional LAL-based endotoxin testing products under this same BLA under different product codes. To Charles River's knowledge, several other manufacturers market naturally-sourced LAL and these companies, like Charles River, do so under an approved BLA.[2]

In recent years, synthetic alternatives to LAL have been marketed for use in endotoxin contamination testing without FDA premarket authorization. These synthetic alternatives include recombinant forms of Factor C that purportedly can be used in place of standard LAL-based assays. When the first recombinant Factor C product (PyroGene) was presented to FDA in 2003, the Agency's Ombudsman asserted that recombinant Factor C products are immune from the PHSA's licensure requirement because they "do not make use of any live animals" and accordingly lack "the possibility of variation inherent in animal-derived products." Letter from Steven H. Unger (FDA Ombudsman) to Andrea E. Chamblee (Cambrex Bio Science Walkersville, Inc. ("Cambrex," now known as Lonza Walkersville, Inc., or "Lonza")), RFD 2002.040 (the "Cambrex Letter"), at 1 (Feb. 24, 2003) (attached as Exh. 1). And even though the manufacturer requested that PyroGene be classified and regulated by FDA as an *in vitro* diagnostic, the FDA Ombudsman asserted that such products ***are not subject to any FDA regulatory oversight at all***. *Id*. ("[N]o premarket submission to either CBER or CDRH will be required.").

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[2] Other companies marketing naturally sourced LAL bacterial endotoxin tests under an approved BLA include Lonza and the Associates of Cape Cod.

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Whatever merit the Cambrex Letter may have had in 2003, it is legally indefensible today. In 2010, Congress for the first time amended the PHSA's definition of "biological product" to include all human disease-preventing products that contain either a "protein" or other component that is "analogous" to a "protein." Biologics Price Competition and Innovation Act of 2009 ("BPCIA"), Pub. L. No. 111-148, § 7002(b), 124 Stat. 119, 814 (2010) (codified as amended at 42 U.S.C. § 262(i)(1)); *see also* Further Consolidated Appropriations Act of 2020, Pub. L. No. 116-94, § 605, 133 Stat. 2534, 3127 (2019). Ever since that amendment, FDA consistently has determined—and in fact has persuaded the courts—that the PHSA's reference to "protein[s]" and their "analog[ues]" precludes any regulatory distinction between products based on their method of manufacture. Indeed, for the purposes of classifying a product to be a "protein" within the ambit of the BPCIA, FDA has argued ***against*** any distinction between naturally derived proteins and laboratory-generated ones, and has successfully convinced courts to rule accordingly. *See Teva Pharms. USA, Inc. v. FDA*, 514 F. Supp. 3d 66, 101-02 (D.D.C. 2020) (affirming FDA's determination that the PHSA "does ***not*** differentiate between natural and synthetic proteins" but instead "treat[s] all qualifying molecules as proteins, ***regardless of mode of manufacture***") (emphasis added). Thus, whether sourced directly from horseshoe crabs or produced using recombinant methods, the PHSA subjects ***all*** proteins and ***all*** their analogues to premarket licensure and post-marketing regulation when used in products that are intended to prevent human diseases or conditions like endotoxemia. *Id.*

Indeed, the intended use of the recombinant Factor C products is to prevent contaminated biological products, pharmaceuticals, and medical devices from being administered to people, because doing so raises serious patient safety issues. Thus, the intended use of these products is "applicable to the prevention, treatment, or cure of a disease or condition of human beings," 42 U.S.C. § 262(i)(1)), just as FDA first recognized in 1973. 38 Fed. Reg. at 1404. The PHSA's intended-use clause has not materially changed since that time, and FDA's continued regulation of Charles River's products confirms that bacterial endotoxin tests fully meet the PHSA's intended-use requirement. The PHSA therefore compels FDA to require pre-market licensure of any Factor C product used for bacterial endotoxin testing irrespective of the specific process used to manufacture that protein, and FDA's 2003 determination that recombinant Factor C products are not subject to the PHSA's licensure requirement directly conflicts with the plain language of the post-BPCIA statute. *See, e.g.*, 5 U.S.C. § 706(2) (directing courts to set aside any agency decision that is "arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law" or "in excess of statutory … limitations"); *see also Genus Med. Techs. LLC v. FDA*, 994 F.3d 631, 644 (D.C. Cir. 2021) ("FDA's decision must be set aside because it was based on an erroneous interpretation of law. *See* 5 U.S.C. § 706(2).").

As explained *infra*, the continued marketing of these unlicensed and unregulated products also puts Americans at risk; indeed, it threatens a recurrence of the contamination-driven outbreaks that first led Congress to pass the precursor to today's PHSA in 1902. A peer-reviewed study comparing the three market-leading recombinant Factor C products (two manufactured by bioMérieux and one by Lonza) with the two market-leading LAL-based assays (one manufactured by Lonza and one by Charles River) establishes that recombinant Factor C assays are inferior by posing an unacceptable risk of returning false negative results in water samples used as inputs for pharmaceutical manufacturing. Regulation of this class of products was designed to avoid the

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patient safety risks highlighted by these results and further underscores the need for mandating licensure.

Against this backdrop; in light of the PHSA's plain text; and pursuant to 21 C.F.R. §§ 10.25 and 10.30, Charles River respectfully requests that FDA:

(1) Formally rescind the Ombudsman's now-obsolete Cambrex Letter;

(2) Declare that any person engaged in the interstate commercial marketing of an end-product endotoxin test for human drugs, biological products, or medical devices which (a) includes recombinant Factor C and (b) has not been granted an effective BLA for its recombinant Factor C product is in violation of 42 U.S.C. § 262(a)(1)(A);

(3) Order any such person(s) to either (a) immediately cease marketing their unlicensed and unlawful recombinant Factor C product(s) in interstate commerce, or (b) submit a complete BLA for such a product within 60 days of the date the Commissioner takes final agency action on this petition; and

(4) Initiate prompt administrative enforcement proceedings against any person who either (a) fails to comply with the terms of the above-requested order, or (b) submits a complete BLA in accordance with the terms of such order, but fails to obtain an effective approval for such BLA within 6 months of its initial submission to FDA.

I. ACTIONS REQUESTED

Charles River respectfully requests that FDA take each of the four actions described in the immediately preceding paragraphs numbered (1)-(4).

II. STATEMENT OF GROUNDS

A. Statutory and Regulatory Background

1. The Public Health Service Act: Origins and Early Enforcement

a. The Biologics Control Act of 1902

In 1902, Congress passed the Biologics Control Act ("BCA"), Pub. L. No. 57-244, 32 Stat. 728, after dozens of children died from exposure to tetanus-contaminated diphtheria antitoxin and smallpox vaccines that were distributed without adequate pre-release testing. *See* T. Coleman, *Early Developments in the Regulation of Biologics*, 71 FOOD & DRUG L.J. 544, 549-51 (2016) (describing the BCA's origins and citing *inter alia* R. DeHovitz, *The 1901 St. Louis Incident: The First Modern Medical Disaster*, 133 PEDIATRICS 964 (2014)). To mitigate the risk of future contamination-driven outbreaks and restore public confidence in the purity of biological products, the original BCA barred any person from engaging in the interstate commercial exchange of

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> any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention and cure of diseases of man, unless … such virus, serum, toxin, antitoxin, or product has been propagated and prepared at an establishment holding an unsuspended and unrevoked license, issued by the Secretary of the Treasury as hereinafter authorized, to propagate and prepare such virus, serum, toxin, antitoxin, or product for sale.

BCA § 1, 32 Stat. at 728. The BCA further established a "Board" that later evolved into the Public Health Service ("PHS"),[3] and directed it to implement the new licensure requirement by implementing regulations "to govern the issue, suspension, and revocation of licenses for the maintenance of establishments for the propagation and preparation of viruses, serums, toxins, antitoxins, and analogous products, applicable to the prevention and cure of diseases of man." *Id*. § 4, 32 Stat. at 729.

From the outset, this early iteration of the PHS faced an array of challenges—perhaps most notable, that it lacked authority to directly regulate the safety or effectiveness of viruses, serums, toxins, antitoxins, and analogous products. Although the BCA had authorized PHS to regulate ***the establishments*** where those complex products were manufactured, it granted PHS no authority to regulate the ***biological products*** themselves. *See id*. § 1, 32 Stat. at 728. That gap put PHS in the undesirable position of potentially having to place the federal government's implicit seal of approval (in the form of a federal license) on scores of biological products that had not been shown to be either safe or effective simply because they were prepared in sanitary conditions.

The early PHS regulators responded to that dilemma by adopting narrow interpretations of the BCA's covered product categories so that the PHS would not be forced to license establishments at which unproven, unsafe or ineffective products were being manufactured. Coleman, 71 FOOD & DRUG L.J. at 567-82 (explaining that "[t]o minimize the number of licensed but ineffective drugs, PHS narrowly interpreted each of the statutory terms describing a product class subject to licensure" and collecting examples).

b. The Public Health Service Act of 1944

In 1944, Congress sought to solve this problem by granting PHS the specific regulatory authorities it lacked under the 1902 statute. *See* PHSA, Pub. L. No. 78-410, 58 Stat. 682 (1944). ***First***, the new PHSA formally established today's commissioned PHS Corps to oversee the production, licensure, and sale of biological products. *Id*. § 203, 58 Stat. at 683-84. ***Second***, Congress expanded the range of biological products subject to premarket licensure to include "arsphenamine or its derivatives (or any other trivalent organic arsenic compound)." *Id*. § 351(a), 58 Stat. at 702. ***Third***, the PHSA for the first time authorized direct federal regulation of biological products by providing that the PHS could license an establishment "only upon a showing that the establishment ***and the products for which a license is desired*** meet standards, designed to insure

[3] For ease of reference, this Petition refers to the board and its successors simply as the PHS.

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the continued ***safety, purity, and potency of such products***." *Id*. § 351(d), 58 Stat. at 702 (emphasis added).

Even so, PHS continued to narrowly construe the range of products subject to licensure. And despite the fact that the PHSA's new reference to "potency" readily encompassed a showing of effectiveness, *see* 21 C.F.R. § 600.3(s) ("The word potency is interpreted to mean the specific ability or capacity of the product … to effect a given result."), PHS likewise construed the statute's new grant of authority narrowly—relying on the statute's legislative history (rather than its plain language) to assert that PHSA was not authorized to regulate the effectiveness of biological products (much less required to do so). *See* Coleman, 71 FOOD & DRUG L.J. at 595-96 & n.284 (citing internal PHS legal records).

2. The Modern PHSA

a. The Public Health Service Amendments of 1970 and 1971 Whistleblower Crisis

In response to ongoing criticism that PHS continued to under-regulate biological products, Congress amended the PHSA in 1970 by "making clear" that the statute's reference to "biological product[s]" encompassed any "vaccine, blood, blood component or derivative, [and] allergenic product." H.R. Conf. Rep. 91-1590, *reprinted in* 116 CONG. REC. H35860, 35866 (daily ed. Oct. 8, 1970) (explaining Congress's rationale for the amendments made by Pub. L. No. 91-515, § 291, 84 Stat. 1297, 1308 (Oct. 30, 1970)). But the PHS's Division of Biologics Standards ("DBS") nonetheless continued to under-enforce the statute until 1971—when it was found that DBS had been "knowingly allowing the distribution of subpotent and ineffective influenza vaccine" despite the 1970 amendments, and the ensuing General Accounting Office ("GAO") investigation found that nearly 30 percent of the products DBS licensed "were not recognized … as being effective." 71 FOOD & DRUG L.J. at 596-97 (citing GAO, *Rep. to the Sen. Subcomm. on Exec. Reorg. & Gov't Res.: Problems Involving the Effectiveness of Vaccines*, 11-15 (Mar. 28, 1972)); *see also* N. Wade, *Division of Biologics Standards: Reaping the Whirlwind*, 180 SCI. 162-64 (Apr. 13, 1973) (detailing the DBS scandal and its fallout).

The resulting public scrutiny prompted the transfer of DBS's functions to FDA. *See* PHS & FDA, *Joint Statement of Organization, Functions, and Delegations of Authority*, 37 Fed. Reg. 12865 (June 29, 1972). And in August 1972, FDA both asserted its authority to regulate the effectiveness of biological products and announced that it planned a comprehensive review of all DBS-licensed biological products for both safety and efficacy. FDA, *Biological Products: Procedures for Review of Safety, Effectiveness, and Labeling—Proposed Rule*, 37 Fed. Reg. 16,679, 16,679 (Aug. 18, 1972) (asserting that "[t]he importance to the American public of safe and effective vaccines, serums, blood, and other analogous biological products cannot be understated" and declaring that "[a]lthough these products have been reviewed for safety in the past, it is concluded that safety of these products should be reviewed again at this time").

The proposed rule was finalized in 1973 and the Agency promptly took steps to subject all previously approved biological products to a rigorous re-review. FDA, *Biological Products: Procedures for Review of Safety, Effectiveness, and Labeling—Final Rule*, 38 Fed. Reg. 4319,

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4319 (Feb. 13, 1973) (asserting that "[t]he agency's overriding purpose is to assure everyone who administers or receives a biological product that he is utilizing a product which is safe and effective for its labeled purpose" and rejecting claims that FDA lacked authority to review biological products for effectiveness on the ground that "[a] biological product whose label purports, represents, or suggests it to be effective and/or safe for certain intended uses, and which is not safe and effective for such uses, is misbranded … and therefore should not and will not be licensed under section 351 of the [PHSA]").

b. Congress Modernizes the PHSA and Expressly Extends Its Coverage to Proteins and Their Analogues.

Twenty-five years later, Congress modernized the statute in 1997 by simplifying the antiquated language of its licensure requirement (which had remained largely unchanged since the 1902 BCA). *See* Food and Drug Modernization Act of 1997 ("FDAMA"), § 123, Pub. L. No. 105-115, 111 Stat. 2299, 2322 (Nov. 21, 1997). As reframed by FDAMA and still in effect today, the statute now provides simply that "[n]o person shall introduce or deliver for introduction into interstate commerce any biological product unless a biologics license … is in effect for the biological product," 42 U.S.C. § 262(a)(1)(A) (current; internal enumeration omitted), and bars FDA from issuing such a license unless it determines that such a "biological product that is the subject of the application is safe, pure, and potent." *Id*. § 262(a)(2)(B)(i)(I) (current). Consistent with the 1970 statute, the post-FDAMA version in turn defined the term "biological product" as "a virus, therapeutic serum, toxin, antitoxin, blood, blood component or derivative, allergenic product, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of a disease or condition of human beings." *Id*. § 262(i) (1998 ed.); *cf. id.* at § 262(a) (1970 to 1997 eds.) (defining "biological product[s]" as "applicable to the prevention, treatment, or cure of diseases or injuries to man").

That update, however, left unclear one issue: the proper regulatory classification of protein-based products that mirror traditional biological products in their complexity but do not fit squarely within the categories set forth in the post-FDAMA statute. As a historical matter, the superficial overlap between the PHSA and the Federal Food, Drug, and Cosmetic Act (the "FDCA," codified in scattered sections of 21 U.S.C.) repeatedly raised questions regarding how the Agency should classify complex products that seemingly could have been regulated either as "drugs" under the FDCA or as "biological products" under the PHSA, and which sometimes were approved under FDCA-governed New Drug Applications (each an "NDA") and other times were licensed under PHSA-governed BLAs. *See Assessing the Impact of a Safe & Equitable Biosimilar Pol'y in the U.S.: Hearing Before the House Subcomm. on Health* (the "BPCIA Hearing"), at 20 (May 2, 2007) (testimony of Dr. J. Woodcock) ("In the U.S., proteins are regulated either as drugs under the [FDCA] or as biological products under the [PHSA]. Whether regulated as drugs or biological products, proteins fit into the category of complex molecules that can be difficult to fully characterize."); *see also* K. Carver *et al*., *An Unofficial Legis. Hist. of the Biologics Price Competition and Innovation Act 2009*, 65 FOOD & DRUG L.J. 671, 684-85 (2010) (noting that FDA had approved bovine-derived and porcine-derived insulin, human growth hormone, and conjugated estrogens under NDAs but "never explained its decision to require NDAs for these products" instead of BLAs).

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As the biotechnology revolution accelerated into the 21st century, FDA's hybrid approach to classifying complex proteins began to pose an increasingly serious dilemma for the American healthcare system. Though the FDCA included an abbreviated pathway for approving generic drugs, *see* Drug Price Competition and Patent Term Restoration Act of 1984 (the "Hatch-Waxman Act" or "Hatch-Waxman"), Pub. L. No. 98-417, 98 Stat. 1585, there was no comparable pathway for follow-on biological products under the PHSA. After years of studying the issue, Congress established a new pathway for the approval of follow-on biological products in 2010—and in the process unified FDA's regulation of complex protein-based products under the PHSA by passing the Biologics Price Competition and Innovation Act of 2009 (the "BPCIA"), Pub. L. No. 111-148, § 7002, 124 Stat. 119, 804-21 (2010).

Three features of the BPCIA are especially notable here. ***First***, the BPCIA amended the PHSA's definition of "biological product" to for the first time include "protein[s]" and their analogues. *Id*. § 7002(b)(1) (codified as amended at 42 U.S.C. § 262(i)(2)). ***Second***, Congress imposed a ten-year deadline for transitioning into the BLA regulatory scheme all products that meet the new, protein-including definition of "biological product" but previously had been approved under NDAs. *Id*. § 7002(e)(4) ("An approved application for a biological product under section 505 of the [FDCA] shall be deemed to be a license for the biological product under such section 351 on the date that is 10 years after the date of enactment of this Act."). And ***third***, the BPCIA made no changes to the "biological product" definition's intended-use clause. *See id*. § 7002(b)(1). Just as it did following FDAMA's passage, the post-BPCIA intended-use clause continues to apply to any covered product (now including proteins and their analogues) that is "applicable to the prevention, treatment, or cure of a disease or condition of human beings." 42 U.S.C. § 262(i)(1).[4]

FDA completed formal notice-and-comment rulemaking to implement the BPCIA's licensure-and-transition requirement for protein-based products shortly before the BPCIA's ten-year transition window expired. FDA, *Definition of the Term "Biological Product,"* 85 Fed. Reg. 10,057, 10,063 (Feb. 21, 2020). As relevant here, the Agency's final regulation now defines "protein" as:

> any alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size. When two or more amino acid chains in an amino acid polymer are associated with each other in a manner that occurs in nature, the size of the amino acid polymer for purposes of this paragraph (h)(6) will be based on the total number of amino acids in those chains, and will not be limited to the number of amino acids in a contiguous sequence.

[4] FDA's implementing regulations parallel the statutory definition of "biological product." *See* 21 C.F.R. § 600.3(h) ("Biological product means a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of a disease or condition of human beings.")

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21 C.F.R. § 600.3(h)(6). Under current law, any product which contains (1) "any alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size," *id*., and (2) is "applicable to the prevention, treatment, or cure of a disease or condition of human beings," *id*. § 600.3(h), therefore is a "biological product" subject to mandatory premarket licensure before it can be lawfully introduced into interstate commerce. 42 U.S.C. § 262(a)(1).

B. Relevant Factual Background

1. Endotoxins, Endotoxemia, and the Rabbit Pyrogen Test

Lipopolysaccharides (commonly called endotoxins) are highly pyrogenic substances located on the outer cell wall of Gram-negative bacteria. C. Raetz, *Bacterial Endotoxins: Extraordinary Lipids That Activate Eucaryotic Signal Transduction*, 175 J. BACTERIOLOGY 5745, 5745 (1993). When those bacteria divide or die, their endotoxins are secreted within a protective envelope (an "outer membrane vesicle") that helps them travel to distal binding sites without being mediated in transit. *See* A. Kulp *et al*., *Biological Functions & Biogenesis of Secreted Bacterial Outer Membrane Vesicles*, 64 ANN. REV. MICROBIOLOGY 163, 163-64, 168-70 (2010). Once endotoxins infect the human bloodstream (a condition known as "endotoxemia"), they can trigger a pathogenic cascade that causes patients to develop high fever and, without early detection and aggressive treatment, can lead to sepsis, septic shock, and even death—particularly in patients already suffering from serious medical conditions. J. Parillo, *Pathogenic Mechanisms of Septic Shock*, 328 NEW ENG. J. MED. 1471, 1471-73 (1993) (detailing endotoxemia's pathogenic cascade and noting that death rates for septic shock patients with endotoxemia are nearly 6 times higher than for septic shock patients without endotoxemia). It therefore is critically important to prevent endotoxic contamination in parenterally administered drugs, biological products, and many medical devices; after all, those products can be vectors for directly introducing endotoxins into the human bloodstream, thereby inducing potentially fatal endotoxemic reactions. *See* S. Fennrich *et al*., *More than 70 Years of Pyrogen Detection: Current State and Future Perspectives*, 44 ALTERNATIVES TO LAB. ANIMALS 239 (2016).

From the early 1940's through the mid-1970's, the standard method of testing pharmaceutical and medical device products for endotoxic contamination was the rabbit pyrogen test. *Id*. at 240-41. In this *in vivo* test, the test substance was injected in the upper ear vein of several live rabbits; an increase in body temperature was deemed as confirming the presence of endotoxins. *Id*. at 240. But that method was not without limitations: False positive results often were obtained due to the use of live animals, and the method otherwise was not suitable for use with products having properties that were not compatible with an injection-based test. *Id*.

2. LAL and Factor C

In the early 1960's, researchers discovered that the blood of horseshoe crabs contains a unique defense mechanism comprised of highly reactive cells (called "amebocytes") which degranulate and induce coagulation after exposure to endotoxins. *See* J. Levin *et al*., *The Role of Endotoxin in the Extracellular Coagulation of Limulus Blood*, 115 BULL. OF JOHNS HOPKINS HOSP. 265 (Sept. 1964) (first detailing the *Limulus* clotting system's response to endotoxins); T. Muta *et al*., *An Endotoxin-Sensitive Serine Protease Zymogen With a Mosaic Structure of Complement-*

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Like, Epidermal Growth Factor-Like, and Lectin-Like Domains, 266 J. BIOLOGICAL CHEM. 6554, 6554 (1991) (identifying amebocytes as the key reactive component in *Limulus* blood); *see also* S. Iwanaga, *Biochemical Principle of Limulus Test for Detecting Bacterial Endotoxins*, 83 PROCS. JAPAN ACAD., SER. B 110, 110 (2007) (noting that the clotting factors within *Limulus* amebocytes can be activated by mere pico or nano grams of endotoxin).

This unique defense mechanism was leveraged into the development of LAL-based alternatives to the rabbit pyrogen test, and the first such products became available in the early 1970s; it did not take long for FDA to recognize LAL's advantages over the rabbit pyrogen test in terms of speed, accuracy, sensitivity, specificity, and economy. 38 Fed. Reg. at 1404 (noting that "results are obtained [more] quickly" with LAL than with the rabbit pyrogen test); *see also* 42 Fed. Reg. at 57,749 ("The use of LAL in testing many biological products and devices has been demonstrated to usually exceed results obtained on the same products with the rabbit pyrogen test. The LAL test is more economical and requires a smaller volume of product for testing than the rabbit pyrogen test, and a large number of tests can be performed by one individual in a single day.").

LAL's mechanism of action—again, the "LAL cascade"—is now well-documented in the literature. As illustrated in Figure 1 below, even minute quantities of endotoxin rapidly trigger successive activations of LAL's Factor C, Factor B, and Pro-clotting Enzyme. *Iwanaga*, 83 PROCS. JAPAN ACAD., SER. B, at 112. Upon contact with endotoxin, Factor C cleaves itself and autoactivates; the activated Factor C then cleaves and activates Factor B; the activated Factor B then cleaves and activates the Pro-clotting Enzyme; and, when coupled with additional substrates, the activated Pro-clotting enzyme causes either the formation of a gel clot (when coupled with natural coagulogen) or generates an observable color change (when coupled with a substrate) that in turn establishes the presence (or confirms the absence) of endotoxin in sampled materials. *See id*.

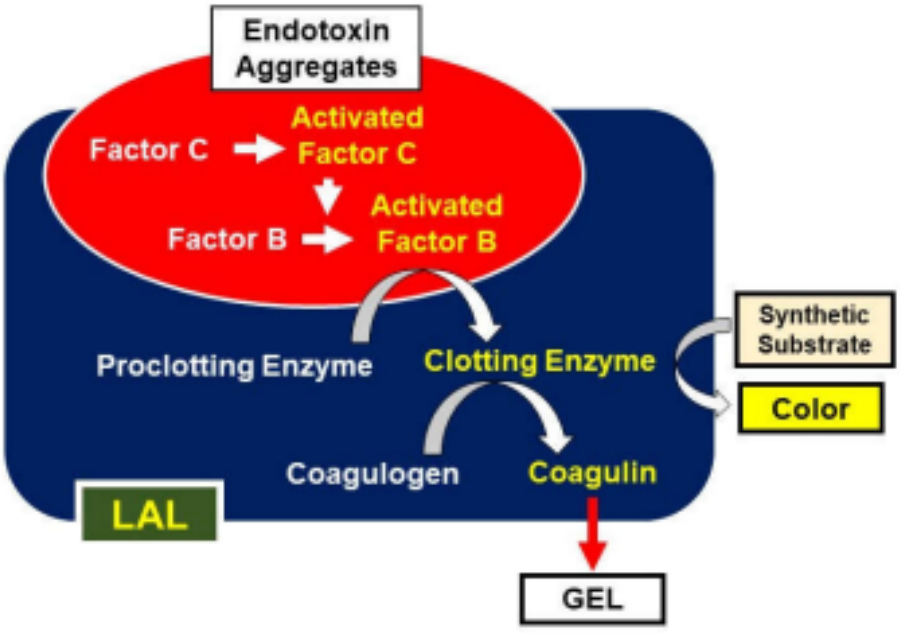

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Fig. 1: The LAL Cascade (reproduced from M. Tsuchiya, *Innovative Mechanism of Limulus Amebocyte Lysate Activation to Achieve Specificity and Sensitivity to Endotoxin; Comparison with Recombinant Factor C Reagents*, 10 INT'L J. DEV. RES. 36751, 36754 (June 2020).

Each of LAL's three active components is important to the accuracy, specificity, and sensitivity of LAL-based end-product endotoxin tests. Nonetheless, Factor C is believed to be LAL's most critical component because endotoxins do not activate Factor B or Pro-Clotting Enzyme on their own. Tsuchiya, 10 INT'L J. DEV. RES. at 36755; *see also* Y. Kobayashi *et al*., *Factor B Is the Second Lipopolysaccharide-Binding Protease Zymogen in the Horseshoe Crab Coagulation Cascade*, 31 J. BIOL. CHEM. 19379 (2015) (establishing that Factor B activates only in the presence of activated Factor C).

Most important for present purposes, Factor C, Factor B, and Pro-Clotting Enzyme are each proteins. With respect to Factor C in particular, as early as 1991, Muta and colleagues sequenced *Limulus*-derived Factor C and concluded that it consists of 994 amino acids with a calculated mass of 109,648 Da. T. Muta *et al*., 266 J. BIOL. CHEM. at 6555. Factor C's amino acids in turn are arranged in a mosaic structure that, as illustrated in Figure 2 below, includes (1) five short consensus repeat ("sushi") units of roughly 60 amino acids each that resemble those "found in more than 20 proteins, mainly in proteins participating the mammalian complement system"; (2) an epidermal growth factor-like domain that likewise resembles complementary domains in other recognized proteins; (3) a unique C-type lectin domain; and (4) a typical serine protease domain that once again is found in other well-characterized proteins. *Id.* Subsequent research establishes that Factor C derived from other horseshoe crab species shares substantially similar features. *See, e.g.*, Ding *et al*., *Molecular Cloning and Sequence Analysis of Factor C cDNA from the Singapore Horseshoe Crab, Carcinoscorpius rotundicauda*, 4 MOLECULAR MARINE BIOLOGY AND BIOTECH. 90, 93 (Apr. 1995) (determining that *Carcinoscorpius*-derived Factor C consists of 1059 amino acids and contains the same defining features as its *Limulus* analogue); *id*. at 94-96 (illustrating *Carcinoscorpius*'s homology with Factor C derived from *Tachypleus tridentatus*).

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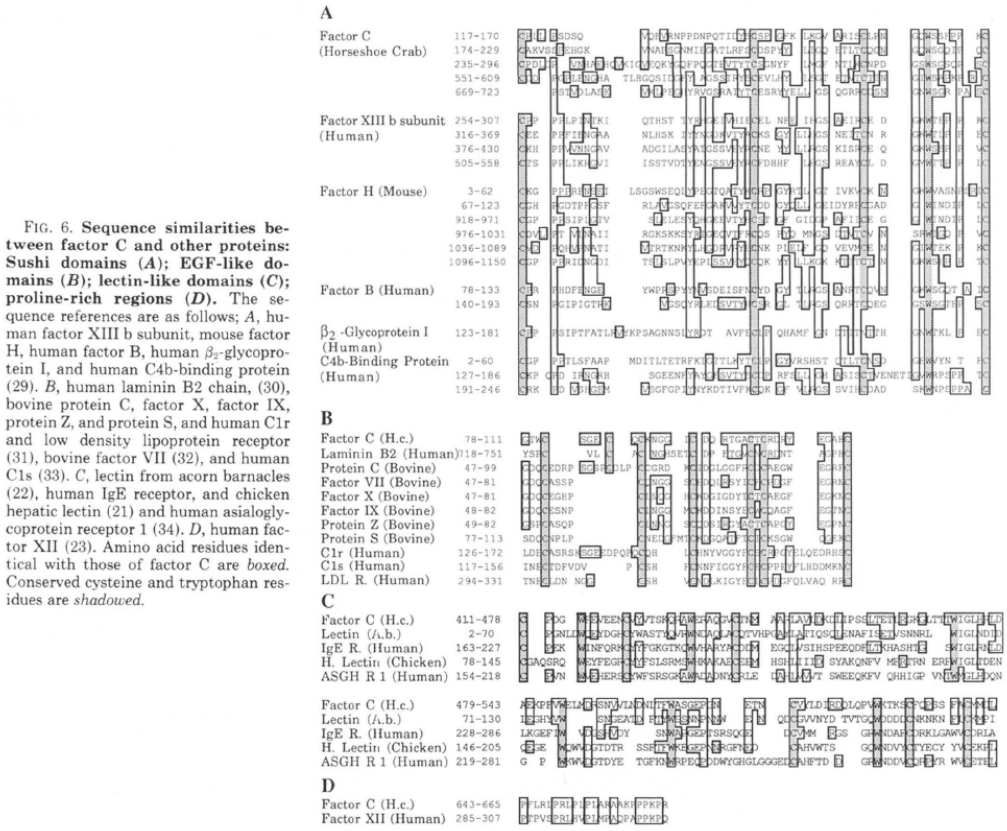

FIG. 6. **Sequence similarities between factor C and other proteins: Sushi domains (*A*); EGF-like domains (*B*); lectin-like domains (*C*); proline-rich regions (*D*).** The sequence references are as follows; *A*, human factor XIII b subunit, mouse factor H, human factor B, human β_2-glycoprotein I, and human C4b-binding protein (29). *B*, human laminin B2 chain, (30), bovine protein C, factor X, factor IX, protein Z, and protein S, and human C1r and low density lipoprotein receptor (31), bovine factor VII (32), and human C1s (33). *C*, lectin from acorn barnacles (22), human IgE receptor, and chicken hepatic lectin (21) and human asialoglycoprotein receptor 1 (34). *D*, human factor XII (23). Amino acid residues identical with those of factor C are *boxed*. Conserved cysteine and tryptophan residues are *shadowed*.

Fig. 2: Sequence and Structural Similarities Between Factor C and Other Known Proteins (reproduced from Muta *et al*., 266 J. BIOL. CHEM. at 6558).

Factor C's endotoxin binding sites are located along two of the protein's roughly 60-amino-acid-long sushi domains (designated S1 and S3). Tan *et al*., *Definition of Endotoxin Binding Sites in Horseshoe Crab Factor C Recombinant Sushi Proteins and Neutralization of Endotoxin by Sushi Peptides*, 14 J. FED. AM. SCI. EXPERIMENTAL BIOLOGY 1801, 1806 (2000). Two features of those sushi domains in turn appear to explain Factor C's high reactivity to endotoxin: the presence of multiple binding sites and their extraordinary positive cooperativity in binding. *Id.* at 1811.

C. FDA's Longstanding Classification of LAL-Based Products as Biological Products Subject to Mandatory Premarket Licensure Under the PHSA

In January 1973, FDA responded to widespread commercial interest in the use of LAL end-product endotoxin tests by publishing its seminal LAL classification decision in the *Federal Register*. 38 Fed. Reg. at 1404. After noting that the post-1970 PHSA covered products containing any "blood, blood component or derivative, … or analogous product," *id*. (quoting 42 U.S.C. § 262(i)), the Agency first concluded that LAL end-product endotoxin tests meet that standard because LAL is "prepared from the circulating blood cells (amebocytes) of the horseshoe crab (*Limulus polyphemus*)." *Id*. It next concluded that LAL products meet the statute's intended-use clause when used "for the detection of bacterial endotoxins (pyrogens) in biological products and other drugs or fluids for parenteral administration to man." *Id*. In light of the fact that

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"administration of fluids containing bacterial endotoxins can produce shock, fever, and death," FDA explained:

> The Commissioner of Food and Drugs, who is charged with administering section 351 of the [PHSA] and the provisions of 21 CFR Part 273, finds that such a product ***is a biological product applicable to the prevention or treatment of disease in man, in that [its] utilization [is] for the detection of bacterial endotoxins to prevent unsafe drugs from being administered [and thereby] renders it subject to section 351 of the [PHSA]***.

Id. (emphasis added).

These findings meant that unlicensed LAL-based end-product endotoxin tests could not immediately be marketed in interstate commerce despite "the value of such a product when employed for the prevention or treatment of disease in man by the detection of bacterial endotoxins to prevent the administration of unsafe drugs." *Id*. In an effort to begin leveraging the benefits of LAL technology as soon as possible, however, the Commissioner's decision pledged that FDA would promulgate standards for licensure and, in the interim, expressly authorized end-product manufacturers to begin using LAL-based assays for in-process testing so long as the product labeling "clearly limits its use to in-process testing … and states that the test is not suitable … as a replacement for the official rabbit pyrogen test." *Id*.

The Agency issued its standards for LAL end-product endotoxin testing of biological products and medical devices in May 1977. *See* FDA, *Biological Products*, 42 Fed. Reg. 23,167 (May 6, 1977) (announcing the release of guidance for industry). And six months later, FDA announced that it had approved the first BLA for such a product under the May 1977 standards. 42 Fed. Reg. at 57,750. Given that approval, the Agency formally authorized (1) biological product manufacturers to begin using licensed LAL assays in lieu of the rabbit pyrogen test so long as they obtain FDA approval of "a product license amendment for each product for which licensed LAL will be used in the official pyrogen test," and (2) device manufacturers to use such tests once they "submit[ted] to the Bureau of Medical Devices data establishing that the LAL test that the manufacturer proposes to use is at least equivalent to the rabbit test, and the manufacturer has obtained written approval to use the LAL test from the director of the Bureau." *Id*.[5]

Since that time, FDA without exception has regulated all naturally-sourced LAL-based end-product endotoxin tests as biological products subject to the PHSA mandatory premarket

[5] The Commissioner's initial decision did not apply to manufacturers of human drugs, pending FDA's development of LAL standards for end-product testing of drugs. *Id*. The Agency issued its first such standards in 1980, *see* FDA, *Human and Veterinary Drugs; Availability of Draft Guidance for Use of Limulus Amebocyte Lysate*, 45 Fed. Reg. 3,668 (Jan. 18, 1980), and the use of LAL-based products to test biological products, pharmaceuticals, and medical devices for endotoxin is now the most prevalent means of clearing all three categories of medical products for release. *See, e.g.*, FDA, *Guidance for Industry—Pyrogen and Endotoxins Testing: Questions and Answer*s, at 2 (June 2012).

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licensure provisions, and their use is now a firmly entrenched feature of drug, biological product, and medical device production. *See, e.g.*, FDA, *Guidance for Industry—Pyrogen and Endotoxins Testing: Questions and Answers* (June 2012) ("For more than 30 years, FDA has accepted the use of a Limulus Amoebocyte Lysate (LAL) test for endotoxins in lieu of the rabbit pyrogens test.").

D. Recombinant Factor C and the Ombudsman's Cambrex Letter

The advent of recombinant DNA technology in the 1980's transformed the pharmaceutical sciences. At the highest level of generality, this technique involves cloning DNA encoding a useful protein; integrating the cloned DNA into engineered expression vectors which in turn are introduced into host cells which can translate the encoded DNA sequences into proteins; and purifying/isolating the protein of interest. *See generally* U.S. Patent No. 5,712,144 (Examples 1-8).

Researchers began work on making recombinantly produced alternatives to the proteins in the LAL cascade, and in 1998, scientists at the National University of Singapore patented a method for engineering recombinant Factor C from one particular horseshoe crab species (*Carcinoscorpius rotundicauda*) for potential use as an alternative to naturally derived LAL-based end-product endotoxin tests. *See generally* U.S. Patent No. 5,712,144.[6] Unlike naturally sourced LAL-based endotoxin tests, however, recombinant Factor C technology by design lacks both the Factor B and Pro-clotting Enzyme found in LAL; as illustrated in Figure 3, it can be coupled with a fluorogenic substrate that is triggered directly by the activated recombinant Factor C, and therefore operates in a single step rather than through LAL's well-characterized and finely tuned multistep cascade.

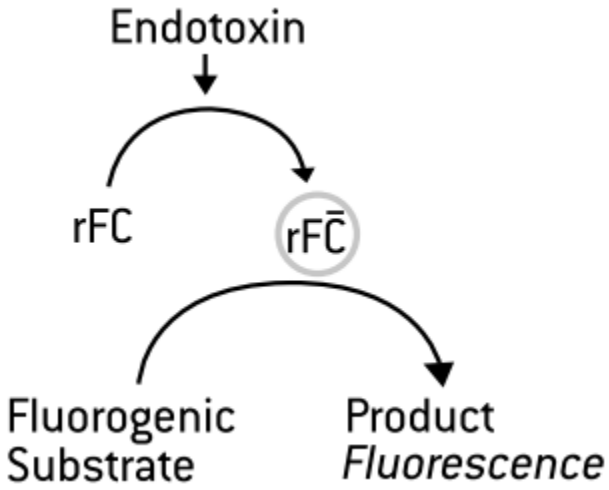

Fig. 3: Recombinant Factor C's Single-Step Activation (reproduced from Lonza's PyroGene Brochure at 4).

[6] Figures 6A-6D of this patent provide the complete DNA sequence and deduced amino acid sequence of Factor C derived from this species of horseshoe crab.

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This technology initially appears to have been licensed to Lonza's predecessor Cambrex in 2000, *see Singapore Scientists Cloned Anti-Clot Factor C Enzyme,* 8 ASIA-PACIFIC BIOTECH NEWS 1011, 1012 (2004) (documenting the license arrangement), and on December 26, 2002, Cambrex submitted a Request for Designation ("RFD") requesting that FDA (1) regulate the resulting product (PyroGene) as "an *in vitro* diagnostic (IVD) test … intended to test products for contamination with endotoxin," and (2) assign lead responsibility for the review to the Center for Devices and Radiological Health ("CDRH"). *See* Cover Letter to RFD 2002.040 (the "PyroGene RFD," attached as Exh. 2); *see also id*. at 8, 10.

Cambrex's RFD initially acknowledged that CBER "currently is the lead on conventional LAL products for endotoxin detection." *Id*. at 10. But it argued that FDA's historical assignment of LAL should not control the assignment of PyroGene because its recombinant Factor C has "less inherent variability [than LAL], and more importantly … blood is not used in the source material." *Id*.; *see also id*. at 11 ("Regardless of the history of LAL regulation in CBER, because this next generation test is not derived from the blood of any animal, and because it relies on distinguishable technology from the *Limulus* amebocyte assay, the initial assignment of LAL to CBER should not unduly influence the assignment of this new test."). Instead, Cambrex argued that its product should be regulated "as *in vitro* diagnostic used to test inert substances." *Id*. at 11. And emphasizing that "CDRH was not yet a functional center" when FDA classified LAL as a biological product subject to CBER's purview, *id*. at 10, Cambrex asserted that "[t]he expertise to review the technology and the indications [now] exists in [t]he new Office of In Vitro Diagnostics in CDRH." *Id*. at 11.

On February 24, 2003, FDA's Ombudsman—who at that time was responsible for resolving intra-agency jurisdictional questions, *see infra* at 20—responded to Cambrex in a private letter decision that Charles River has obtained under the Freedom of Information Act. *See* Cambrex Ltr. at 1. The Ombudsman's letter denied Cambrex's request on the ground that PyroGene "does not require premarket approval" ***at all***, whether as a biological product under the PHSA or as an IVD under the Agency's IVD regulations. *Id*. With respect to the former, the Ombudsman's letter found that PyroGene was not a biological product subject to premarket licensure under the PHSA because it "does not make use of any live animals" and thus lacks "the possibility of variation in animal-derived products." *Id*. With regard to the latter, the Cambrex Letter found that the product was not subject to regulation as an IVD because it was intended "to test for endotoxin contamination in other products and not man or animals, is not intended to qualify blood or blood products, and is not intended for use in patient management." *Id*. at 1; *see also* 21 C.F.R. § 809.3 (defining an IVD as a product "intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, … [*via*] the collection, preparation, and examination of specimens taken from the human body"). Finally, the FDA Ombudsman informed Cambrex that its immunity from regulation was contingent on the product labeling's inclusion of "[a] statement specifically excluding the use of this test for clinical diagnosis, patient management, and qualification of blood or blood products" (*i.e*., indications that would trigger regulation as an IVD), and cautioned that future "[p]remarket approval will be required" if the company later intended PyroGene for use as an IVD—*i.e*., "for clinical diagnosis or patient management, or to qualify blood or blood products." Cambrex Ltr. at 2.

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Since that time, several other companies have begun marketing recombinant Factor C products for use in end-product endotoxin testing. But—in apparent reliance on the Ombudsman's 2003 Cambrex Letter—neither Cambrex, its successors, or the other recombinant Factor C manufacturers appears to have submitted a BLA or other premarket application for FDA review (much less secured FDA approval for such a license or application).

E. ARGUMENT

1. Recombinant Factor C End-Product Endotoxin Tests Are Biological Products Subject to Mandatory Premarket Licensure Under the Post-BPCIA Version of the PHSA.

The PHSA subjects every "biological product" to mandatory premarket licensure through the BLA pathway. 42 U.S.C. § 262(a)(1)(A) ("No person shall introduce or deliver for introduction into interstate commerce any biological product unless a biologics license … is in effect for the biological product.") (internal enumeration omitted). And it establishes a clear test defining what a "biological product" is: The product must (1) contain "a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound)," *id*. § 262(i)(1), and (2) be "applicable to the prevention, treatment, or cure of a disease or condition of human beings." *Id*. Recombinant Factor C products intended for end-product endotoxin testing satisfy both prongs of the statute's definition. As such, FDA must mandate that the manufacturers of these products obtain premarket authorization from the Agency *via* an approved BLA prior to commercialization.

a. Recombinant Factor C Is a Protein.

Recombinant Factor C meets the first prong of the statute's "biological product" definition because it indisputably is a "protein." The Agency's BPCIA-implementing regulations define the term "protein" as "any alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size," 21 C.F.R. § 600.3(h)(6), and recombinant Factor C meets that bright-line standard. ***First***, all forms of Factor C—whether derived directly from natural specimens or bio-engineered through recombinant DNA technology, and regardless of which horseshoe crab species supplies the starting source material or is used to build a recombinant DNA template—are comprised of a polymerized peptide chain (*i.e*., a polymer) that is far greater than 40 alpha-amino acids in size.[7] As detailed *supra*, the scientific literature unambiguously confirms that Factor C exceeds the Agency's 40-unit numerical threshold for alpha amino acids; depending on the particular species of horseshoe crab from which it is derived, Factor C's complete sequence ranges from ***just under 1000 amino acids*** to ***nearly 1100 amino acids*** in size. *Supra* at 11-12 (determining the alpha amino acid structures of Factor C in multiple horseshoe crab species; citing

[7] Factor C and recombinant Factor C comprise a polymer of naturally occurring alpha-amino acids. *See, e.g*., U.S. Patent No. 5,712,144, Figures 6A-6D (disclosing the complete sequences of polymerized alpha amino acids in recombinant Factor C derived from *Carcinoscorpius rotundicauda*).

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Muta *et al*., 266 J. BIOLOGICAL CHEMISTRY at 6554; Ding *et al*., 4 MOLECULAR MARINE BIOLOGY & BIOTECH. at 93-96).[8]

Second, recombinant Factor C meets the Agency's "specific, defined sequence" requirement. Indeed, that is true of ***all*** recombinant DNA products, regardless of their size; the whole point of recombinant biotechnology is to reproduce specifically defined amino acid sequences from a single DNA template, without variation, time and again. *Supra* at 14 (describing the production process for recombinant DNA products). The specific and defined full amino acid sequence of the Factor C protein from *C. rotundicauda*, and the DNA sequence encoding the amino acid sequence, is provided in Figures 6A-6D of U.S. Patent No. No. 5,712,144.

The fact that recombinant Factor C is made from a synthetic process that theoretically results in less variability in the product—a factor cited in the Ombudsman's decision regarding the PyroGene product in the Cambrex Letter—provides no legal basis for drawing a distinction relative to naturally derived LAL products under the PHSA. Even before the BPCIA's enactment, FDA's regulations made clear that recombinant products which otherwise meet the statute's definition of "biological product" were subject to mandatory premarket licensure under the PHSA. *See, e.g.*, 21 C.F.R. § 601.2(a)(4) (promulgated *via* FDA, *Biological Products Regulated Under Section 351 of the Public Health Service Act; Implementation of Biologics License; Elimination of Establishment License and Product License—Final Rule*, 64 Fed. Reg. 56,441 (Oct. 20, 1999)). The Agency's BPCIA-implementing regulation further prevents any distinctions among protein-based products based on their method of manufacture. By its plain terms, FDA's definition of "protein" applies to "***any***" product that meets the defined-sequence and 40-unit-size requirements, and therefore applies to ***all proteins, irrespective of their method of manufacture***. *See* 21 C.F.R. § 600.3(h)(6) (emphasis added).

That is no accident. After FDA first proposed its "clear, bright-line rule" covering "***any*** alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size" in 2018, *see* FDA, *Definition of the Term "Biological Product"—Proposed Rule*, 83 Fed. Reg. 63,817, 63,820 (Dec. 12, 2018) (emphasis added), at least one commenter asked that FDA instead adopt a definition that would have been "principally focused on the [product's] method of manufacture" instead of its embedded amino acid structure. 85 Fed. Reg. at 10,060. The Agency expressly and unequivocally rejected that proposal:

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[8] Manufacturers of recombinant Factor C products ***recognize*** that recombinant Factor C is a "protein," as the term is normally used in the scientific literature. *See, e.g.*, Lonza, *PyroGene® Recombinant Factor C Assay* (the "PyroGene® Brochure"), at 4 (2021, attached as Exh. 3) ("The PyroGene® rFC contains ***a recombinant protein***, Factor C, derived from the horseshoe crab's defensive enzyme clotting cascade which is used in traditional bacterial endotoxin tests.") (emphasis added); bioMérieux, *Endozyme® II GO: Endotoxin Testing Made Faster, Easier, and Sustainable* (the "Endozyme® II GO Brochure"), at 2 (2020, attached as Exh. 4) ("The rFC in ENDOZYME II GO consists of ***a single recombinant protein*** and a small fluorescent peptide.") (same).

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> [A]dopting an interpretation that focused on the method of manufacture could improperly incentivize product developers to choose a suboptimal method of manufacturing a product … based on a perceived regulatory advantage under a particular regulatory scheme. It is FDA's view that the optimal policy for determining which products are subject to regulation under the PHS Act is to apply a bright-line rule that provides regulatory certainty. Thus, in order to provide regulatory certainty and provide a bright-line interpretation of the term 'protein,' we are focusing on the number of amino acids in the amino acid polymer ***(irrespective of the method of manufacture)***.

Id. (emphasis added).

The circumstances giving rise to this Petition illustrate why the Agency's concerns were well-founded. In stark contrast to Charles River—whose naturally derived end-product endotoxin tests are subject to mandatory premarket licensure and comprehensive postmarketing regulation that includes inspections, reporting obligations, and the need to apply for and obtain FDA's prior authorization for virtually any product- or process-based improvement—the Company's recombinant Factor C competitors are free to market their products without direct FDA regulation, because their products are not limited by the terms of an effective BLA or subject to the PHSA's postmarketing requirements. That is precisely the kind of "regulatory advantage" that the Agency's unqualified regulation of "any" protein claimed to eschew, *see id*., and there is no legitimate basis for distinguishing among proteins depending on their method of manufacture.

In addition, FDA's unqualified regulation of all proteins, regardless of their mode of manufacture, has withstood legal challenge and has been adopted by the courts. Shortly after the Agency implemented its post-BPCIA regulation, its approach was challenged by a product sponsor who asserted that FDA's defined-sequence requirement impermissibly distinguished between proteins depending on their method of manufacture. *See Teva*, 514 F. Supp. 3d at 97. The court squarely rejected the sponsor's argument—holding ***not only*** that the Agency's regulations draw no distinction based on method of manufacture, ***but also*** that the BPCIA recognizes no such distinction: "[C]ontrary to Teva's representations, neither the statute nor the rule ever distinguished between naturally derived and chemically synthesized proteins…. Rather, it treated all qualifying molecules as proteins, regardless of mode of manufacture." *Id*. at 101. Given the plain language of the statute and the Agency's post-BPCIA regulations; the well-characterized features of Factor C, as established in the scientific literature; FDA's explicit rejection of a proposal to distinguish between protein-based products depending on their method of manufacture; and the Agency's successful defense of that position in court, there can be no serious question that recombinant Factor C is a "protein" for purposes of the PHSA.

b. End-Product Endotoxin Tests Meet the Statute's Intended-Use Clause Regardless of Their Method of Manufacture.

Every Factor C-containing product intended to test finished biological products for endotoxic contamination likewise satisfies the intended-use clause of the PHSA's "biological product" definition, just as naturally derived LAL products do. That test requires that the product at issue be "applicable to the prevention, treatment, or cure of a disease or condition of human

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beings," 42 U.S.C. § 262(i)(1), and therefore focuses solely on the product's utility—not how it was manufactured before being put to its intended use. End-product endotoxin tests that include naturally sourced LAL and recombinant Factor C both meet that standard, because both types of tests have precisely the same intended use: to prevent endotoxemia that is caused by exposure to contaminated drugs, biological products, and medical devices.

As explained previously, endotoxemia is a serious "disease or condition of human beings" that has been known for more than a century to cause high fever, sepsis, shock, and even death (each of which is a "condition of human beings" in its own right). *Supra* at 9; *see also* Disease, MERRIAM-WEBSTER DICTIONARY ONLINE, https://www.merriam-webster.com/dictionary/disease (defining "disease" as "a condition of the living animal … that impairs normal functioning and is typically manifested by distinguishing signs and symptoms" and "condition" as "a state of being … usually [a] defective state of health"); OXFORD ENG. DICTIONARY (June 2021 ed.) (defining "disease" as "[a] condition of the body, or of some part or organ of the body, in which its functions are disturbed or deranged … an illness, ailment, malady, disorder" and "condition" as "[a] state of health, esp. one which is poor or abnormal; a malady or sickness"). The whole point of testing finished biological products, pharmaceuticals, and medical devices for endotoxic contamination before they are released for human use is ***to prevent human beings*** from succumbing to endotoxemia and the litany of dangerous medical conditions it inflicts on the body. *Supra* at 9-10, 12-13. The manufacturers of recombinant Factor C products likewise recognize that the intended use of their product is applicable to the prevent, treatment, or cure of a disease condition in people. *See* bioMérieux, *10 Reasons to Choose Recombinant Factor C for Bacterial Endotoxin Testing*, at 1 (2020) (the "bioMérieux Marketing Brochure," attached as Exh. 5) ("LPS, also known as bacterial endotoxins, can elicit a severe response in the immune system, resulting in fever, hypotension, nausea, shock, and sepsis. Severe reactions to bacterial endotoxins can be fatal, and as such, great care must be taken to ensure that they don't find their way into medical products making contact with a patient's bloodstream or cerebral fluid").

This same disease-preventing use is why the Commissioner of Food of Drugs had little trouble recognizing that LAL-based end-product endotoxin tests meet the statute's test for mandatory premarket licensure requirement when the Agency definitively resolved this question nearly 50 years ago:

> It is well known that the administration of fluids containing bacterial endotoxins can produce shock, fever, and death. The Commissioner of Food and Drugs, who is charged with administering section 351 of the [PHSA] and the provisions of 21 CFR Part 273, finds that such a product ***is a biological product applicable to the prevention or treatment of disease in man, in that utilization for the detection of bacterial endotoxins to prevent unsafe drugs from being administered*** … renders it subject to section 351 of the Public Health Service Act.

38 Fed. Reg. at 1404 (emphasis added). Apart from updating the intended-use clause to use gender-neutral language (*i.e.*, replacing the phrase "diseases or injuries to man," 42 U.S.C. § 262(a) (1970), with "disease or condition of human beings," *id.* § 262(i) (current)), Congress has not substantively altered the intended-use clause since the Commissioner's decision; there accordingly is no basis for reaching a different conclusion now. *See, e.g.*, *Lorillard, Div. of Loew's*

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Theaters Inc. v. Pons, 434 U.S. 575, 580 (1978) ("Congress is presumed to be aware of an administrative or judicial interpretation of a statute and to adopt that interpretation when it re-enacts a statute without change.") (collecting cases); *see also CFTC v. Schor*, 478 U.S. 833, 846 (1986) ("[W]hen Congress revisits a statute giving rise to a longstanding administrative interpretation without pertinent change, the 'congressional failure to revise or repeal the agency's interpretation is persuasive evidence that the interpretation is the one intended by Congress.'") (quoting *NLRB v. Bell Aerospace Co*., 416 U.S. 267, 275 (1974)).

The 2003 Cambrex Letter does not support a different interpretation of the PHSA, and any reliance on it to argue otherwise is misplaced.[9] The Ombudsman's discussion of PyroGene's intended use as a "test for endotoxin contamination in other products and not man or animals [and not] to qualify blood or blood products [or] for use in patient management" did not purport to interpret ***the PHSA***, but instead answered the specific and narrow question presented by the manufacturer Cambrex; namely, whether PyroGene qualified ***as an IVD***. *See* Cambrex Ltr. at 1. After all, the Cambrex Letter already had observed that PyroGene was a recombinant Factor C product that by design "does not make use of any live animals" and therefore lacks "the possibility of variation in animal-derived products," *id*.—facts that in 2003 were viewed as arguably sufficient to rule out PyroGene's status as a "biological product" subject to the PHSA, which at that time applied (as relevant here) only to "blood, blood component[s] or derivative[s]" and so arguably did not cover proteins which, are not derived directly from blood (like recombinant Factor C). *See* 42 U.S.C. § 262(i) (2003).

The Agency's longstanding IVD regulation, by contrast, did implicate the intended-use issues considered by the Ombudsman; it applied to products that were "intended for use ***in the diagnosis of disease or other conditions***, including a determination of the state of health, … [*via*] the collection, preparation, and examination of specimens taken from the human body." 21 C.F.R. § 809.3. Given Cambrex's request that PyroGene be regulated ***as an IVD***, the Ombudsman's recognition that PyroGene was not intended to diagnose endotoxemia in patients (*i.e*., to determine whether their blood had been infected by endotoxins), but instead was meant only to test end-products for endotoxic contamination in order to prevent endotoxemia, speaks only to the issue of whether PyroGene should be regulated as an IVD. It did ***not*** represent the Ombudsman's position

[9] In October 2002, the Medical Device User Fee and Modernization Act ("MDUFMA"), Pub. L. No. 107-250, 116 Stat. 1588 (2002), ordered FDA to establish a new Office under the Commissioner's purview in order to resolve intra-agency jurisdictional questions and ensure "consistent and appropriate postmarket regulation of like products subject to the same statutory requirements to the extent permitted by law." *Id*. § 204, 116 Stat. at 1611 (currently codified at 21 U.S.C. § 353(g)(8)). That re-assignment of functions was a direct response to complaints that the existing process did not provide adequate "predictability, transparency, clarity, communication, speed, and fairness in the review process." H. Manresa *et al*., *Combination Products and the FDA: Issues and Answers*, 2 BIOTECH. HEALTHCARE 41, 44 (Feb. 2005). Given MDUMFA's 60-day deadline for transferring responsibility for such decisions to OCP, it is at best unclear whether the Ombudsman even had lawful authority to issue the Cambrex Letter at the time he did so (*i.e*., on Feb. 24, 2003, some four months after MDUMFA's enactment).

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that end-product endotoxin tests are outside ***the PHSA's intended-use clause***, but instead explained the Ombudsman's conclusion that PyroGene ***is not an IVD*** subject to premarket approval by CDRH because it is not a diagnostic tool that operates directly on human blood, tissue, or other samples.

It hardly could be otherwise. As noted above, the Commissioner definitively construed the PHSA's intended-use clause in 1973 and determined that it applied to LAL products used for end-product endotoxin testing, 38 Fed. Reg. at 1404, and FDA to this day continues to regulate Charles River's LAL-based end-product endotoxin tests as biological products subject to premarket licensure under the PHSA and comprehensive postmarketing regulation that includes inspections and reporting obligations. PyroGene and the other recombinant Factor C-based end-product endotoxin tests currently being marketed in interstate commerce have precisely the same intended use that led FDA to regulate naturally sourced LAL products as biological products—namely, to ensure that finished biological products, pharmaceuticals, and medical devices are not contaminated with endotoxins, in order to prevent the potentially fatal risks that endotoxin-contaminated biological products, pharmaceuticals, and medical devices pose to human health. Again, nothing in the PHSA's intended-use clause turns on whether a given product is derived from natural specimens or produced through recombinant DNA technology, and whatever merit there may have been to the Ombudsman's decision that PyroGene was immune from premarket licensure ***in 2003*** because it was not directly blood-derived, that fact is irrelevant ***today*** given the BPCIA's 2010 extension of the PHSA's definition of "biological product" to include all "protein[s]" regardless of their method of manufacture.

At bottom, recombinant Factor C products used to test finished biological products, pharmaceuticals, and medical devices for the presence of endotoxins are "biological products" within the plain meaning of today's PHSA. The continued marketing of those products in interstate commerce without an effective BLA therefore is patently unlawful. 42 U.S.C. § 262(a)(1)(A) ("No person shall introduce or deliver for introduction into interstate commerce any biological product unless a biologics license under this subsection or subsection (k) is in effect for the biological product.") (internal enumeration omitted). The Agency must take action to align its regulatory treatment of these products with the post-BPCIA statute, and Charles River accordingly requests that the Agency act promptly to ensure that the manufacturers of these products take all necessary steps to bring their conduct into compliance with the PHSA's unambiguous licensure requirement.

2. FDA's Failure to Regulate Recombinant Factor C Tests Jeopardizes Public Health.

As explained, the post-BPCIA version of the PHSA and FDA's BPCIA-implementing regulations leave the Agency no discretion to subject end-product endotoxin tests containing Factor C to different regulatory standards depending on their method of manufacture. Even if the Agency did have discretion (which it does not), patient safety concerns strongly weigh in favor of regulating endotoxin tests that contain recombinant Factor C the same way FDA has always regulated the naturally derived LAL-based products—through the BLA pathway, by applying the Agency's experience and expertise to ensure these products are safe, pure, potent, and manufactured, processed, packed, and held in facilities that comply with FDA's well-established

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safety standards. *See* 42 U.S.C. §§ 262(a)(2)(C)(i). Given that the whole point of the original BCA was to prevent contamination-driven outbreaks, and after 120 years of legislative reforms that progressively strengthened and expanded the reach of the PHSA, there can be no justification for exemption of recombinant Factor C products from direct regulatory oversight. *Supra* at 4-7 (detailing the PHSA's history from the 1901 St. Louis Incident through the 1971 DBS vaccine scandal).

The patient health and safety risks posed by these products are more than merely theoretical. Recently published, peer-reviewed data comparing the three market-leading recombinant Factor C products (two manufactured by bioMérieux and one by Lonza) with the two market-leading LAL-based assays (one manufactured by Lonza and one by Charles River) now leave no doubt that recombinant Factor C assays are inferior—and indeed pose an unacceptable risk of returning false negative results in real-world conditions involving purified water used to produce pharmaceuticals and biologics. *See* J. Dubczak *et al*., *Evaluation of Limulus Amebocyte Lysate & Recombinant Endotoxin Alternative Assays for an Assessment of Endotoxin Detection Specificity*, 159 EUR. J. OF PHARM. SCIS. 105716, at 1 (2021) (attached as Exh. 6). Importantly, this new data was generated using natural environmental endotoxins (rather than purified endotoxins that do not exist in the real world) present in samples sourced directly from water systems in pharmaceutical facilities that manufacture parental medications. *Id*. at 2. The results are unmistakable: As shown in Fig. 4 (Figure 1 of the reference), the recombinant Factor C products on average exhibited roughly 40% of the average reactivity exhibited by the LAL-based assays (an experimental recombinant full-cascade formulation (rLAL) developed by Charles River exhibited roughly 60% relative reactivity).

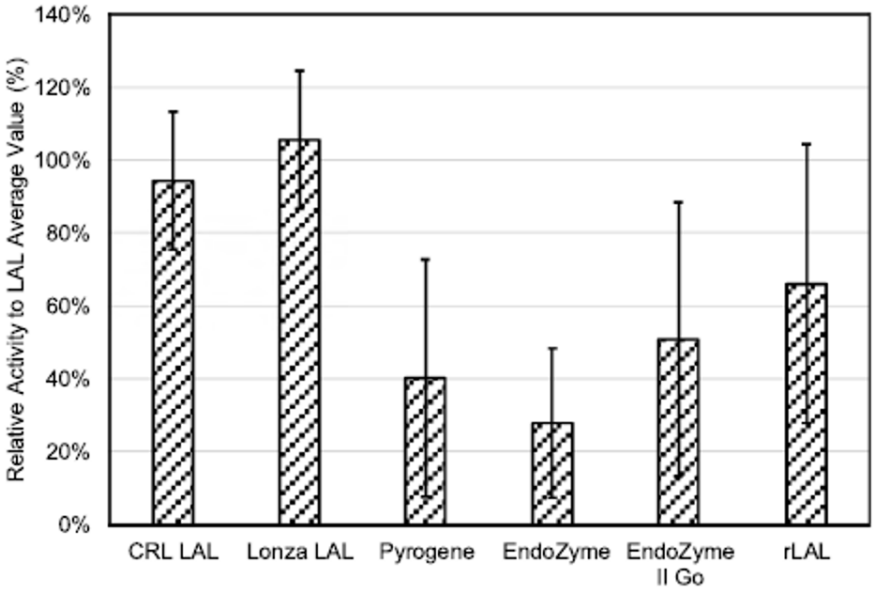

Fig. 4. Recoverable Endotoxin Activity of LAL and Recombinant Factor C Assays (reproduced from Dubczak *et al*., 159 EUR. J. OF PHARM. SCIS. 105716, at 4).

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The average underperformance of these recombinant Factor C products is concerning enough. But the raw data reveals an even more dire picture: It reflects that the various recombinant Factor C products ***underestimated*** the quantity of natural endotoxin present in real-world samples ***by more than 50 percent*** in ***between 65 and 95 percent*** of cases—meaning that in the ***overwhelming majority*** of cases, recombinant Factor C products detected ***less than half*** of the natural endotoxin in these real-world samples (again, Charles River's experimental formulation significantly outperformed). *Id*. at 4. The extraordinary failure rate of these recombinant Factor C products not only is statistically significant (all p-values were < 0.0001), *id*. at 5-6, but establishes the inferiority of recombinant Factor C products under USP's neutral and unbiased standard for the validating the comparability of endotoxin test formulations. *See, e.g*., USP, *Use of Recombinant Animal-Free Reagents in the Bacterial Endotoxins Test*, at https://www.usp.org/covid-19/treatment-and-prevention/rfc-summary (last visited July 28, 2022) (establishing a comparability protocol requiring that "[t]he measured activity of a sample containing endotoxins using a recombinant reagent method should fall within 50%-200% of the measured activity in the same sample tested using natural lysate").

Recombinant Reagent	Number of Samples Tested	Number of Samples <50% of the Average LAL Result	Percentage of Samples <50% of the Average LAL Result
Pyrogene	128	100	78%
EndoZyme II	128	122	95%
EndoZyme II Go	128	83	65%
rLAL	128	50	39%

Fig. 5. Systematic Underperformance by Recombinant Factor C Assays (reproduced from *id*.).

In contrast, as shown in Fig. 4 above, the two regulated LAL-based assays performed relatively comparably and each exhibited reactivity that was significantly higher than the recombinant Factor C products. The picture painted by this data could not be clearer: the bacterial endotoxin test products regulated by FDA performed significantly better than the unregulated recombinant Factor C assays. In addition, compliance with USP standards was not enough to ensure comparability across the different products.

Two additional points bear note. ***First***, the manufacturers of recombinant Factor C products sometimes seek to explain away their products' exceedingly poor performance by asserting that traditional LAL-based assays may overestimate the presence of endotoxins because of their reactivity to β-glucans, which allegedly can result in false positives but supposedly do not react with isolated Factor C protein. *See, e.g*., bioMérieux Marketing Brochure at 13 ("LAL assays give false-positive results when exposed to β-glucans."). This recent data, however, (1) demonstrates that this influence is likely only in the presence of extreme quantities of β-glucans that are not generally observed in real-world conditions, *id*. at 4-5 (detailing the β-glucan levels in water samples obtained directly from pharmaceutical manufacturing facilities), and (2) further confirms that recombinant Factor C products continue to fail at extraordinarily high rates ***even***

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when β-glucan presence is factored in. *Id*. at 5. As a result, the most likely explanation for the severe underperformance of recombinant Factor C products is that they in fact are inferior to the industry-standard LAL products that have been validated for more than fifty years—almost certainly because they lack the specificity-enhancing signal amplification that results from the interaction of activated Factor C with Factor B in the traditional LAL cascade. *See, e.g*., Tsuchiya, 10 INT'L J. DEV. RES. at 36752 (explaining how "[t]he specificity of LAL to endotoxin is achieved by the cooperation between Factor C and Factor B on LPS aggregates").

Second, and perhaps most important, ***false positives*** are not the issue. ***False negatives*** are, because those are the errors that jeopardize human health. From a regulatory perspective, then, the relevant concern is not whether LAL-based endotoxin test products might err on the side of caution; when it comes to preventing the release of contaminated product lots that can sicken thousands of patients at a time, caution generally is considered to be a good thing—which is why USP's standard for end-product endotoxin test specificity provides an acceptability criterion of 50% to 200% of known endotoxin concentration, and therefore tolerates far greater over-prediction of endotoxin levels than it does under-prediction. *See* USP <85> Bacterial Endotoxins Test (finalized Dec. 1, 2012). Instead, the relevant regulatory question is whether unlicensed recombinant Factor C products, which have never been submitted to the Agency for regulatory review, might pose risks to the millions of Americans who depend on parenterally administered medications and the medical devices used to administer them. After all, the PHSA asks whether biological products are safe and effective for their intended use—not whether they are ***too safe*** or ***too effective*** in preventing avoidable hospitalizations and deaths due to endotoxic contamination.

Finally, Charles River wishes to emphasize that nothing in this Petition requires the Agency to resolve the serious safety questions raised by these unlicensed products in the context of this docket. The key point instead is that these issues ought to be resolved in the usual way—through the Agency's rigorous, evidence-based review of a properly filed BLA, as the statute unambiguously requires and the serious safety questions presented by recombinant Factor C products plainly warrant. Given the significant underperformance of recombinant Factor C products in real-world conditions, Charles River respectfully submits that it is time for FDA to conform its regulatory actions to the post-BPCIA statute by compelling the prompt submission of BLAs for all current (and any future) recombinant Factor C end-product endotoxin tests.

F. CONCLUSION

For the foregoing reasons, FDA should (1) rescind the now-obsolete Cambrex Letter; (2) declare that any person engaged in the interstate commercial marketing of an end-product endotoxin test for human drugs, biological products, or medical devices which (a) includes recombinant Factor C and (b) has not been granted effective biologics license is in violation of 42 U.S.C. § 262(a)(1)(A); (3) order any such persons to either (a) immediately cease marketing their unlawful recombinant Factor C product(s) in interstate commerce, or (b) submit a complete BLA for such a product within 60 days of the date the Commissioner takes final agency action on this petition; and (4) initiate prompt administrative enforcement proceedings against any person who either (a) fails to comply with the terms of the above-requested order, or (b) submits a complete BLA in accordance with the terms of such order, but fails to obtain an effective approval for such BLA within 6 months of its submission to FDA.

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III. ENVIRONMENTAL IMPACT

The undersigned claims a categorical exclusion from the requirements for an Environmental Assessment under 21 C.F.R. §§ 25.30(a)-(b), 25.31(a)-(c) & (h), and to the best of petitioner's knowledge, no extraordinary circumstances exist.

IV. ECONOMIC IMPACT

An economic impact statement will be submitted at the request of the Commissioner.

V. 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 petitioner which are unfavorable to the petition.

Respectfully submitted,

DocuSigned by:
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