

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CHARLES RIVER LABORATORIES, INC.,
Petitioner,

v.

SEIKAGAKU CORPORATION,
Patent Owner.

Case No. PGR2025-00023

Patent No. 11,959,109

**PATENT OWNER RESPONSE
TO PETITION FOR POST-GRANT REVIEW OF
U.S. PATENT NO. 11,959,109**

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I. INTRODUCTION

Patent Owner Seikagaku Corp. (“Patent Owner” or “SKK”) submits this Response to the Petition for Post-Grant Review of Claims 1-9 of U.S. Patent No. 11,959,109 (“the ’109 Patent”) (EX1001) filed by Petitioner Charles River Laboratories (“Petitioner” or “CRL”). Patent Owner has disclaimed Claim 10 (*see* EX2001), therefore only Claims 1-9 are at issue.

Petitioner asserts the following grounds for invalidity against Claims 1-9 (the “Challenged Claims”): lack of written description (Ground 1); lack of enablement (Ground 2); and anticipation by U.S. Publication No. 2019/0241629 (“the ’629 Publication”) (EX1015) (Ground 3). The ’629 Publication was published on August 8, 2019, and is not prior art if the ’109 Patent is entitled to the December 10, 2013 filing date of its ultimate parent application, no. 14/650,767 (“the ’767 Application”). Accordingly, Petitioner advanced Ground 3 as a contingent argument premised on “Patent Owner pivot[ing] from the priority claim of the ’109 patent and argue[ing] that its specification as filed on July 3, 2023... adequately describes and enables such claims.” Pet. at 60.

Patent Owner has not pivoted. Ground 3 fails because the ’629 Publication is not prior art; the Challenged Claims are entitled to the December 10, 2013 filing date of the ’767 Application for the reasons discussed herein. Grounds 1 and 2 fail either for the same reason as Ground 3, or for failure of proof because Petitioner failed to

address lack of written description and enablement as of the July 3, 2023 filing date of the '109 Patent.

The Board should deny the Petition on all grounds.

II. OVERVIEW OF THE '109 PATENT AND TECHNOLOGY

The '109 Patent is titled “Recombinant Factor C and Method for Producing the Same, and Method for Measuring Endotoxin.” EX1001. Independent Claim 1, the sole independent claim of the '109 Patent after Patent Owner’s disclaimer of Claim 10 (EX2001), is directed to a method for producing an endotoxin assay agent comprising three recombinant proteins expressed by mammalian cells: Factor C, Factor B, and Pro-clotting enzyme. These proteins are found in horseshoe crab amoebocytes. Claim 1 specifies Factor C is from two out of the four living species of horseshoe crab and the source of the Factor B and Pro-clotting enzyme is not specified.

The '109 Patent issued from U.S. Patent Application No. 18/346,420, which is a continuation of U.S. Patent Application No. 17/554,128, filed December 17, 2021, which is a continuation of U.S. Patent Application No. 16/399,386 (issued as U.S. Patent No. 11,236,318), filed April 30, 2019, which is a continuation of U.S. Patent Application No. 15/983,725 (issued as U.S. Patent No. 10,982,202), filed May 18, 2018, which is a continuation of U.S. Patent Application No. 14/650,767 (“the '767 Application,” which issued as U.S. Patent No. 10,144,923), filed June 9,

2015, which is a National Stage Entry of PCT/JP2013/083082, filed December 10, 2013. The '109 Patent further claims foreign priority to JP 2012-269840, filed on December 10, 2012.

A. Horseshoe Crab Coagulation Cascade

There are four living species of horseshoe crab: *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*, all of which have similar morphological characteristics and physiological coagulation systems. EX1001 at 13:58-62; EX2058 at 1-2; EX2062 ¶¶18-21. The horseshoe crab is a primitive organism that evolved slowly, hence they are often described as “living fossils.” EX2065 at 21, 25; EX1038 at 6¹; EX2062 ¶18.

In 1964, Levin and Bang identified that the amebocytes of horseshoe crab blood forms a gel when exposed to endotoxin, leading to the development of the *Limulus* amoebocyte lysate (“LAL”) assay for endotoxin detection. EX2066 at 3; EX2062 ¶19. Endotoxin is a lipopolysaccharide (“LPS”) present in the outer membrane of Gram-negative bacteria that “even in a very small amount... causes various pathological conditions” following “bacterial infection.” EX1001 at 1:40-42; EX2062 ¶19. In humans, exposure to endotoxin via injectable drugs, vaccines,

¹ Petitioner did not add pagination to many exhibits, including EX1038. For those exhibits, citations are to the page numbers of the original publication.

or medical devices results in critical medical conditions, including sepsis, which can lead to organ failure and death. EX2005 at 5. Thus, detection of endotoxin is critical in pharmaceutical manufacturing to establish the safety of injectable products and medical devices. *Id.*; EX1001 at 1:45-47. The FDA has accepted the use of the LAL assay for endotoxins in lieu of the rabbit pyrogens test since 1987. EX2084 at 5; EX2062 ¶19, 48.

The coagulation cascade utilized by the LAL assay is part of the horseshoe crab's biological defense mechanism against infections, and this immune mechanism is critical for survival. EX2037 at 1²; EX2024 at 11. By 1992, the mechanism of the horseshoe crab coagulation cascade had largely been elucidated, and its enzyme components identified. *See* EX2067 at 2; EX2037 at 2-3; EX2062 ¶20. A sequential activation of three serine protease zymogens—Factor C, Factor B, and Pro-clotting enzyme—causes the polymerization of coagulogen, forming a coagulin clot. *Id.* The first serine protease zymogen in the cascade is Factor C. *Id.* Endotoxin activates Factor C to form Activated Factor C (“Factor $\bar{C}$ ”). *Id.* Factor $\bar{C}$ in turn activates Factor B to form Activated Factor B (“Factor $\bar{B}$ ”). *Id.* Factor $\bar{B}$

² EX2037 is “Non-Patent Document 1” incorporated by reference in the ’109 Patent. EX1001 at 2:64-65.

then activates Pro-clotting enzyme to form clotting enzyme, which cleaves coagulogen to form coagulin. *Id.* This activation cascade is depicted in Figure 1.

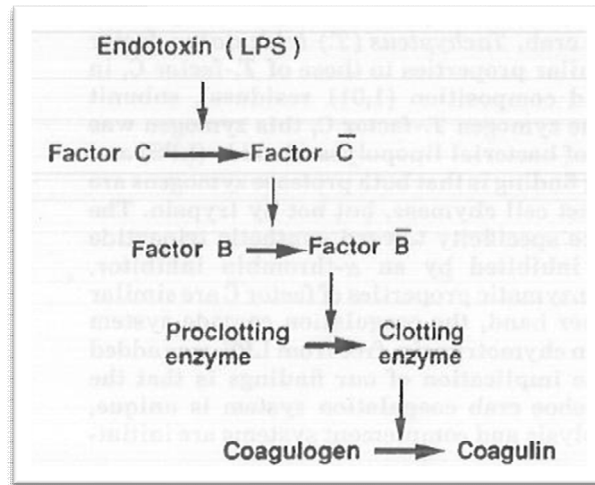


Figure 1. Mechanism for coagulation cascade of horseshoe crab hemocyte lysate. EX1025 at 151, Fig. 1.

Each of the four species of horseshoe crab utilizes the same coagulation cascade in response to endotoxin exposure. *See* EX1009 at 1:17-26, Fig. 1; EX2038 at 2; EX2062 ¶21.

B. Isolation and Characterization of the Coagulation Cascade Proteins

Factor C, Factor B and Pro-clotting enzyme have been characterized structurally and functionally, and share similarities in their domain structures, as illustrated below.

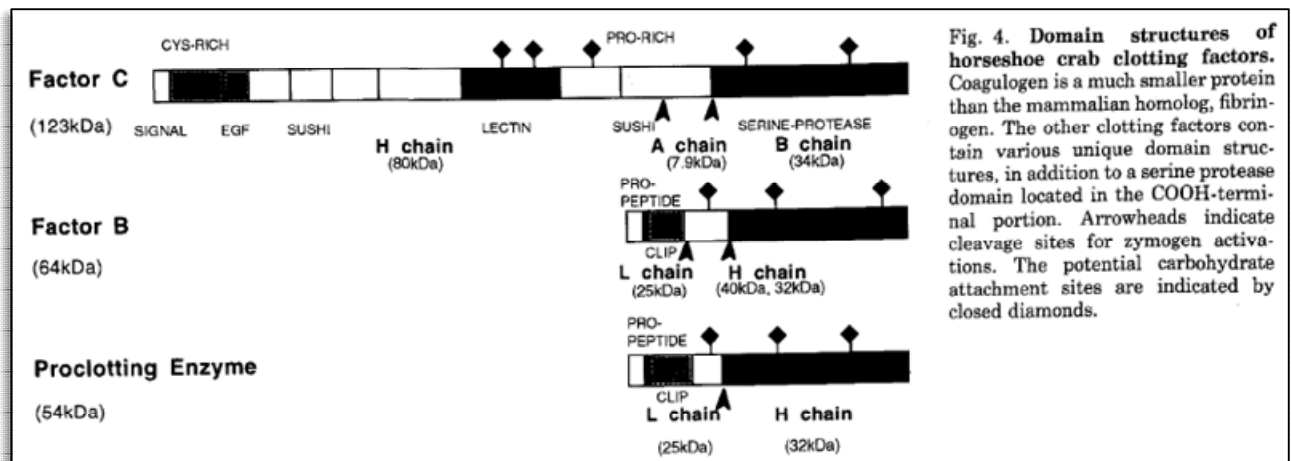


Figure 2. Domain structures of horseshoe crab clotting factors. EX2039 at 6, Fig. 4. Arrowheads indicate cleavage sites and diamonds indicate potential glycosylation sites.

1. Factor C

Factor C is the first serine protease zymogen in the coagulation cascade of all known horseshoe crabs. The sequence and biochemical properties for native Factor C in *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda* has been studied extensively. As a result of these studies, by December 2013, it was clear that Factor C was highly conserved and functionally identical across these species. EX2062 ¶23.

Factor C was first purified from *Tachypleus tridentatus* in 1986 by Nakamura et al. EX2068 at 1.³ Nakamura determined that Factor C is a glycoprotein consisting

³ EX2068 is incorporated by reference as “Non-Patent Document 2” in the ’109 Patent. EX1001 at 2:66-67.

of two polypeptide chains—a heavy chain and a light chain. *Id.* at 7; EX2062 ¶24. When Factor C is activated by endotoxin, a limited proteolysis of the light chain occurs into A chain and B chain via a Phe-Ile cleavage. EX2069 at 1. Thus, Factor C̄ consists of three chains—heavy chain, A chain, and B chain—linked via disulfide bonds.

In 1991, Muta et al. published the *Tachypleus tridentatus* cDNA and amino acid sequence and characterized its domains. EX1010 at 6554; EX2062 ¶25. Muta determined that Factor C includes several domains: a signal peptide, a Cys-rich region, an EGF-like domain, five sushi domains,⁴ one lectin-like domain, one Pro-rich region which is located within the fifth sushi domain, and one serine protease domain. *Id.* at 6558; EX2062 ¶25. Figure 2 shows a schematic representation of these domains.

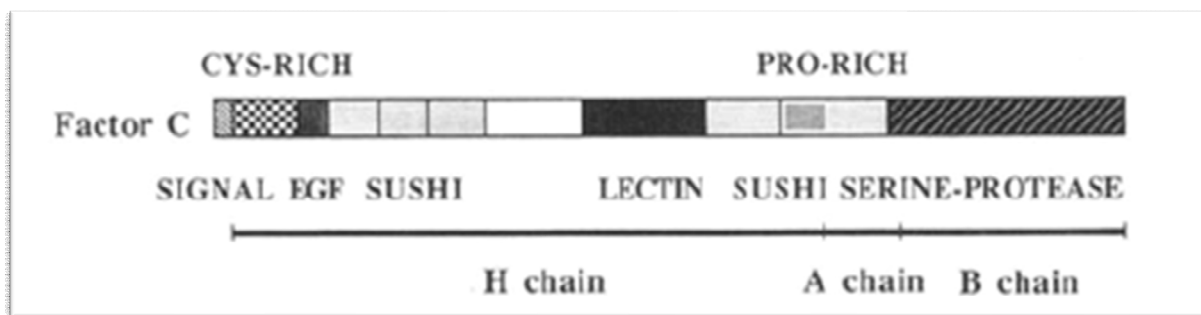


Figure 2. The domain structure of *Tachypleus tridentatus* Factor C. EX1010, Fig. 7.

⁴ Sushi or short consensus repeat domains are found in many mammalian complement system proteins. EX1010 at Abstract.

Muta identified the catalytic triad of Factor C, which was that of “a typical serine protease,” reporting that Factor $\bar{C}$ “has similar specificity toward synthetic fluorogenic peptide substrates as α -thrombin” and has a conserved substrate-binding site. *Id.* at 6557. Muta also identified that the sequence of Factor C was related to mammalian complement factors. EX1010 at 6557-6558 and Fig. 6; EX2062 ¶26.

In 1991, Tokunaga purified *Limulus polyphemus* Factor C (“LpFC”) from amoebocyte lysate. EX1025. Like *Tachypleus tridentatus* Factor C (“TtFC”), the *Limulus* protein consists of a heavy chain and a light chain that, when activated by endotoxin is cleaved into an A chain and B chain. *Id.* at 151. Tokunaga reported that both proteins are rapidly activated by α -chymotrypsin, and the activated proteins showed α -thrombin-like specificity towards synthetic tripeptide substrates and were inhibited by an α -thrombin inhibitor. *Id.* at Abstract, 153-154.

Tokunaga conducted an amino acid sequence analysis and reported that “the individual amino acid compositions of the two proteins and of their chain subunits were indistinguishable.” *Id.* at 151. Although Tokunaga did not disclose the cDNA or full amino acid sequence of the *Limulus polyphemus* Factor C, a partial N-terminal sequence for each of the Factor $\bar{C}$ chains showed a “close resemblance” to the corresponding *Tachypleus tridentatus* sequence. *Id.* at 151-152; EX2062 ¶¶29-30.

Importantly, the B chain of *Limulus* Factor C begins with an Ile (highlighted in yellow below), suggesting that, like *T. tridentatus* Factor C, it includes the Phe-Ile cleavage site between the A chain and B chain; EX2062 ¶28.

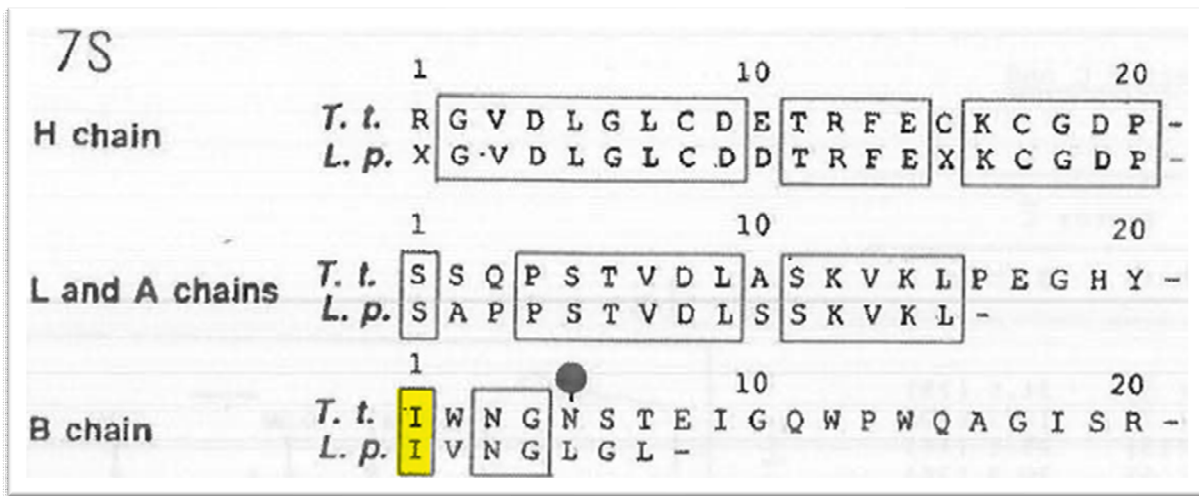


Figure 3. Comparison of the N-terminal sequences of heavy, A chain, and B chain of LpFC and TtFC.⁵ EX1022 at 157.

⁵ Post-priority date publications revealed that the sequences Tokunaga reported for the B chain contained discrepancies that decreased its apparent identity to the *T. tridentatus* sequence. The first 7 amino acids of the B chain are IVNGNST. See EX1015 at 18-23 (SEQ ID NOS: 2 and 4). Thus, *L. polyphemus* Factor C in fact includes the same potential glycosylation site as *T. tridentatus* at these positions. EX2062 ¶31 n.3. Tokunaga's reported sequences for the H chain and L and A chains are a 100% match with SEQ ID NOS: 2 and 4. EX1015 at 18-23.

Tokunaga concluded that “the gross structure of [LpFC] must resemble that of known [TtFC]” and indicates that they “are identical protein entities, both immunologically and biochemically.” *Id.* The LPS binding domain of *Tachypleus tridentatus* Factor C has also been identified and determined to be conserved in other mammalian LPS-recognizing proteins. EX2040 at Abstract.

In 1995, the cDNA and amino acid sequence of *Carcinoscorpius rotundicauda* Factor C were published. EX2008 at 1; *see also* EX1009 at Example 4. *Carcinoscorpius rotundicauda* Factor C, like the *Tachypleus tridentatus* Factor C, consists of a heavy chain and a light chain. EX2008 at 1-2. Upon activation by endotoxin, like *Tachypleus tridentatus* Factor C, the *Carcinoscorpius rotundicauda* Factor C undergoes limited proteolysis of its light chain into A chain and B chain upon endotoxin activation. *Id.* at 5, Fig. 5; EX2062 ¶27.

The amino acid sequence of *C. rotundicauda* Factor C is over 97% identical to the *Tachypleus tridentatus* Factor C. EX1009 at Example 6 (reporting 97.7% identity between the two sequences, with most of the dissimilarities in the 3’ untranslated region); *see also* EX2008 at 8; EX2062 ¶28. Both Factor C proteins include the same domains, and importantly the same Phe-Ile cleavage site between the A chain and B chain. EX2008 at 8; EX2062 ¶28. Ding confirmed that the *Carcinoscorpius rotundicauda* Factor C is, like *Tachypleus tridentatus* Factor C, catalytically similar to trypsin-type serine proteases. EX1009 at Example 7.

As early as 2001, the amino acid composition of *Limulus polyphemus* Factor C had been determined and compared to the sequence of *Carcinoscorpius rotundicauda* Factor C. EX2047 at 21 ([¶0134]) (“[t]ryptic peptide sequencing revealed that the Limulus factor C sequence closely matched the Asian species, with >90% sequence identity.”); *see also* EX2006 at 9, n.2. Other publications disclosed a similar level of sequence identity and similarity in 2006 and 2009. EX2048 at 4 (“the Factor C of *Carcinoscorpius rotundicauda* has 89.3% homology with that of *Limulus polyphemus*”)⁶; EX2070 at 2 (“The rFC protein derived from *C. rotundicauda* shares a 90.5% identical amino acid sequence with the native Factor C protein derived from *L. polyphemus*.”); EX2062 ¶¶33-34.

Thus, not only had *Limulus polyphemus* Factor C been biochemically characterized and shown to be functionally, immunologically, and structurally equivalent to *Tachypleus tridentatus* Factor C, its amino acid sequence was known to have high identity to *Carcinoscorpius rotundicauda* Factor C, and by extension to *Tachypleus tridentatus* Factor C as well. EX2062 ¶¶34.

The full cDNA sequence of *Limulus polyphemus* Factor C was not published contemporaneously with the foregoing reports. However, researchers at the

⁶ The authors defined “homology” to mean sequence identity in the preceding sentence. EX2048 at 4.

University of Connecticut sequenced and deposited on October 31, 2012 (i) a 188 Mb *Limulus polyphemus* cDNA amoebocyte transcriptome under accession number PRJNA178588; and (ii) a 4 Gb shotgun sequence of the *Limulus polyphemus* genome under BioProject accession number PRJNA178587.⁷ EX2072 (PRJNA178588); EX2071 (PRJNA178587); EX2062 ¶35.

BioProject PRJNA178588 is linked to Sequence Read Archive (“SRA”) record SRX202160 and data run SRR610305, which were published as of October

⁷ According to the NCBI: “[a] BioProject is a collection of biological data related to a single initiative originating from a single organization or from a consortium. A BioProject record provides users a single place to find links to the diverse data generated for that project and deposited into the archival databases maintained by members of the INSDC. Typical examples of a BioProject include a multiisolate project for sequencing multiple strains of a bacterial species, or a monoisolate project for the genome and transcriptome of a particular organism.” Further, “BioProject registration is required as part of data deposit to several NCBI primary data archives including SRA [Sequence Read Archive], TSA [Transcriptome Shotgun Assembly] and WGS [Whole Genome Shotgun]. Typically, a BioProject is registered first or during the submission of a genome assembly being submitted to WGS.” EX2076.

31, 2013. EX2073 (SRX202160); EX2074 (SRR610305). The SRA records associated with the genomic DNA deposit was also published as of the same date. EX2075 (SRX202152 and SRX202154). As of December 10, 2013, a keyword search for “limulus polyphemus” of NCBI’s BioProject or SRA databases would have found these BioProjects or their associated sequence deposits. EX2062 ¶¶35-37

2. Factor B and Pro-clotting enzyme

Factor B and Pro-clotting enzyme from *Tachypleus tridentatus* were cloned and sequenced in 1993 and 1990, respectively. EX2002; EX2003.

Factor B is a single chain glycoprotein consisting of 400 amino acids. EX2002 at 1. Upon activation by Factor $\bar{C}$, Factor B is cleaved into a heavy chain and light chain and releases an activation peptide. *Id.* The cleavage site associated with Factor B activation is between Ile-124 and Ile-125. *Id.* at 3. Factor $\bar{B}$ contains a “typical trypsin-type serine protease domain with active site triads, histidine, aspartic acid, and serine and a conserved primary substrate binding site, Asp-31.” *Id.*; EX2062 ¶38.

Pro-clotting enzyme is a single chain glycoprotein that, when activated by Factor $\bar{B}$ is converted into clotting enzyme, a two-chain protein comprising a heavy chain and light chain. EX2003 at 1; EX2067 at 8. The cleavage site associated with

Pro-clotting enzyme activation is Arg-98-Ile-99. EX2003 at 4; EX2067 at 8. The active site triad is His-143-Asp-199-Ser-297. *Id.*; EX2062 ¶39.

Factor B and Pro-clotting enzyme are similar to each other in terms of amino acid sequence and structure, each containing a CLIP domain and serine protease domain. EX2038 at 3-4 and Fig. 4 (reproduced in relevant part below as Figure 4); EX2039 at 2-3. It was hypothesized as early as 1992 that the CLIP domain in Factor B and Pro-clotting enzyme is a common structural element across invertebrate serine protease zymogens. EX2067 at 10. EX2062 ¶40.

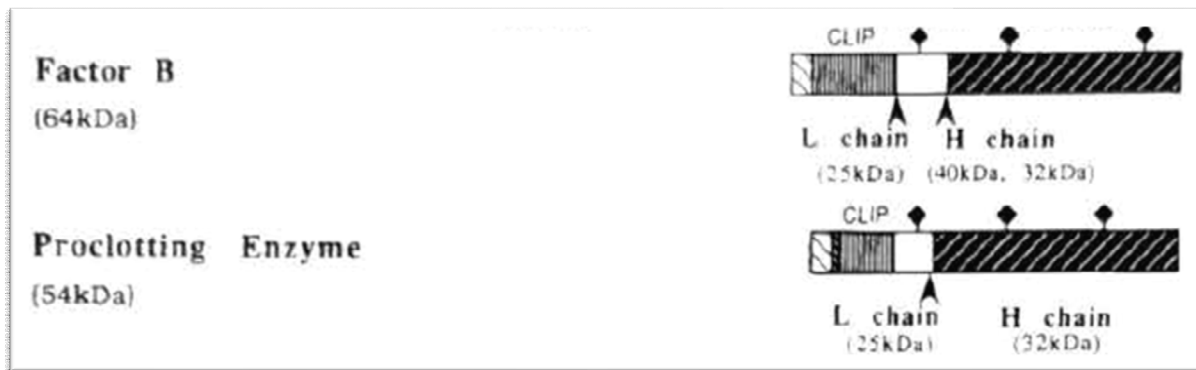


Figure 4. Domain structure of *T. tridentatus* Factor B and Pro-clotting Enzyme. EX2038, Fig. 4. The arrowheads indicate cleavage sites for zymogen activation and diamonds indicate potential glycosylation sites.

The serine protease domains of Factor C, Factor B and Pro-clotting enzyme also share significant sequence similarity with the serine protease domain of Factor C, with all three factors having an identical serine protease active site triad. EX2062 ¶41; EX2038 at Fig. 3 (reproduced below as Figure 5):

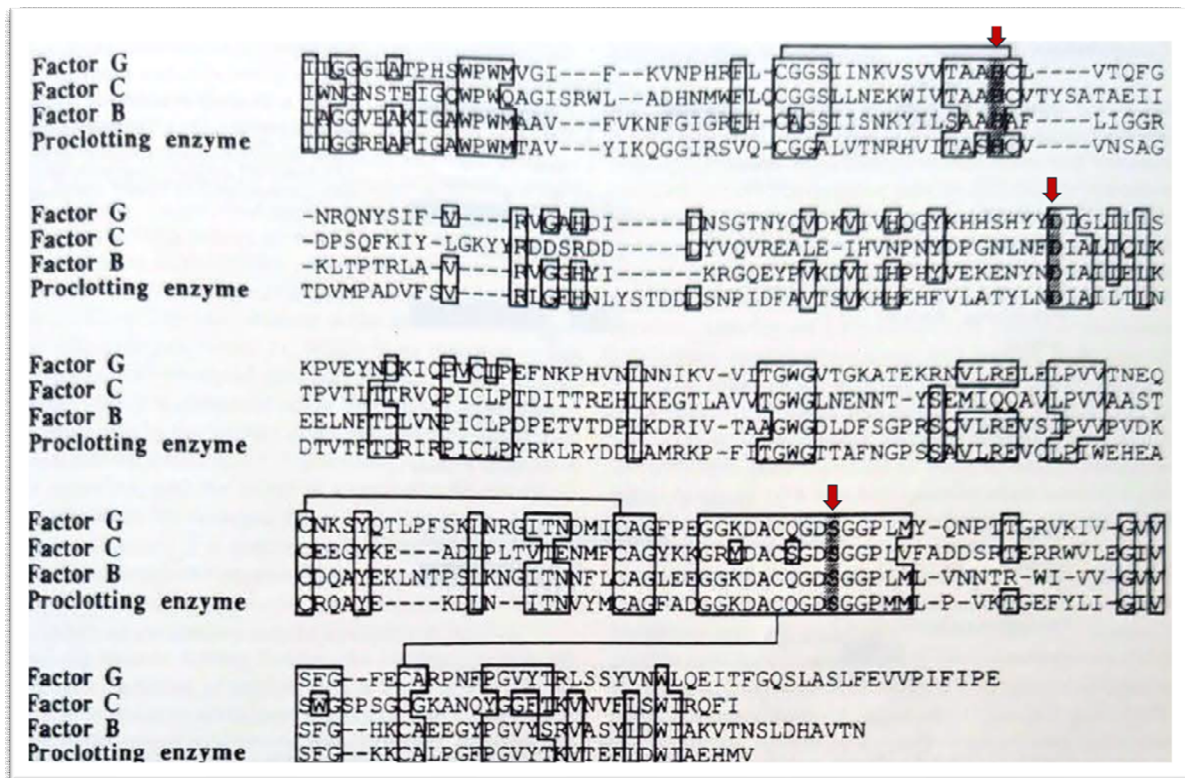


Figure 5. Sequence Alignment of serine protease domains of *T. tridentatus* coagulation cascade proteins with the residues of the active triad indicated with arrows.⁸ EX2038, Fig. 3.

3. Coagulogen

The final protein in the native clotting cascade is coagulogen. Coagulogen is a fibrinogen-like substance. EX2080 at 1; EX2067 at 3. It is cleaved by clotting enzyme (i.e., activated Pro-clotting enzyme) to become coagulin, which forms an insoluble gel, or clot. *Id.* Coagulogen is not used in in chromogenic version of the

⁸ Factor G is activated by (1,3)- β -D-glucan, a major cell wall constituent of yeast and other fungi and cleaves Pro-clotting enzyme to clotting enzyme. EX2038 at 1.

LAL assay; its place at the end of the cascade is replaced by a detection substrate.

EX2070 at 1; EX2062 ¶42.

The amino acid and cDNA sequences of coagulogen from *Limulus polyphemus* was determined in 1984 and 1986, respectively. EX1001 at 24:26-30; EX2080 at 4, Fig. 15 (reproduced below); EX2081. The cleavage sites in coagulogen, *i.e.*, the substrate sites of Pro-clotting enzyme were known. EX2080 at Fig. 15; EX2062 ¶43.

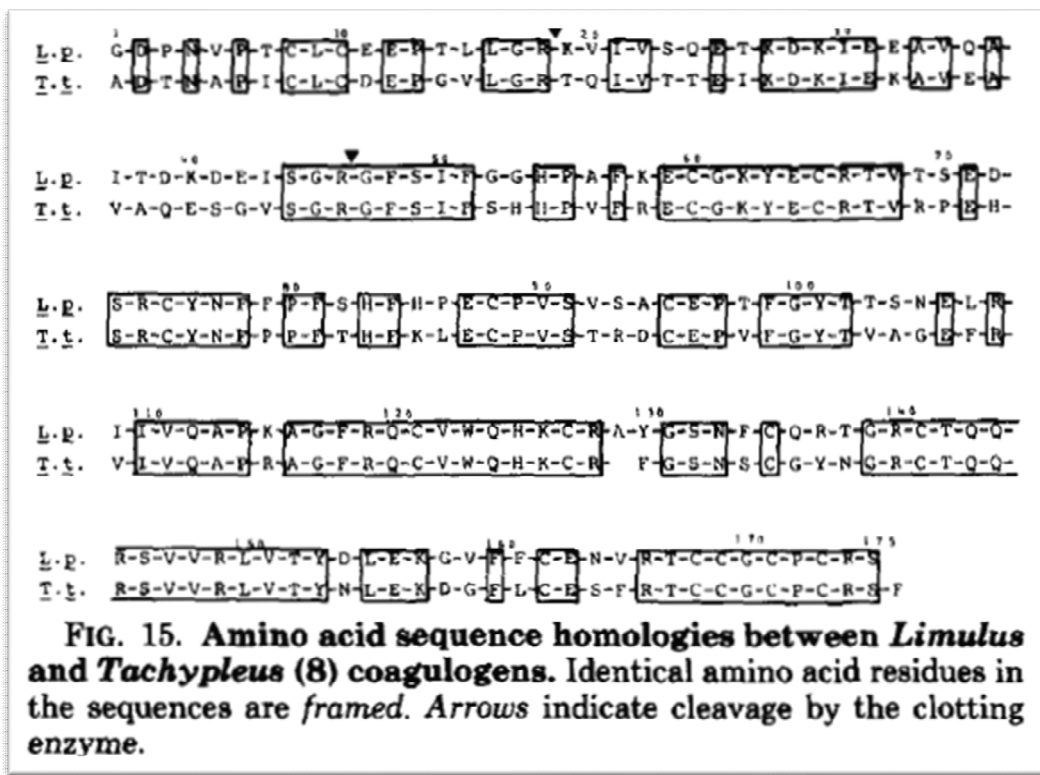


Figure 6. Amino acid sequence comparison between *Limulus polyphemus* and *Tachyplesus tridentatus* coagulogens, with cleavage sites indicated by arrows. EX2080, Fig. 15.

Miyata characterized the amino acid sequence homology (similarity) of the coagulogen proteins in these two species as “remarkable” and suggested the two proteins had “very similar conformations.” EX2080 at 5; *see also* EX2067 at 4-5. Consistent with this early observation, substrates with the same sequence—Leu-Gly-Arg—were used in assays employing *Limulus polyphemus* or *Tachypleus tridentatus* amoebocyte lysate. *See* EX1017 at 68-70 ([0072]) (providing examples of peptides suitable for detecting activated Factor C and Pro-clotting enzyme); EX2067 at 11; EX2055 at [0034], [0214] (providing examples of peptides used in synthetic chromogenic substrates, and stating Boc-LGR-pNA is an “optimum substrate in conventional LAL reaction”); EX2067 at 11 (discussing substrates suitable for detecting activity of each of Pro-clotting enzyme, Factor B, and Factor C). EX2062 ¶44.

C. Functional Interchangeability

Not only was it known very early on that the structure and biochemistry of these four proteins were similar to each other and, in the case of Factor C and coagulogen, highly similar across species, it was also known in the art that the horseshoe crab coagulation systems were indistinguishable across species in terms of their function. EX2062 ¶45; *see, e.g.*, EX1025 at 153 (stating in 1991 that “[t]hese findings also lead to the idea that the hemocytes of *L. polyphemus* probably contain a coagulation system similar to that established for the *T. tridentatus* hemocytes.”);

see also EX2005 at 2 (stating LAL is commercially manufactured from *L. polyphemus* and *T. tridentatus* amebocytes and “can also be obtained from the other two species”) and 4 (Fig. 2 describing “Consensus biochemistry of the LAL reaction”).

The U.S. Pharmacopeia created a chapter on bacterial endotoxin testing using a *Limulus polyphemus* lysate-based reagent and validated an endotoxin reference standard. EX2062 ¶46; EX2066 at 10-11. The European Pharmacopoeia and Japanese Pharmacopoeia followed suit but also allowed use of lysates from *Tachypleus tridentatus*. *Id.* at 13-14. These chapters were harmonized in 2012. EX2051; EX2052; EX2053. The harmonized chapters allowed use of lysate from both species of crab, indicating that these lysates will “yield the same results and the same accept/reject decision [will be] reached.” EX2082 at 2.

Coagulation cascade proteins from different horseshoe crab species have successfully been used to reconstruct the endotoxin detection cascade. EX2050 at 35-38 ([0171]-[0182]); EX2023 at 4 ([0009]). For example, recombinant Factor C from *C. rotundicauda* and recombinant Factor B and Pro-clotting enzyme from *T. tridentatus* could be used to reconstruct the endotoxin detection cascade in 2008. EX2050 at 22, 33-38 ([0110], [0171]-[0182]). Thus, it was known in the art as of December 2013 that the endotoxin-detecting function of the cascade was preserved even when proteins from different horseshoe crab species were used together.

EX2062 ¶47. A post-priority date publication confirmed that native *Tachypleus tridentatus* Factor C could activate recombinant *Limulus polyphemus* Factor B and vice versa. EX2085 at 28-29 ([0177]-[0186]).

D. LAL Assays

In 1987, the FDA issued a guidance that allowed the LAL assay to be used as the final pyrogen test instead of the traditional rabbit pyrogen test. EX2066 at 10. The extensive demand for the LAL reagent, as well as the corresponding TAL reagent derived from *Tachypleus tridentatus* amoebocyte lysate led to concerns that over-harvesting of horseshoe crab species in Asia and America may drive these organisms to potential extinction. EX2062 ¶48; EX2024 at 12-13.

Production and use of recombinant proteins can provide an unlimited source of the horseshoe crab coagulation factors and thus avoids the need to capture and bleed horseshoe crabs to make the amoebocyte lysate. EX1001 at 2:26-34. There was also a desire for endotoxin-specific assays and to reduce the potential for lot-to-lot variations. EX2048 at 2; EX2024 at 12; EX2062 ¶49. In 2012, FDA withdrew its 1987 Guidance and issued a new one. EX2084 at 5. The 2012 guidance acknowledged that recombinant factor C-based assays could be an acceptable alternative to the native lysate reagent. *Id.* at 8; EX2062 ¶49.

A commercial product employing recombinant *C. rotundicauda* Factor C was commercially launched in 2004. EX2024 at 16 (discussing PyroGene); *see also*

EX2023 at 4 ([0010]). Other products using *Tachypleus tridentatus* Factor C followed. EX2027; EX2083 at 5; EX2062 ¶50. The difference in the pathways of the amoebocyte-based LAL assay and the recombinant Factor C assay is shown below:

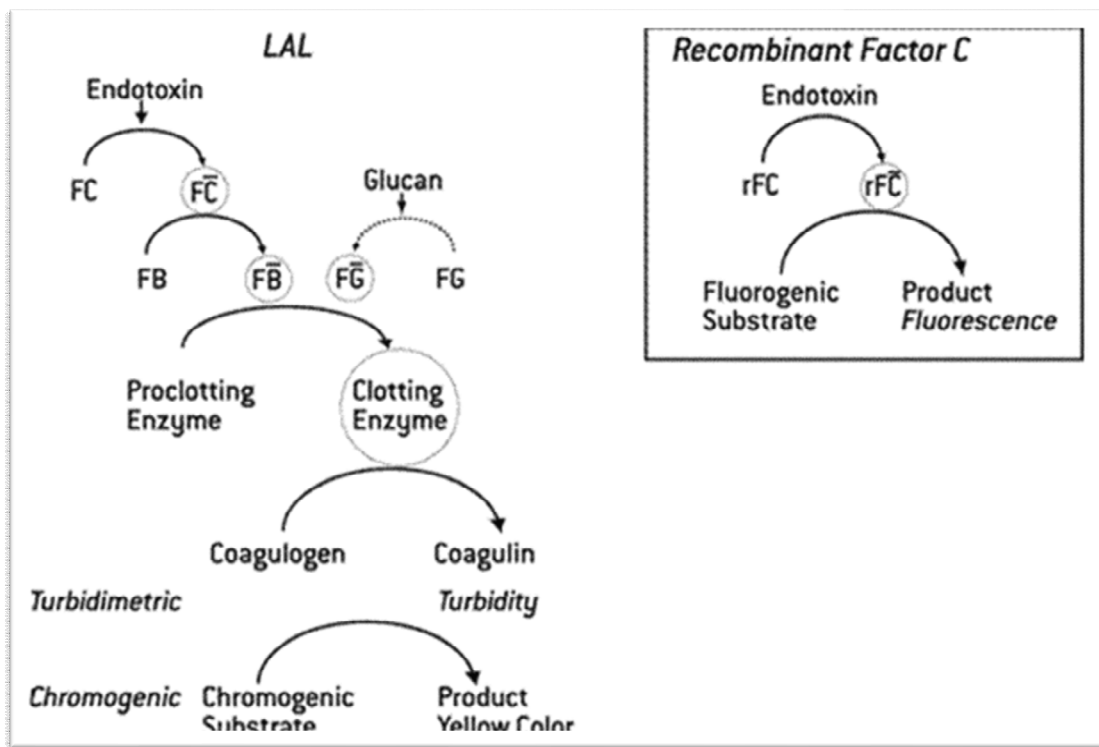


Figure 7: Reactions involved in the LAL and recombinant Factor C assays. EX2070 at 2, Fig. 1.

A fully recombinant assay using Factor C, Factor B, and Pro-clotting enzyme expressed in insect cells was also disclosed as of 2013 and later marketed by Patent Owner. EX2050 at 35-38 ([0171]-[0182]); EX2023 at 4 ([0009]); EX2083 at 4. Assays using recombinant Factor C were found to yield comparable results to native lysates. See EX2048 at 4; EX2022 at 6; EX2062 ¶51; see also EX2083 at 5-88

(surveying literature and concluding the publications showed “rFC and LAL produce comparable results” and further studies “are likely to arrive at the same conclusion... as long as the study accounts for the specificity of LAL with respect to beta glucans.”).

III. PERSON OF ORDINARY SKILL IN THE ART

Petitioner proposes a high level of ordinary skill in the art, namely a person with “a Ph.D. degree in biological, chemical or biochemical engineering, molecular biology, or biochemistry and at least two years of experience in the field of utilizing or assessing horseshoe crab zymogen proteins (lysate, purified native and/or recombinantly produced) for endotoxin detection and quantitation.” Pet. at 22. According to Petitioner, more education could substitute for experience to a certain extent, and vice versa. *Id.*

Patent Owner disagrees with Petitioner’s definition of a person of ordinary skill in the art (“POSA”). Factors that may be considered in determining the level of ordinary skill in the art may include the educational level of the inventor; the type of problems encountered in the art; prior art solutions to those problems; rapidity with which innovations are made; sophistication of the technology; and educational level of active workers in the field. *See, e.g., Daiichi Sankyo Co., Ltd. v. Apotex, Inc.*, 501 F.3d 1254, 1256 (Fed. Cir. 2007).

The field of the '109 Patent is the cloning and recombinant expression of horseshoe crab serine protease zymogens and determining their activity. EX1001 at 1:30-34. This field requires experience and education in molecular biology and biochemistry. EX2062 ¶56. For example, inventor Shun-ichiro Kawabata has a Ph.D. in biology and experience in biochemistry. EX2086. Determining the gene and amino acid sequences of proteins, expressing them in various types of host cells, and assessing their properties were the type of problems encountered in the art, which were solved using molecular biology techniques. EX2062 ¶57. Thus, experience and knowledge in biochemistry and molecular biology was essential to those working in the field. *Id.* Education and experience in biological, chemical, or biochemical engineering, which relate to developing industrial scale processes for protein production, is not required. *Id.*

Accordingly, Patent Owner proposes that a POSA would have a Ph.D. in molecular biology, biochemistry, or enzymology and at least two years of experience with cloning genes, protein expression and characterization. EX2062 ¶58. A POSA may collaborate with others, including those with specific experience in conducting structure-function analyses of enzyme function, evaluating horseshoe crab zymogen proteins individually, or in a cascade reaction for endotoxin detection and quantitation. *Id.* However, the Challenged Claims are valid under either parties' proposed definitions.

IV. CLAIM CONSTRUCTION

Petitioner does not argue that any terms require construction. Patent Owner agrees that the terms can be afforded their plain and ordinary meaning. However, Petitioner has raised an issue concerning claim construction that requires resolution by the Board. Petitioner’s written description and enablement arguments import limitations into the Challenged Claims, namely the steps of mixing with a test specimen and detection substrate, and that a cascade reaction occur. “One of the cardinal sins of patent law [is] reading a limitation from the written description into the claims.” *Phillips v. AWH Corp.*, 415 F.3d 13013, 1320 (Fed. Cir. 2005).

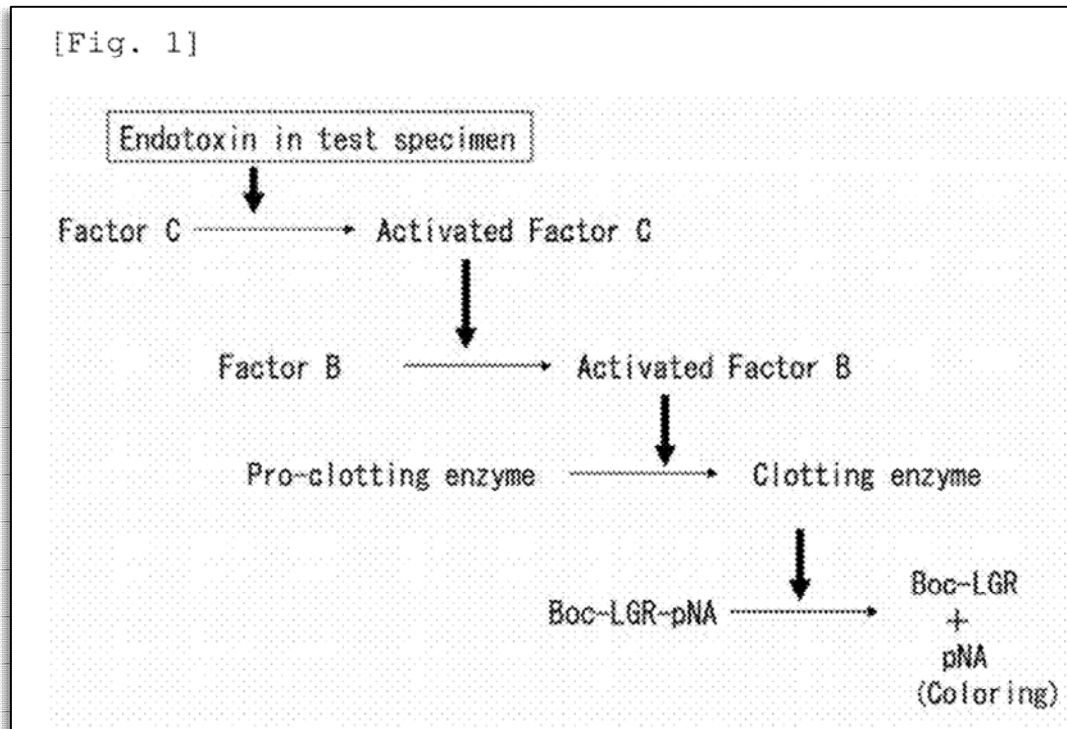
For example, Petitioner argues that “an adequate written description would have to either disclose representative examples of the possible *working cascades* of various proteins or some accurate structural feature of the claimed proteins that allow them to *function in the claimed assay as a whole*.” Pet. at 35 (emphasis added). Petitioner then extensively criticizes the specification for failing to describe ways of making or producing “a functioning endotoxin detection assay” or “making an endotoxin detection assay... to act in a functioning cascade for the detection of endotoxin.” *Id.* at 25, 29, 31, 32, 34, 36; *see also id.* at 33 (“a functioning enzymatic cascade to detect endotoxin as claimed”).

Petitioner’s enablement arguments are based on the same premise—that Claim 1 requires the additional steps associated with using the endotoxin assay agent

produced by the recited steps to measure endotoxin. *See, e.g.*, Pet. at 40 (arguing that a POSA would have to “engage in random trial-and-error discovery to assemble functional claimed assays”); *see also id.* at 41 (“all of the challenged claims require that the claimed combination of recombinantly produced proteins result in a functional assay”), 43 (“functioning endotoxin assay agent” or “functioning endotoxin assay”), 44 (“work as a cascade”), 46 (components must be “able to detect endotoxin”), 48 (“functioning endotoxin-detection assay”) and 50 (claims require a “functional assay capable of detecting endotoxin”).

However, Claim 1 is directed to a method for producing an endotoxin assay agent. Claim 1 ***does not require*** the agent to be combined with a test specimen and a detection substrate, or that a coagulation cascade take place—all of which would be required to ***use*** the agent to measure endotoxin. EX2062 ¶60. All that Claim 1 requires is the expression of Factor C protein that has the activity of Factor C from two of the four species of horseshoe crab into the culture supernatant by Chinese Hamster Ovary or Human Embryonic Kidney cells, expression of Factor B and Pro-clotting enzyme having activity in mammalian cells and producing an endotoxin assay agent comprising these three proteins. EX1001 at cl. 1. Notably, the endotoxin assay agent produced by the claimed method could only generate a cascade reaction ***after*** it is mixed with an endotoxin-containing test specimen and a detection substrate, but these additional components and steps are not recited in

Claim 1. EX2062 ¶61; *see, e.g.*, EX1001 at 1:66-2:21; *see also* EX1001 at Fig. 1, below:



The specification discusses the endotoxin assay method separately from the endotoxin assay agent. EX1001 at 26:4-49. The specification also makes clear that mixing the agent with a test specimen containing endotoxin is required for a cascade reaction and endotoxin measurement. *Id.*

A comparison of Claims 1 and 10 illustrates Petitioner's error:

Claim 1	Claim 10
A method for <u>producing an endotoxin assay agent</u> comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method	A method for <u>measuring endotoxin in a test specimen</u> , the method comprising:

comprising:	
expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C in the culture supernatant, wherein the recovered horseshoe Factor C has activity of Factor C, and wherein the horseshoe crab Factor C is a <i>Tachypleus tridentatus</i> Factor C or a <i>Limulus polyphemus</i> Factor C,	expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, secreting the horseshoe crab Factor C into an extracellular culture supernatant, and recovering the horseshoe crab Factor C in the culture supernatant, wherein the recovered horseshoe Factor C has activity of Factor C, and wherein the horseshoe crab Factor C is a <i>Tachypleus tridentatus</i> Factor C or a <i>Limulus polyphemus</i> Factor C,
expressing the horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,	expressing the horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,
expressing the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme in a mammalian cell as a host cell, and	expressing the horseshoe crab Pro-clotting enzyme having activity of Proclotting enzyme in a mammalian cell as a host cell, and
producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,	producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,
	<u>mixing the endotoxin assay agent and a test specimen, and measuring progress of a cascade reaction,</u>
wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the	wherein the host cell for expressing the horseshoe crab Factor C, the host cell for expressing the

horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.	horseshoe crab Factor B, and the host cell for expressing the horseshoe crab Pro-clotting enzyme may be the same or different.
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Claims 1 and 10 are similar, but the language of Claim 10 clearly sets forth the additional steps required to use the endotoxin assay agent to measure endotoxin in a test specimen. In contrast, Claim 1 does not include any language concerning a test specimen or a cascade reaction. The activity of each individual component may be determined separately. EX2067 at 11 (discussing substrates suitable for detecting activity of each of Pro-clotting enzyme, Factor B, and Factor C); EX1025 at 153; EX2002, EX2003 at 4; EX2062 ¶61. The entirety of Petitioner’s arguments is based on improperly importing these additional components and steps, along with measuring the ensuing cascade reaction, into Claim 1, and should be rejected on this basis alone.

“[W]hen a patent claim ‘does not contain a certain limitation and another claim does, that limitation cannot be read into the former claim in determining either validity or infringement.’” *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1326 (Fed. Cir. 2003) (declining to read limitation exogenous DNA into claim where a different claim expressly included that limitation). While the endotoxin assay agent resulting from the method of Claim 1 is intended for use in an endotoxin

detection assay, Claim 1 is not directed to the act of carrying out such an assay to detect endotoxin. There is no basis in the language of Claim 1 to import these additional components and steps from the specification as Petitioner has done. *Id.* at 1325 (“The danger of improperly importing a limitation is even greater when the purported limitation is based upon a term not appearing in the claim.”). Accordingly, the Board should not read these additional limitations into the Challenged Claims or require Patent Owner to describe or enable them. A specification need only provide written description and enablement support for what is actually *claimed*. *In re Entresto*, 125 F.4th 1090, 1097, 98 (Fed Cir. 2025) (citing *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1564 (Fed. Cir. 1991) and *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610 (2023)).

V. GROUND 1 SHOULD BE REJECTED

Written description is assessed as of the filing date. *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010) (*en banc*). That is because “each patented advance has a novel relationship with the state of the art from which it emerges.” *Id.* In the event an application is not entitled to an earlier filing date, the effective filing date is “the actual filing date of the application for the patent containing a claim to the invention.” 35 U.S.C. § 100(i)(1)(A).

If the Board find the claims of the ’109 Patent are entitled to the earlier December 10, 2013 filing date, it should reject Ground 1 for the same reasons set

forth with respect to written description in Section VIII.A, below. If the Board finds the Challenged Claims are not entitled to this priority date, it should reject Ground 1 because Petitioner has failed to provide any evidence or argument that the Challenged Claims lack written description support as of the July 3, 2023 filing date of the '420 application which issued as the '109 Patent. All of Petitioner's arguments and evidence concern the December 10, 2013 timeframe. Pet. at 23-24; EX1003 ¶¶50-51, 59.

VI. GROUND 2 SHOULD BE REJECTED

Like written description, enablement is determined as of the effective filing date of the application. *ALZA Corp. v. Andrx Pharms. LLC*, 603 F.3d 935, 940 (Fed. Cir. 2010). In the event an application is not entitled to an earlier filing date, the effective filing date is “the actual filing date of the application for the patent containing a claim to the invention.” 35 U.S.C. § 100(i)(1)(A).

If the Board find the claims of the '109 Patent are entitled to the earlier December 10, 2013 filing date, it should reject Ground 2 for the same reasons set forth below in Section VIII.B, below. If the Board finds the Challenged Claims are not entitled to this priority date, it should reject Ground 2 because Petitioner has failed to provide any evidence or argument that the Challenged Claims lack enablement as of the July 3, 2023 filing date of the '420 application which issued as

the '109 Patent. All of Petitioner's arguments and evidence concern the December 10, 2013 timeframe. Pet. at 24; EX1003 ¶¶50-51.

VII. GROUND 3 SHOULD BE REJECTED

The Challenged Claims are directed to a method of making an endotoxin assay agent comprising three components: Factor C, Factor B, Pro-clotting enzyme. The Factor C protein is from *Limulus polyphemus* or *Tachypleus tridentatus* and is expressed in either a Chinese Hamster Ovary ("CHO") cell or a Human Embryonic Kidney ("HEK") cell. The Factor B and Pro-clotting enzyme may be from any of the four species of horseshoe crab and may be expressed in any mammalian cell. Thus, the Challenged Claims encompass at most ten distinct proteins. EX2062 ¶¶99-104. Claim 4 requires only six distinct proteins – from either *Limulus polyphemus* or *Tachypleus tridentatus*. *Id.*

The Challenged Claims are entitled to claim priority to the December 10, 2013 filing date of PCT/JP2013/083082 because the '767 Application provides written description support and enables the Challenged Claims.

A. The '767 Application Provides Sufficient Written Description Support for the Challenged Claims

"Ascertaining the required possession" for written description "starts with an accurate understanding of the claimed invention and then proceeds to an objective inquiring into the four corners of the specification." *BASF Plant Sci., LP v.*

Commonwealth Sci. and Indus. Rsch. Org., 28 F.4th 1247, 1264 (Fed. Cir. 2022) (internal citations and quotations omitted). Here, Petitioner’s expert proceeded from an inaccurate understanding of the claimed invention, as discussed in Section IV, above, and the Board can reject Petitioner’s written description arguments and evidence on that basis alone. Even if the Board agrees with Petitioner that the Challenged Claims have a functional requirement, the ’767 Application provides sufficient written description support.

Petitioner’s primary argument is based on the inventors’ lack of actual possession of an assay agent from the Challenged Claims. *See* Pet. at 29-33. But the Federal Circuit has instructed: “(1) examples are not necessary to support the adequacy of a written description; (2) the written description standard may be met (as it is here) even where actual reduction to practice of an invention is absent; and (3) there is no *per se* rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure.” *Falko-Gunter Falkner v. Inglis*, 448 F.3d 1357, 1366 (Fed. Cir. 2006). Thus, neither experimental data demonstrating effectiveness, nor a theory or explanation of how or why a claimed composition will be effective, nor actual reduction to practice are required for written description support. *Nuvo Pharms. (Ireland) Designated Activity Co. v. Dr. Reddy’s Lab ’ys, Inc.*, 923 F.3d 1368, 1380 (Fed. Cir. 2019).

“What is required to meet the written description requirement ‘varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence.’” *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1335 (Fed. Cir. 2021). “A patentee may rely on information that is well-known in the art to the extent it informs how a relevant artisan would reasonably understand what is actually described in the specification.” *BASF Plant Sci.*, 28 F.4th at 1264. Where, as here, there is extensive knowledge in the art, the specification need only provide direction that “reasonably leads persons skilled in the art to the later claimed compound[s].” *See In re Edwards*, 568 F.2d 1349, 1352 (C.C.P.A. 1978).

The '767 Application satisfies the written description requirement because a POSA as of December 10, 2013 would have understood the inventors to have possessed the sequences necessary to carry out the Challenged Claims, as well as by disclosing a structure-function relationship and representative species. EX2062 ¶¶68-93, 105-135.

1. The '767 Application shows possession of *Limulus polyphemus* Factor C by the inventors

The '767 Application refers to proteins from *Limulus polyphemus* multiple times. *See* EX1017 at 30-31, 56 ([0020], [0046]). A POSA would have understood

that the inventors considered the *Limulus polyphemus* proteins to be a part of their invention. EX2062 ¶¶68, 73.

The '767 Application also discloses the cDNA and amino acid sequences of Factor C from *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* as SEQ ID NOs: 1 and 2 and SEQ ID NOs: 3 and 4. EX1017 at 98 ([0156]). The information available from public sources would allow a POSA to arrive at the cDNA and amino acid sequences of *Limulus polyphemus* Factor C through routine experimentation. EX2062 ¶¶68-89.

In 1991, researchers published a partial amino acid sequence of *Limulus polyphemus* Factor C, determined its full amino acid composition, and found that that composition was identical to the *Tachypleus tridentatus* protein. EX1025 at 151; EX2062 ¶122. And, as early as 2001, tryptic peptide sequencing was used to determine that the percentage sequence identity of *Limulus polyphemus* Factor C to *Carcinoscorpius rotundicauda* Factor C was more than 90%. EX2047 ¶[0134]; see also EX2048 at 4; EX2070 at 1614; EX2006 at 9, n.2. Thus, the majority of the amino acid sequence was known and available before December 2013. EX2062 ¶122.

Researchers at the University of Connecticut sequenced and deposited on October 31, 2012 (i) a 188 Mb *Limulus polyphemus* cDNA amoebocyte transcriptome under BioProject accession number PRJNA178588; and (ii) a 4 Gb

shotgun sequence of the *Limulus polyphemus* genome under BioProject accession number PRJNA178587. EX2072 (PRJNA178588); EX2071 (PRJNA178587). The Sequence Read Archive (“SRA”) record SRX202160 and run SRR610305 linked to the transcriptome deposit was published as of October 31, 2013. EX2073; EX2074; EX2078; EX2062 ¶¶35, 69. The SRA records associated with the genomic DNA deposit was also published as of the same date. EX2075; EX2062 ¶¶36, 69. As of December 10, 2013, a keyword search for “limulus polyphemus” of NCBI’s BioProject database would have found these BioProjects and their associated sequence deposits. EX2062 ¶¶36, 69.

The data in the SRA records included short and intermediate cDNA sequence fragments or reads from which cDNA sequences of *Limulus polyphemus* genes can be reassembled using the NCBI’s Basic Local Alignment Search Tool (“BLAST”) and CD-Search and known sequences. EX2062 ¶¶37, 70. In particular, a large majority of the cDNA sequences of *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme could be reassembled and identified by querying the data using the *Tachypleus tridentatus* sequences disclosed in the ’767 Application, SEQ ID Nos: 1,5, 7. EX1017 at 98 ([0156]); EX2062 ¶¶37, 69-72, 79-81; *see also* EX2004 ¶¶18-21; EX2006 ¶¶59-73, 76-77.

Petitioner’s argument that the inventors “did not and could not have described” the “gene and sequence information for Factor C, Factor B, and Pro-

clotting enzyme” from *Limulus polyphemus* because those sequences had not been disclosed, Pet. at 31, ignores that they could be obtained from public sources and through routine experimentation as of December 10, 2013. EX2062 ¶¶68-93.

In particular, the amoebocyte transcriptome data run SRR610305 was published and publicly available months before December 10, 2013. EX2074; EX2062 ¶¶36, 69. When used as a query against the 2013 deposits, the *Tachypleus tridentatus* sequences disclosed in the ’767 Application allowed a POSA to reassemble the full-length or nearly full-length open reading frame (“ORF”) sequences of the corresponding *Limulus polyphemus* genes. EX2062 ¶¶78, 80; EX2004 ¶¶18-26.

Petitioner’s argument that a POSA would not have recognized the inventors possessed or described the sequences of the *Limulus polyphemus* proteins also ignores that a POSA could also have used a common technique called RACE or a variation thereof to use *Tachypleus tridentatus* sequence information published in the early 1990s to design primers to obtain the complete *Limulus polyphemus* cDNA sequences. EX2062 ¶¶74-81; *see also* EX2004 ¶¶30-43; EX2006 ¶¶58-73. RACE was a commonplace technique used since 1988 to rapidly generate full-length cDNA clones based on partial sequence information. EX2062 ¶74; EX2004 ¶¶7-10, 35-37; EX2004 at 103-179 (product inserts for commercial kits); *see also* Section VII.A.5, below (several *Limulus* genes cloned using RACE in the 1990s).

A POSA could also have isolated these sequences by probing a phage library using the *Tachypleus tridentatus* sequence information, the same way the cDNA sequence of *Carcinoscorpius rotundicauda* Factor C was determined in the mid-1990s. EX2062 ¶¶92, 137, 164; EX2004 ¶¶11-16; EX2006 ¶¶80-90; EX1009 at 5:1-13, 10:12-16.

To the extent Petitioner argues that the starting material needed to conduct RACE PCR or create a cDNA library, *i.e.*, samples of *Limulus polyphemus* blood, was not available to a POSA (*see* EX1065 at 182-184), this argument is untimely, speculative, and unsupported. Petitioner did not advance this argument in the Petition and has not identified any evidence of unavailability. To Patent Owner's knowledge, none of the evidence of record in this proceeding mention any difficulties in obtaining such samples. Indeed, that horseshoe crabs were routinely captured and bled was the exact problem the invention seeks to solve. EX1001 at 2:26-34; *see also* EX1011 at 20-21; EX2066 at 3-4, 6-7, 16.

Written description does not require that the *Limulus polyphemus* sequences be set forth in the specification or incorporated by reference. *Falko-Gunter*, 448 F.3d at 1367-68 (“When the prior art includes the nucleotide information” or accessible sources provide the necessary sequence information “satisfaction of the written description requirement does not require either the recitation or incorporation by reference... of such genes and sequences.”). The public information along with

the disclosures in the '767 Application would have allowed a POSA to arrive at the sequence without undue burden and constitutes sufficient written description of the *Limulus polyphemus* sequences, and constructive possession of the claimed method. *See BASF Plant Sci.*, 28 F.4th at 1267.

Notably, Petitioner obtained a patent claiming priority to June 2010 that claims methods of preparing *Limulus* amebocyte lysates, which the specification defines as including recombinantly produced cascade proteins, including Factor G. EX2046 at 5:19-24 and cl. 1; EX2062 ¶93. Factor G comprises two subunits, alpha and beta. *See* EX2038 at 4-5, Fig. 4; EX2062 ¶93. The sequence of Factor G-subunit alpha was not published in an article until 2011, although the sequence was submitted to NCBI's GenBank database in 2010. EX2062 ¶93 and n.8; EX2113. Nevertheless, Petitioner's patent does not disclose nucleic acid or amino acid sequences for Factor G. EX2046. Thus, Petitioner's position that written description demands disclosure of the precise sequences for the coagulation cascade factors is undermined by its own patent and positions taken before the Patent Office in obtaining that patent.

The '767 Application discloses detailed directives on how to express functional Factor C in CHO and HEK293 cells and instructs that the same methods could be applied to express Factor B and Pro-clotting enzyme. EX1017 at 63, 87-88 ([0057], [0108]-[0115]); EX2062 ¶¶94-97. The '767 Application also describes

a functional endotoxin assay agent comprising recombinant Factor C, Factor B, and Pro-clotting enzyme. EX1017 at 94-95 ([0129]-[0130]); EX2062 ¶¶66. The '767 Application also discloses methods of determining the activity of the individual zymogens. EX1017 at 67-70 ([0070]-[0072]); EX1025 at 153; EX2003 at 4; EX2067 at 11; EX2062 ¶¶168.

A POSA would have understood from the disclosures of the '767 Application and the existing scientific information and knowledge in the field that the inventors possessed the method of producing an endotoxin assay agent recited in the Challenged Claims. EX2062 ¶¶66-68, 94, 105-106, 111-113, 135, 143-146; *see also* EX2006 ¶¶48, 50-54. “Because actual reduction to practice is not a requirement of possession, delay in actual production [of the claimed invention] does not negate constructive possession at the time of filing. *BASF Plant Sci.*, 28 F.4th at 1267; *see also Centocor Ortho Biotech, Inc. v. Abbott Lab’ys*, 636 F.3d 1341, 1353 (Fed. Cir. 2011) (a disclosure that allows one of skill in the art to visualize or recognize the claimed proteins demonstrates “constructive possession”).

Similar to the claims in *Amgen v. Hoechst Marion*, which were directed to producing erythropoietin in vertebrate and mammalian cells, “the claim terms at issue here are not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend.” 314 F.3d at 1332 (affirming that specification’s description of producing the claimed protein in two species of cells

adequately supported claims covering production using all vertebrate and mammalian cells because one of ordinary skill in the art could “visualize or recognize the identity” of those cells); *see also Invitrogen Corp. v. Clontech Lab ’ys, Inc.*, 429 F.3d 1052, 1073 (Fed. Cir. 2005) (finding sufficient written description where “sequences of RT genes were known and members of the RT [reverse transcriptase] gene family shared significant homologies from one species of RT to another” and “the written description teaches that the invention can be applied to RT genes of other retroviruses”).

2. The genus is not large

Petitioner calculates the genus as comprising 3,456 possible combinations of 54 distinct proteins. Pet. at 28, 33-34. However, this calculation includes assumptions that unjustifiably increase the number of possible combinations. The Board should not credit Petitioner’s artificial inflation of the number of possible combinations encompassed by the Challenged Claims.

- a. The same amino acid sequence expressed by different mammalian cells should not be counted as distinct proteins or “options”*

Petitioner counts different mammalian expression systems as distinct proteins or “options.” But regardless of the cell line, the amino acid sequence of the protein will be the same. Further, expression vectors are typically agnostic as to the particular mammalian cell line. For example, the pCI-Neo vector referenced in the

'767 Application (EX1017 at 85 ([0108]) may be used in many mammalian cell lines. EX2089. Similarly, the specification states that pCA7 is a “vector for expression in mammalian cells”—plural. EX1017 at 87-88 ([0114]). A POSA would understand that they only need to make one vector for each Factor C, Factor B, and Pro-clotting enzyme protein – a total of ten vectors – to practice claim 1 across many mammalian cells lines. EX2062 ¶100.

In view of the '767 Application disclosing successful production of Factor C in two mammalian cell lines, a POSA would have understood this to be highly predictive of successful expression of Factor B and Pro-clotting enzyme in mammalian cells. EX2004 ¶¶44-47; EX2006 ¶53; EX2062 ¶¶163-164. Absent evidence that the particular expression system has an effect, there is no basis for Petitioner's assumption that proteins made by different mammalian cells would be functionally or structurally distinct.⁹ Thus, different mammalian cells expressing the same amino acid sequence should not be counted as distinct proteins or “options.” *See Amgen v. Hoechst Marion*, 314 F.3d at 1335 (affirming written description and enablement of claims directed to erythropoietin purified from mammalian cells grown in culture because “an ordinarily skilled artisan would infer

⁹ Unlike Factor B and Pro-clotting enzyme, there is evidence that expression system matters for Factor C. *See* EX1001 at 34:48-58.

from [two] examples that similar outcomes could be expected from all mammalian cells since all mammalian cells produce and secrete hormones like EPO by means of the same fundamental processes”).

b. Different recovery methods do not create different proteins or “options”

Petitioner also counts two recovery methods as distinct proteins and options. Pet. at 28. This rests on the unsupported assumption that Factor B and Pro-clotting enzyme would be expressed differently to the extent of requiring different recovery methods in different mammalian cell lines. To the contrary, given the similarities between these two proteins in terms of sequence and structure, a skilled artisan would expect them to be secreted similarly.¹⁰ EX2062 ¶¶ 38-40, 102. Yet Petitioner has not identified any evidence that Factor B or Pro-clotting enzyme would have issues with expression or secretion in any mammalian cell line.

In addition to lacking evidentiary support, treating these methods as a distinct species is fundamentally illogical. The two “recovery” methods included in

¹⁰ A post-priority date reference from authors including the inventors confirms that Factor B is secreted into the culture medium when expressed in a mammalian cell line (HEK293 GnTI-) using techniques similar to those disclosed in the ’109 Patent for Factor C. EX1007 at 19380; EX1001 at 31:45-32:5.

Petitioner's calculation (secreted and not secreted) should not be included in the calculation because they are typically mutually exclusive. Regardless, it would be a simple matter for a POSA to determine whether the Factor B and Pro-clotting enzyme proteins are secreted or remain intracellular. *See, e.g.*, EX1017 at 90 ([0120]; EX2062 ¶102. Secreted or not, it is still the same the same sequence of amino acids and the same protein, not two different "options." *Id.* Petitioner has presented no evidence that the secretion (or not) of Factor B and Pro-clotting enzyme would result in functional differences such that they should be considered distinct proteins or options.

In view of the '767 Application's disclosure that insect cells secrete Factor C, Factor B and Pro-clotting enzyme, and mammalian cells secrete Factor C, a POSA would have expected Factor B and Pro-clotting enzyme to also be secreted in mammalian cell expression systems. EX2090 at 516; EX2062 ¶102. If, however, Factor B or Pro-clotting enzyme remained inside the mammalian cells, a POSA would have been capable of harvesting the proteins by cell lysis and determining their activity. *See, e.g.*, EX2013 at 2; EX2062 ¶102.

The invention here is directed to functional Factor C that is expressed into the supernatant by mammalian cells—an accomplishment not described in the prior art—not general techniques for expressing recombinant proteins. Mammalian expression systems and protein recovery methods were already well explored in the

relevant art and are not part of the claimed invention. Accordingly, even if Petitioner's unproven assumptions that the function and recovery of Factor B and Pro-clotting enzyme depend on the particular expression system are credited, they should not be included as distinct species in the calculation of the genus size. *See Ajinomoto Co. v. Int'l Trade Comm'n*, 932 F.3d 1342, 1359 (Fed. Cir. 2019) (upholding genus claim because the invention was identifying a particular gene and discovering that it conferred certain properties when its expression was amplified or enhanced, not the well-known techniques for performing the amplification or expression enhancement, explaining "here, the genus of more potent promoters was already well explored in the relevant art"); *see also Amgen v. Hoechst Marion*, 314 F.3d at 1333 ("[U]nder our precedent the patentee need only describe the invention as claimed, and need not describe an unclaimed method of making the claimed product."). A specification need only provide written description support for what is actually *claimed*. *In re Entresto*, 125 F.4th at 1097. The methods of recovering Factor B and Pro-clotting enzyme are not recited in the claims, and thus need not be described.

Correcting for these two unjustified assumptions reduces Petitioner's calculation of 3,456 possible combinations to 32 for claim 1, as shown below in an annotated version of Petitioner's table:

Challenged Claims 1 and 10				
	Expression System	Horseshoe Crab Species	Recovery	Options
Factor B	~~"Mammalian"~~ ~~(could include)~~ ~~at least:~~ ~~CHO~~ ~~DG44~~ ~~CHO K1~~ ~~HEK293~~	<u>Tachypleus tridentatus</u> <u>Tachypleus gigas</u> <u>Limulus polyphemus</u> <u>Carcinoscorpius rotundicauda</u>	~~Secreted into extra-cellular culture supernatant~~ ~~Not secreted (i.e., lyse cells and recover)~~	At least 24 options 4 options
Factor C	~~CHO~~ ~~DG44~~ ~~CHO K1~~ ~~HEK293~~	<u>Tachypleus tridentatus</u> <u>Limulus polyphemus</u>	~~Secreted into extra-cellular culture supernatant~~	At least 6 options 2 options
Pro-Clotting Enzyme	~~"Mammalian"~~ ~~(could include)~~ ~~at least:~~ ~~CHO~~ ~~DG44~~ ~~CHO K1~~ ~~HEK293~~	<u>Tachypleus tridentatus</u> <u>Tachypleus gigas</u> <u>Limulus polyphemus</u> <u>Carcinoscorpius rotundicauda</u>	~~Secreted into extra-cellular culture supernatant~~ ~~Not secreted (i.e., lyse cells and recover)~~	At least 24 options 4 options
				At least 3,456 32 possible combinations

Thus, claim 1 does not “broadly recite[] a genus of thousands of possible methods for producing the claimed endotoxin assay agent” as Dr. Jain asserts. EX2062 ¶104; *cf.* EX1003 ¶¶58, 70. Claim 1 is relatively narrow, encompassing only ten distinct proteins. EX2062 ¶104. Claim 4 is even narrower, encompassing only six distinct proteins. *Id.*

3. The specification discloses a representative number of species

Petitioner’s focus on the purported lack of a working example of making an assay out of proteins expressed by mammalian cells is incorrect. The law is clear that working examples are not required. *Falko-Gunter*, 558 F.3d at 1366. Petitioner’s representative species arguments rely on an incorrect view of what constitutes a species of the claimed invention.

Written description analyses are highly fact specific. *Ariad*, 598 F.3d at 1383. Petitioner relies on *Boston Scientific Corp. v. Johnson & Johnson*, 647 F.3d 1353 (Fed. Cir. 2011) and *Carnegie Mellon University v. Hoffmann-La Roche Inc.*, 541 F.3d 1115 (Fed. Cir. 2008). Pet. at 30. But those cases are highly inapposite to the facts here. The genus in *Boston Scientific* was at least in the tens of thousands, and “potentially limitless.” 647 F.3d at 1364. In *Carnegie Mellon*, only three out of thousands of potential genes had been cloned. 541 F.3d at 1126.

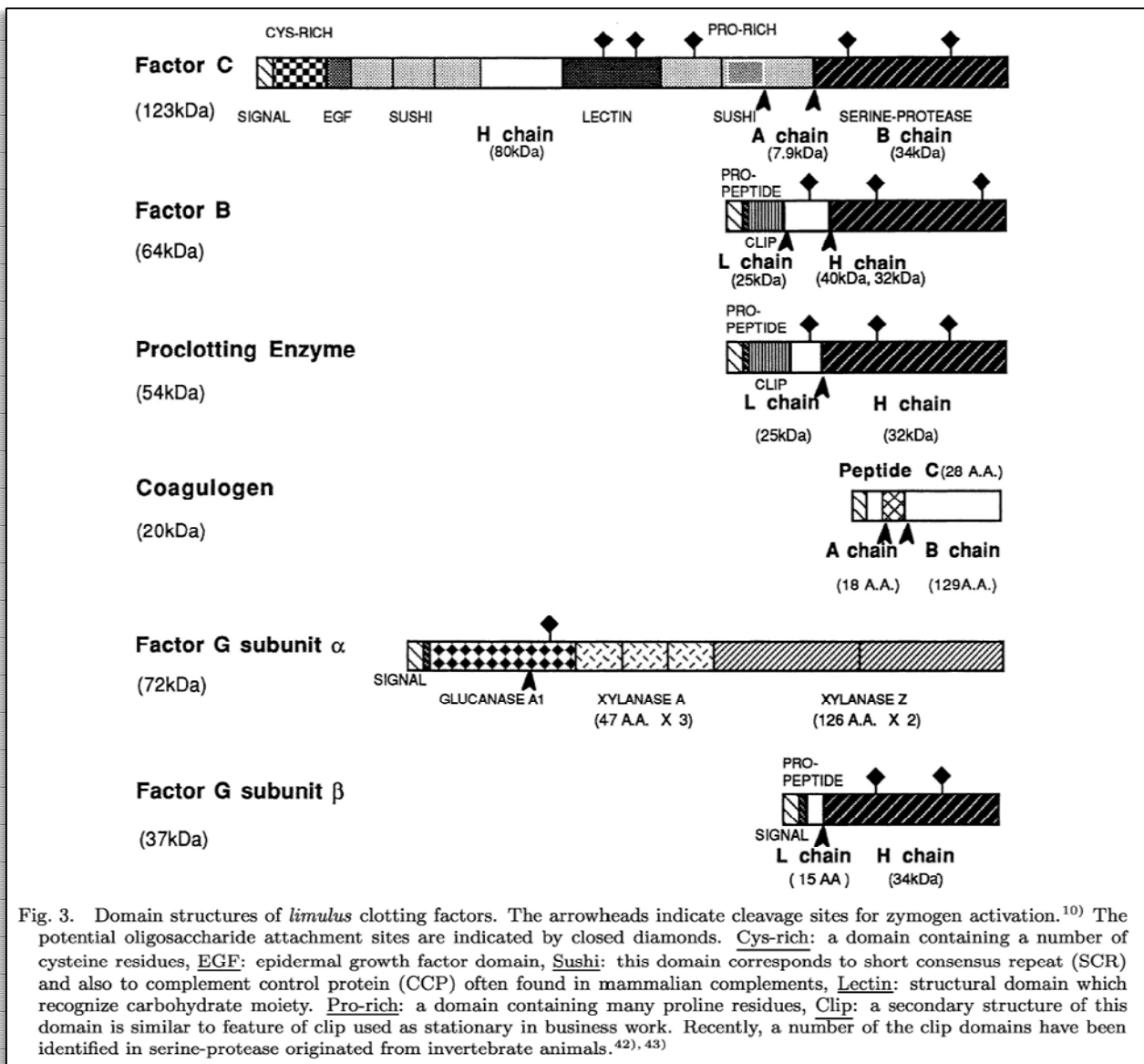
In contrast, here, a POSA only needs ten distinct proteins to practice the Challenged Claims: two Factor C proteins, four Factor B proteins, and four Pro-clotting enzymes. EX2062 ¶105. The specification discloses the cDNA and amino acid sequences of each of these proteins from *Tachypleus tridentatus*. EX1017 at 108 ([0156]). Those sequences are representative of the gene and amino acid sequences of Factor C, Factor B, and Pro-clotting enzyme from *Limulus polyphemus* and, in the case of Factor B and Pro-clotting enzyme, the other two species of horseshoe crab, because of the known or expected high similarity across species and their known functional similarity. EX2062 ¶¶45, 105-106. Thus, the '767 Application discloses a representative number of the ten proteins required by claim 1 and of the six proteins required by claim 4.

4. A structure-function correlation was known as of December 10, 2013

Petitioner argues that the '767 Application fails to describe a common “structural feature of the claimed proteins that allow them to function in the claimed assay as a whole.” Pet. at 35. Petitioner’s arguments improperly ignore the disclosures of the '767 Application as well as the extensive knowledge in the art concerning the three proteins. The written description requirement can be met “when the art has established a correlation between structure and function.” *Ariad*, 598 F.3d at 1350. That is the situation here.

As discussed, the proteins involved in the horseshoe crab coagulation cascade were isolated and characterized beginning in the mid-1980s—*four decades* ago. EX2062 ¶¶22-34, 38-44; *see also* EX1010; EX1011 at pp. 22-31 (citing prior art references); EX1017; EX1025. Accordingly, by December 10, 2013, there was a wealth of existing scientific information about the horseshoe crab coagulation cascade and the common functions, sequences, and structures of Factor C, Factor B, and Pro-clotting enzyme. EX2062 ¶¶22-34, 38-44; EX2059 at 2-6; EX2057 at 3-6; EX2058 at 4-5 and Fig. 5; *see also* EX1002 at Fig. 8-9. Petitioner’s arguments based on taxonomic and geographical separation of the horseshoe crab species and purported lack of common structural features are overly generalized and fail to refute the more specific publications concerning the three zymogen proteins at issue. EX2062 ¶¶107-112; *cf.* Pet. at 34.

By way of example of the knowledge of the structural and functional features of the three proteins, Figure 3 from EX2057 is reproduced below:



Further, the '767 Application's disclosure that both insect cells and mammalian cells expression functional Factor C is significant. EX2062 ¶112. Factor C expressed by insect cells contains no (α -2,3)-linked terminal sialic acid while the latter does. EX1017 at 91-95 [0125]-[0131]; EX1003 ¶¶177-180; EX1019 at 2 ("While native mammalian glycoproteins often have *complex* type *N*-glycans

with terminal sialic acids, insect cell-derived recombinant glycoproteins usually have much simpler side chains, known as *paucimannosidic N*-glycans, at sites normally occupied by complex, terminally sialylated structures.”); EX2062 ¶112. This disclosure informs a POSA that a wide range of glycan forms are tolerated on Factor C with apparently little to no impact on its functionality in the cascade, and likely Factor B and Pro-clotting enzyme as well. EX2062 ¶112.

a. Factor C

The ’767 Application discloses the cDNA and amino acid sequences of Factor C from *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* as SEQ ID NOs: 1 and 2 and SEQ ID NOs: 3 and 4. EX1017 at 98 ([0156]). The ’767 Application also provides an alignment of their amino acid sequences showing their 97.7% overall identity. EX1017 at 123-124 (Figs. 8-9); EX2062 ¶113. The ’767 Application also discloses that, despite the small differences in sequence, the two Factor C proteins were functionally identical in terms of being activated by LPS and activating Factor B. *Id.*

More specifically, one of the two “Patent Documents” incorporated by reference in the ’757 Application is WO 2008/004674 (“WO ’674”). EX1017 at 14 ([0012]). WO ’674 discloses mixing and matching recombinant *Carcinoscorpius* Factor C with recombinant *Tachypleus tridentatus* Factor B and Pro-clotting enzyme expressed in bacteria, yeast, and insect cells. EX2050 ¶¶[0110], [0171]-[0182].

The cDNA and amino acid sequences of *Limulus polyphemus* Factor C had not been published in continuous or full-length form as of December 10, 2013. However, the nearly complete cDNA sequence could be assembled from the transcriptome data that was publicly available by October 31, 2013 by querying with *Tachypleus tridentatus* Factor C. EX2062 ¶¶68-70. The amino acid sequence of *Limulus polyphemus* Factor C was reported by multiple sources to be around 90% homologous to *Carcinoscorpius rotundicauda* Factor C. EX2047 ¶[0134] (“Tryptic peptide sequencing revealed that the Limulus factor C sequence closely matched the Asian species, with >90% sequence identity.”); EX2048 at 4 (“An unpublished study found that the Factor C of *Carcinoscorpius* has 89.3% homology with that of *Limulus polyphemus*, [the] North American horse shoe crab Factor C”); EX2070 at 2 (“The rFC protein derived from *Carcinoscorpius* shares a 90.5% identical amino acid sequence with the native Factor C protein derived from *Limulus polyphemus* (unpublished data).”); EX2062 ¶113.

In view of the high similarity between: i) the *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* Factor C proteins and ii) the *Carcinoscorpius rotundicauda* and *Limulus polyphemus* Factor C proteins, a POSA would have understood that the *Tachypleus tridentatus* and *Limulus polyphemus* Factor C proteins would also be highly similar. EX2062 ¶¶114-120; *see also* EX2060 at 7 (reporting that peptides obtained from *Limulus polyphemus* hemolymph were

homologous to proteins from other crab species, including “Limulus clotting Factor C” from *Tachypleus tridentatus*). A POSA could have confirmed this understanding by querying the *Limulus polyphemus* transcriptome data with the Factor C sequences provided by the ’767 Application to identify the full *Limulus polyphemus* Factor C sequence. EX2062 ¶¶114-120. A sequence alignment and analysis would have revealed an extremely high degree of similarity—98.9%—across the three species. EX2062 ¶115; EX2094; EX2114; *see also* EX1011 at 44-46. The predicted 3D structures of the two proteins reveals that key functional domains of Factor C are highly conserved, reinforcing a POSA’s belief that the enzymes for each species would most likely have indistinguishable functions. EX2062 ¶¶117-120; EX2112.

Even if a POSA did not perform the sequence alignment and analysis, they would have believed that Factor C has a high degree of functional similarity across species. EX2062 ¶121. In 1991, Tokunaga et al. reported that the “immunological and biochemical properties” of *Limulus polyphemus* Factor C were “indistinguishable” from *Tachypleus tridentatus* Factor C, including their amino acid compositions, and that they were “identical protein entities.” EX1025 at 151, 152, 153; EX2062 ¶122. Tokunaga’s data showed that both Factor C zymogens were rapidly activated by LPS and lipid A analogue over a similar time course. EX1025 at 151, 153, Fig. 2, Suppl. Figs. 6-8; EX2062 ¶122. The proteins were so similar in function that the authors discontinued their comparative studies and

proceed with activation and substrate specific experiments mainly with the *Tachypleus tridentatus* protein because it was more readily available. EX1025 at 152; EX2062 ¶122.

Later reports reinforced the functional similarity across species. EX2062 ¶¶124-126; EX1025 at 153 (reporting that the Factor C proteins from *Limulus polyphemus* and *Tachypleus tridentatus* were “indistinguishable” in terms of “immunological and biochemical properties”); EX2048 at 4 (reporting that recombinant *Carcinoscorpius* Factor C and native *Limulus polyphemus* Factor C “are highly comparable” in terms of detecting endotoxin); EX2024 at 15-17; EX2096; EX2022 at 7; EX2024 at 14-16; EX2070 at 9 (“The rFC procedure for the detection of gram-negative bacterial endotoxin has been shown to be equivalent to LAL in its ability to quantifiably measure endotoxin and has a quantitative range comparable to that of quantitative photometric LAL procedures”).

Further, the predicted 3D structures of the two proteins reveals that key functional domains of Factor C are highly conserved across species. EX2062 ¶120. This would reinforce a POSA’s belief and conclusion that the enzymes for each species would most likely have indistinguishable functions. EX2062 ¶120; EX2112.

In sum, a POSA in December 2013 viewing all of the information available and known would have had a high degree of confidence that the *Limulus polyphemus* and *Tachypleus tridentatus* Factor C proteins are functionally identical and

interchangeable. EX2054 at 3; EX2050 at 35-38 ([0110], [0171]-[0182]) (reporting that recombinant *Carcinoscorpius rotundicauda* Factor C activated recombinant *Tachypleus tridentatus* Factor B); EX2062 ¶112.

b. Factor B and Pro-clotting enzyme

Factor B and Pro-clotting enzyme from *Tachypleus tridentatus* were cloned and sequenced in 1993 and 1990, respectively. EX2062 ¶¶38-39; EX2002; EX2003. Factor B and Pro-clotting enzyme are similar to each other in terms of amino acid sequence and structure, each containing a CLIP domain and serine protease domain. EX2062 ¶40; EX2038 at 3-4; EX2039 at 2-3 and Fig. 4. These two domains are conserved across arthropods and insects. *See, e.g.*, EX2088 at 2; EX2097 at 7-9; EX2062 ¶127.

Factor B and Pro-clotting enzyme also share significant sequence similarity with the serine protease domain of Factor C, with all three factors having an identical serine protease active site triad. EX2038 at Fig. 3; EX2062 ¶40. It was suggested as early as 1992 that the CLIP domain of Factor B and Pro-clotting enzyme was a common structural feature of serine protease zymogens across all invertebrates. EX2067 at 9-10; EX2062 ¶40.

Sequence analysis reveals that the *Tachypleus tridentatus* and *Limulus polyphemus* Factor B proteins are 95.7% similar and the Pro-clotting enzymes are 94.7% similar. EX2062 ¶¶133-134; EX2094. With respect to Pro-clotting enzyme,

it was known that coagulogen—its target—was remarkably similar across species. EX2080 at 4-5, Fig. 15; EX2062 ¶131. The majority of the non-conservative substitutions are outside of the conserved CLIP and serine protease domains. EX2062 ¶¶133-134; EX2094. The predicted 3D structures of these two domains are essentially indistinguishable. EX2062 ¶¶133-134. Thus, the non-conservative substitutions across the species do not affect their ability to bind and activate coagulation (or a detection substrate), emphasizing that these differences are not relevant to their fundamental function.

c. Functional interchangeability

There was extensive evidence in the art that the coagulation cascades were functionally interchangeable across horseshoe crab species.

Commercial *Limulus* amebocyte lysate is manufactured from amebocytes such as *Limulus polyphemus* and *Tachypleus tridentatus*. EX2005 at 1. LAL can also be obtained from *Tachypleus gigas* and *Carcinoscorpius*. *Id.* at 2. Notably, the U.S. Pharmacopeia chapter on bacterial endotoxin testing describes amoeocyte lysate as “a lyophilized product obtained from the lysate of amoeocytes... from the

horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*).”¹¹ EX2051 at 1; EX2062 ¶45. The corresponding chapters in the European Pharmacopoeia and Japanese Pharmacopoeia were harmonized with the USP in 2012.¹² EX2052 at 1; EX2053 at 4; EX2062 ¶46. Thus, these compendia reflect the understanding in the art that the coagulation cascade and its constituent proteins are functionally identical between *Limulus polyphemus* and *Tachypleus tridentatus*.

It had also been established well before December 2013 that assays using on recombinant *Carcinoscorpius* Factor C and native LAL were interchangeable for measuring endotoxin. EX2062 ¶113; EX2048 at 4 (“LAL and rFC gave comparable results” and “are highly comparable”); EX2022 at 7 (reporting that *Limulus*

¹¹ The U.S. Pharmacopeia “works closely with government agencies, ministries, and regulatory authorities around the world to help provide standards of identity, strength, quality, and purity that can help safeguard the global supply of medicines, dietary supplements, and food ingredients. USP standards are legally recognized in the U.S. and elsewhere, and are used in more than 140 countries.” See <https://www.usp.org/about/legal-recognition> (last accessed June 11, 2025).

¹² Harmonization means that “a pharmaceutical substance or product tested by the document’s harmonized procedure yields the same results and the same accept/reject decision is reached.” EX2082.

polyphemus lysate and recombinant *Carcinoscorpius* Factor C showed “a very strong positive correlation” and “returned similar values” in quantitative endotoxin detection assays).

Petitioner has admitted the assumption in the field was that “Factor C enzymes from all horseshoe crab genera... will behave similarly.” See EX2054 at 3 (“both rFC assays rely on the underlying assumption that the Factor C enzymes from all horseshoe crab genera (*limulus*, *carcinoscorpius* and *tachypleus*) will behave similarly to all of the proteins that are found within an aqueous amoebocyte extract” and “[d]ata from initial studies confirmed that both versions of rFC bind to and detect reference standard endotoxin and control standard endotoxin, which was hypothesized based on the similarities among Factor C enzymes from the three horseshoe crab species” (citing a publication from 2000)); EX2062 ¶125.

Petitioner makes much of the unpredictability of mixing and matching proteins from different horseshoe crab species (see Pet. at 26, 31, 32, 35, 55) into a functional coagulation cascade but fails to acknowledge that the ’767 Application references Patent Owner’s prior disclosures that functional cascade reactions could be created by mixing and matching components from different horseshoe crab species. EX1017 at 14 ([0012]); EX2050 ¶¶[0110], [0171]-[0182]; EX2062 ¶¶47, 51, 126. Thus, successful “mixing and matching” was disclosed in the ’767 Application and known in the art.

In sum, considering the data together, a POSA would conclude that Factor C, Factor B, and Pro-clotting enzyme from each species would most likely behave in an indistinguishable manner in the coagulation cascade. EX2062 ¶135.

5. There was no struggle in the field to sequence or characterize *Limulus polyphemus* genes and proteins

Petitioner alleges that “the field had struggled to sequence and structurally and functionally characterize the *Limulus polyphemus* species of horseshoe crab” in general, citing the ’629 Publication. Pet. at 32, 42. However, publications in the field from as early as 1986 show that there was no such struggle, and certainly any struggle that may have existed in those earlier days of molecular biology had been overcome by December 2013. EX2062 ¶¶136-137.

The numerous peer-reviewed publications as of December 2013 reporting on the sequence and characterization of *Limulus polyphemus* genes and proteins, including those involved in the innate immune system generally and coagulation in particular (C-reactive protein, agglutination-aggregation factor, endotoxin-binding protein, limunectin, anti-lipopolysaccharide and coagulogen), refute Dr. Jain’s assertion. EX2062 ¶¶136-137. A few are listed below; none mention any difficulties particular to *Limulus polyphemus*:

- The amino acid sequence *Limulus polyphemus* coagulogen had been elucidated and cDNA cloned by 1986. EX2038 at 3 (citing papers from

1984 and 1986).

- The amino acid sequence and structure of the *Limulus* C-reactive protein was published in 1986. EX2098.
- The complete amino acid sequence of anti-lipopolysaccharide factor from *Limulus polyphemus* was determined in 1987. EX2099.
- An endotoxin-binding protein with protease inhibitory activity was purified and its cDNA cloned in 1991. EX2100.
- A protein called limunectin was isolated from *Limulus polyphemus* amoebocytes and its cDNA cloned from a lambda phage cDNA library in 1991. EX2101.
- A hemagglutinin and amebocyte aggregation factor from *Limulus polyphemus* was isolated, characterized, and its cDNA cloned in 1992. EX2102.
- The amino acid, DNA sequence, and recombinant expression of an endotoxin binding and neutralizing protein from *Limulus polyphemus* was reported in 1992. EX2103 at 11 (Figs. 15-16).
- The complete cDNA sequence of an *Limulus polyphemus* arginine kinase was determined and analyzed in 1995. EX2104.
- A GTP binding protein subunit of *Limulus polyphemus* was cloned in

1996. EX2105.

- A cyclic nucleotide-gated ion channel cDNA from *Limulus polyphemus* was cloned in 1999. EX2106.
- A protein kinase C from *Limulus polyphemus* was cloned in 2003 (using RACE). EX2107.
- A protein regulating eye development was cloned in 2008 (using RACE-PCR). EX2108 at 2.
- The cDNA sequence of *L. polyphemus* factor G-subunit alpha was cloned in 2011 (using RACE-PCR with a primer designed based on the corresponding *T. tachypleus* sequence). EX2113.
- A search of the NCBI GenBank database for *Limulus polyphemus* sequences released before December 10, 2013 yielded 8707 entries. EX2109.

The above publications include genes and proteins involved in the innate immune system and coagulation cascade. EX2038 at 3 (coagulogen); EX2098 (C-reactive protein involved in the innate immune system); EX2099 (anti-LPS factor); EX2102 (hemagglutinin and amebocyte aggregation factor); EX2062 ¶137; *see also* EX2006 at 9, n.2.

Petitioner points to statements in the '629 Publication that the gene and amino acid sequences for *Limulus polyphemus* zymogen proteins “could not be achieved

for over 20 years after the same information became available for other species of horseshoe crab.” EX1003 ¶133. However, these statements do not establish that it was difficult or impossible to determine the gene and amino acid sequences for *Limulus polyphemus* zymogen proteins as of **December** 2013, since all of the *Tachypleus tridentatus* sequences were published by **October** 1993. See EX2062 ¶138; EX1010 (1991 for Factor C); EX2002 (1993 for Factor B); EX2003 (1990 for Pro-clotting enzyme); EX1065 at 47-48. Further, publications reflect that sequence information had been obtained for *Limulus polyphemus* Factor C well before 2013. EX1025; EX2047; EX2048; EX2070. Other than EX1025, Dr. Jain does not address these publications in the context of his opinions concerning a purported struggle in the field. EX1003 at 5-6; EX2062 at 198-199 (discussing EX2047 and EX2048); EX2062 ¶138. Experts with experience in cloning genes and analyzing sequences, Professors Frohman and Schaefer, both disagree with the statements in the ’629 Publication. Petitioner and Dr. Jain rely on. EX2004 ¶¶40-43; EX2062 ¶140.

Finally, the technique in the ’629 Publication for obtaining the cDNA sequences of *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme used standard primers and protocols for RACE-PCR. EX2062 ¶139; see also EX2006 ¶35. Thus, the statements in the ’629 Publication do not establish the existence of a struggle in the field that precludes a finding of adequate written description.

6. Claims 2-9

Claims 2-4 narrow the scope of claim 1. Therefore, a POSA would have understood the inventors to possess claims 2-4 for the same reasons as claim 1. EX2062 ¶141. Claims 5-6 recite characteristics of the horseshoe crab Factor C. The '767 Application describes how to determine these properties. EX1017 at 100-107 ([0141]-[0156]). Because possession of a protein results in possession of its properties, a POSA would understand the inventors to possess claims 5-6. *Accord* EX2111 ¶32; EX2062 ¶142. The '767 Application expressly discloses filtering the culture supernatant, as recited in claim 7, and formulation of the endotoxin assay agent into a solid, liquid or a gel, as recited in claim 8. EX1017 at 70, 87-89 ([0074], [0112], [0115]). Accordingly, a POSA would understand the inventors possessed claims 7 and 8. EX2062 ¶143. Finally, the '767 Application expressly discloses that the three proteins may be separately expressed in individually established host cells, as recited in claim 9. EX1017 at 67 ([0068]); EX2062 ¶144.

B. The '767 Application Enables the Challenged Claims

“[A] specification may call for a reasonable amount of experimentation to make and use a patented invention. What is reasonable in any case will depend on the nature of the invention and the underlying art.” *Amgen Inc. v. Sanofi*, 598 U.S. at 612. As with written description, “a patent does not need to guarantee that the invention works” or be reduced to practice in working examples for enablement.

Alcon Rsch. Ltd. v. Barr Lab'ys, Inc., 745 F.3d 1180, 1189 (Fed. Cir. 2014) (citing *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 61 (1998)). Where there are no examples “[t]he burden is on one challenging validity to show by clear and convincing evidence that the prophetic examples together with other parts of the specification are not enabling.” *Id.* at 1189-1190 (quoting *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984)). Nor is it “a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works.” *Id.* at 1190 (citing *Newman v. Quigg*, 877 F.2d 1575, 1581-82 (Fed. Cir. 1989)). The enablement requirement is met “if the description enables any mode of making and using the invention.” *Invitrogen*, 429 F.3d at 1071.

As with written description, Petitioner’s expert proceeded from an inaccurate understanding of the claimed invention, as discussed in Section IV, above, and the Board can reject Petitioner’s enablement arguments and evidence on that basis alone. Even if the Board agrees with Petitioner that the Challenged Claims have a functional requirement, the ’767 Application is enabling. In view of the extensive knowledge in the field, the high level of skill in the art, and the disclosures in the specification, generating the ten sequences required to practice the Challenged Claims in a variety of mammalian cell lines and testing those proteins for activity would not require undue experimentation. Thus, the Challenged Claims are enabled.

However, even if the Challenged Claims require generating and testing 3,456 distinct combinations of proteins for the ability to detect endotoxin in a sample as Petitioner contends—which they do not—that amount of testing was reasonable for this field.

As discussed below, the *Wands* factors do not support a lack of enablement even under Petitioner’s incorrect view of the Challenged Claims.

1. Breadth of the claims (factor 8)

Petitioner relies on *Idenix Pharmaceuticals LLC v. Gilead Sciences Inc.*, 941 F.3d 1149 (Fed. Cir. 2019) and *Enzo Life Sciences, Inc. v. Roche Molecular Systems, Inc.*, 928 F.3d 1340 (Fed. Cir. 2019). Pet. at 46-47. But those cases are inapposite. In *Idenix*, the candidate chemical compounds numbered “likely [] millions or at least many, many thousands.” 941 F.3d at 1157. And in *Enzo*, the claims “place[d] almost no limitation on the structure of the claimed polynucleotide.” 928 F.3d at 1346-47.

As discussed above, Petitioner has exaggerated the breadth of the claims. The Challenged Claims encompass only 32 combinations of ten and discloses three of those proteins. EX2062 ¶¶99-103. Even accepting the claimed genus comprises 3,456 species, that is far narrower than the claims addressed in *Idenix* and *Enzo*. Further, there are specific structural limitations on the recombinant proteins, namely their identity and source, and for some, their sequence. Petitioner acknowledges

this. *See, e.g.*, Pet. at 26 (“For Factor C, the recombinant protein must come from either *Tachypleus tridentatus* or *Limulus polyphemus*... For Factor B and Pro-clotting enzyme the recombinant protein can come from any species of horseshoe crab...”).

Nor do the Challenged Claims seek to “monopolize an entire class of things defined by their function” like the claims at issue in *Amgen, Inc. v. Sanofi*, 598 U.S. 594, 613 (2023). The Challenged Claims are directed to specific combinations of proteins encoded by ten distinct amino acid sequences expressed in mammalian cells. Importantly, unlike in *Amgen*, there are not “a ‘vast’ number of additional proteins” that have not been described. *Cf. Amgen v. Sanofi*, 598 U.S. at 613.

2. Nature of the invention, state of the prior art, and unpredictability (factors 4, 5, 7)

Petitioner argues that “a significant amount of research and experimentation as required to generate a particular protein, with the ability to detect endotoxin, across a number of different expression systems.” Pet. at 42-43. Even assuming the Challenged Claims require a cascade reaction, Petitioner has not provided any evidence that any individual component would be inoperable or would require undue experimentation to obtain. To the contrary, the evidence reflects the individual sequences and proteins could have been obtained through routine experimentation.

EX2004 ¶¶4-48; EX2062 ¶¶68, 70, 89, 140; *see also* EX2006 ¶¶28-33, 36, 58-59, 62-63, 68, 70-73, 76-77, 79, 86-87, 90, 138-140.

Instead of particularized evidence, Petitioner relies on (i) general assertions from its expert that are not specific to the particular recombinant proteins recited in the claims; and (ii) a 1997 prior art reference describing difficulties expressing Factor C in COS-1 cells. *See* Pet. at 42-43; EX1003 ¶¶89-92. But these arguments ignore the '767 Application's disclosure that the inventors had successfully expressed recombinant Factor C in the mammalian host cells recited in the claims. *See, e.g.*, EX1017 at 85-88 ([0107]-[0115]). Further, expressing Factor C in COS-1 cells is outside the scope of the Challenged Claims.

Similarly, Petitioner can point to only the absence of reports of expression of recombinant Factor B and Pro-clotting enzyme in mammalian expression systems, which is not evidence of difficulties or failure (or undue experimentation).¹³ Pet. at 43; EX1003 ¶¶91-92; EX1065 at 159. Thus, the '767 Application's instruction that techniques used for production of Factor C in mammalian cells may be applied "mutatis mutandis" to the production of Factor B and Pro-clotting enzyme provides

¹³ As the largest by far of the three proteins, that Factor C was not secreted when expressed by COS-1 cells is not indicative that Factor B or Pro-clotting enzyme would behave similarly when expressed in that cell line. EX2062 ¶149.

sufficient guidance to a POSA to express those proteins without undue experimentation. EX1017 at 63 ([0057]). Petitioner's expert conceded that a POSA following the examples of the '629 Publication—which, are nearly identical to those in the '767 Application as discussed below—was *not* undue experimentation. EX1065 at 195; *see* Section VII.B.4, below.

According to Petitioner, there are four sources of Factor C, Factor B, and Pro-clotting enzyme, namely, the four species of horseshoe crab. Pet. at 28. The Challenged Claims are limited to two of those sources of Factor C: *Tachypleus tridentatus* and *Limulus polyphemus*. The specification provides the sequence for one out of two of those species for Factor C, and one out of the four species' Factor B and Pro-clotting enzyme genes. Two experts in the field agree that the '767 Application discloses sequences that enabled a POSA to obtain the *Limulus polyphemus* Factor C sequence using standard techniques. EX2062 ¶¶68-71, 74-88, 147; EX2004 ¶¶4-43. The evidence also establishes that the *Tachypleus tridentatus* sequences disclosed in the '767 Application enable a POSA to obtain the sequences of Factor B and Pro-clotting enzyme from the other three horseshoe crab species using the same techniques. EX2062 ¶¶68-71, 74-89, 147.

Petitioners fault the '767 Application for failing to describe a single example of the three proteins assembled into a cascade. Pet. at 33 (citing *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 918, 926 (Fed. Cir. 2004)). Even if the

Challenged Claims required a cascade reaction, this argument is misplaced because, unlike *University of Rochester*, the specification here discloses precisely what proteins should be used in the claimed methods and provides extensive instructions and examples on how to make those proteins and test them for activity. EX1017 at 87-88, 93-95 ([0108]-[0115], [0129]-[0131]). The act of assembly is trivial. And, in view of the information in the art about the functional similarity and even interchangeability across species, a POSA would expect the cascade to function after assembly. EX2062 ¶¶108-112. This is not the type of “research assignment” requiring “painstaking experimentation” discussed in *Amgen v. Sanofi*, but rather routine experimentation.

3. Level of skill in the art (factor 6)

Petitioner concedes the level of skill in the art is high, requiring a PhD and at least two years of experience. Pet at 44. For such skilled artisans in 2013, designing, expressing, and testing recombinant proteins was routine. EX2062 ¶¶163-164. The Frohman Declaration is additional evidence that making recombinant Factor C would not have been difficult for persons working in the field. EX2004 ¶¶44-47. Notably, the only references Petitioner cites in support of the premise that “designing, expressing, and testing recombinant proteins is laborious, time-consuming, complicated, and highly unpredictable” are outdated or inapposite. Pet. at 44 (citing EX1031 and EX1006); EX2062 ¶¶148, 151.

EX1031 is outdated. It is from 2004, and therefore does not reflect the state of art of expressing recombinant proteins as of December 2013. EX2062 ¶¶148, 151. Similarly, EX1026 is from 1997. Although researchers from another group reported in 1997 that *Carciniscorpius rotundicauda* Factor C expressed in COS-1 cells was associated with cellular debris (EX1026 at 360), the '767 Application describes in detail how to express functional, secreted, recombinant *Tachypleus tridentatus* Factor C from two exemplary mammalian cell lines. EX2062 ¶149. Petitioner has simply not provided any evidence to support its argument (Pet. at 44-45) that making recombinant Factor C, Factor B, and Pro-clotting enzyme proteins would have been difficult or unpredictable in 2013.

EX1006 is a reference concerning whether sequence similarity with proteins of known functions could be used to predict the function of an uncharacterized protein. EX1006 at 2, 6 (“What our model helps assess is the continuous range of likelihoods that a protein has a particular function based on its sequence similarity to a protein of known function” where the number of possible functions was “over 8000”); EX2062 ¶151. This reference is irrelevant because the three recited proteins were well characterized in function, homologous across horseshoe crab species, and structurally similar to each other. EX2038 at 3-6; EX2057 at 3-4, 5-6; EX2058 at 2-6; EX2062 ¶151. Thus, EX1006 is not probative of unpredictability under the circumstances here because the functions of Factor C, Factor B, and Pro-clotting

enzyme were already well-known. EX2062 ¶151. There is no need to attempt to predict function. *Id.* In sum, Petitioner’s evidence is not specific and not persuasive that the art of producing recombinant Factor C, Factor B, and Pro-clotting enzyme in mammalian host cells was beyond the capabilities of a person of ordinary skill in the art as of 2013, highly unpredictable or highly speculative. *Cf.* Pet. at 44-45.

Petitioner relies on *ALZA Corp. v. Andrx Pharmaceuticals, LLC*, 603 F.3d 935 (Fed. Cir. 2010) for the premise that “reaching the full scope of claim 1 would require a higher than ordinary level of skill in the relevant art.” Pet. at 45. But in *ALZA*, the patent owner had tried and *failed* to make the claimed dosage form. 603 F.3d at 942. Here, inventors *succeeded* using standard cloning and expression techniques. EX1001; EX1015; *see also* Pet. at 85 (asserting that “POSAs *did carry out* the experiments described in the disclosure and examples of the ’629 Publication without undue experimentation.”) (emphasis original).

**4. Guidance, working examples, degree of experimentation
(factors 1-3)**

Petitioner’s arguments concerning these *Wands* factors are premised entirely on importing the requirement of a functioning assay into the Challenged Claims. Pet. at 46. Even if the Challenged Claims do require a functioning assay, there is sufficient guidance in the ’767 Application.

a. Expression of Factor B and Pro-clotting enzyme

As discussed, the '767 Application expressly states that the descriptions of the production of Factor C in mammalian cells may be applied “mutatis mutandis” to the production of Factor B and Pro-clotting enzyme. EX1001 at 22:27-42. As discussed above, there were no known issues with expressing Factor B and Pro-clotting enzyme in mammalian cells, and a post-priority date reference from the inventors confirmed they did not experience any such issues. EX1007; EX2062 ¶156. Thus, Petitioner has failed to present any evidence that a person of ordinary skill in the art could not express Factor B and Pro-clotting enzyme following the teachings of the '767 Application without undue experimentation. EX2062 ¶157. Indeed, with respect to the '629 Publication, Petitioner argues that a POSA would be able to follow its “detailed directives” to assemble an endotoxin detection assay agent within the scope of the claims without undue experimentation. Pet. at 85-86. This assertion belies Petitioner’s arguments that the Challenged Claims lack enablement.

Notably, the instructions on constructing expression vectors and expressing the zymogen proteins in mammalian cells in the '629 Publication is far *less* detailed than those provided in the '767 Application. *Compare* EX1017 at 87-88 ([0108]-[0115]) *with* EX1015 at [0350]-[0351]; EX2062 ¶¶158-159.

If the '629 Publication discloses each and every limitation of the Challenged Claims and is enabling as Petition argues, then the '767 Application is also enabling

for the Challenged Claims. There is no significant difference between their disclosures concerning how the recited proteins may be expressed in mammalian cells, and as discussed *supra* in Section VII.A.1, obtaining the sequences for the recited proteins was routine experimentation.

The '767 Application teaches use of the same pCA7 vector as the '629 Publication. EX1017 at 54-55; EX1001 at 19:28-31, 31:49-56. The '767 Application discloses that the recombinant Factor C is secreted into the culture supernatant. EX1017 at 85-88 ([0107]-[0115]); EX1001 at 19:60-62, 31:27-32. While the '629 Publication describes use of the ExpiCHO Expression System, there is no indication that use of this particular expression system was critical rather than merely a convenience. EX1015 ¶[0350]. Indeed, the '767 Application indicates that it was *not* critical, having achieved successful expression of Factor C in multiple mammalian cell lines without it. EX1017 at 85-88 ([0107]-[0115]); EX1001 at 30:32-32:5; *see also id.* at 19:26-46. Based on the examples in the '767 Application, a POSA could have readily used various mammalian host cells to test the expression Factor B and Pro-clotting enzyme. EX2062 ¶96.

It was routine as of December 2013 for POSAs seeking to express recombinant proteins to test expression in multiple systems and ultimately select their particular expression system based on project-specific goals and requirements. *See, e.g.*, EX2110 at 2-3; *see also* EX1011 at 84-96; EX2062 ¶163. Insect cells are

widely used for recombinant protein production, including glycoproteins. EX1019 at 8; EX2062 ¶163. The '767 Application's disclosure that Factor B and Pro-clotting enzyme were successfully expressed in insect cells also supports these proteins could be successfully expressed in mammalian cells without undue experimentation. *See, e.g.*, EX1019 at 8 ("a significant body of accumulated evidence supports the idea that at least some insects, insect tissues, and/or insect cells have the potential to produce complex, terminally sialylated *N*-glycans."); EX2062 ¶164.

Further, Dr. Jain fails to consider the significance of the '767 Application's disclosure that both insect (Sf9) and mammalian (CHO DG44 and HEK293) cells express functional Factor C even though Factor C expressed by the former contains no (α -2,3)-linked terminal sialic acid while the latter does. EX1017 at 91-95 [0125]-[0131]; EX1003 ¶¶177, 180; EX1019 at 2; EX2062 ¶165. This disclosure is significant because informs a POSA that a wide range of glycan forms are tolerated on the horseshoe crab clotting cascade proteins, with apparently little to no impact on their functionality as enzymes in the cascade. EX2062 ¶165. Thus, a POSA would further conclude that the choice of mammalian cell line would have little meaningful impact on the production of functional clotting cascade proteins. *Id.*

The '767 Application discloses results for assays using recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme for detecting endotoxin. EX1017 at 93-95 ([0128] -[0130]); EX1001 at 34:6-47. This is sufficient

guidance for a POSA to assemble the endotoxin assay agent and to measure endotoxin as recited in the Challenged Claims. For example, the '629 Publication, which Petitioner contends is enabling for assembling a recombinant cascade reaction, *refers back to* the disclosures of the '767 Application. EX1015 at [0361]-[0362] (WO 2014/092079 is the international publication of the '767 Application); EX2062 ¶159.

Even if the Challenged Claims required testing over 3,000 combinations for endotoxin detection activity, that number of assays constitutes routine experimentation in this field. The LAL assay was ubiquitous as of the priority date and high-throughput methods for measuring endotoxin were widely known. *See* EX1001 at 34 (describing activity measurement in microliter volumes); EX2047 ¶¶[0138]-[0140] (describing a one-step endotoxin assay carried out in a 96-well plate); EX2024 at Fig. 9.6; EX2062 ¶¶167-168. In view of the disclosures in the specification, the high level of skill and knowledge, this would have been routine

The only difference between the enabling disclosure of the '629 Publication and the '767 Application is that the former expressly provides the sequences of *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme. Pet. at 62; EX1065 at 192-193, 195. As discussed in Section VII.A.1 above, the '767 Application and publicly available information including published sequences and data deposits would have allowed a POSA to obtain the *Limulus polyphemus*

sequences using routine techniques. *See also* EX2062 ¶¶68-89. A POSA would have similarly been able to obtain the sequences of Factor B and Pro-clotting enzyme from the remaining two species of crab using RACE-PCR and the *Tachypleus tridentatus* sequences. EX2062 ¶¶90-92.

The Challenged Claims are enabling for the expression of Factor B and Pro-clotting enzyme in mammalian cells. *See Amgen v. Hoechst Marion*, 314 F.3d at 1335 (affirming that examples describing expression of the claimed protein in CHO and COS-1 cells were sufficient to enable claims directed to expression in any mammalian cell based on testimony that “similar outcomes could be expected from other mammalian cells since all mammalian cells produce and secrete hormones like [erythropoietin] by means of the same fundamental processes”).

b. The extent of experimentation required is not undue

The quantity of experimentation required to practice the Challenged Claims was not undue for this field. As of December 2013, both LAL and recombinant Factor C assays were carried out on numerous samples in parallel, so testing ten proteins for activity would not have been undue. *See* EX2070 at 2 (describing analytical procedure for recombinant Factor C); EX2066 at 7-8 (discussing automated methods), 14-15 (discussing methodology); EX1001 at 34:6-29; EX2047 ¶¶[0138]-[0140] (describing a one-step endotoxin assay carried out in a 96-well plate); EX2062 ¶¶167-168.

After expressing the recombinant Factor C, Factor B and Pro-clotting enzyme proteins, determining whether they each have activity according to the guidance provided in the specification (*see, e.g.*, EX1001 at 15:66-16:9, 21:6-16, 21:43-53, 25:4-37, 34:1-47) would have taken a reasonable amount of experimentation in view of what was common in the art. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988) (“[t]he test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine”); *Falko-Gunter*, 448 F.3d at 1365 (“The mere fact that the experimentation may have been difficult and time consuming does not mandate a conclusion that such experimentation would have been considered to be ‘undue’ in” a particular field.); EX2062 ¶¶165-166.

5. Claims 2-9

Claims 2-9 narrow the scope of claim 1, reducing the extent of experimentation needed to practice the full scope of the claims. Therefore, they are enabled by the '767 Application for the same reasons that claim 1 is enabled. EX2062 ¶169.

Claims 2-3 reduce the number of mammalian cells for expressing Factor B and Pro-clotting enzyme to two; the same two used in the '767 Application to express Factor C. Claim 4 reduces the number of proteins within the scope of the claims from ten to six, significantly reducing the amount of routine experimentation

needed to generate, test individually for function, and combine these proteins into an endotoxin assay agent. *Id.*

Claims 5-6 recite features of Factor C. The '767 Application includes extensive description of how to determine the sialic acid content and molecular weight of Factor C. EX1017 at 100-107 ([0141]-[0156]). EX2062 ¶170. Such experiments would have been routine for a POSA in view of the '767 Application. *Id.* Thus, claims 5-6 are enabled.

With respect claims 7-8, Petitioner does not argue that a POSA would not have been capable of filtering the culture supernatant comprising the horseshoe crab Factor C as recited in claim 7, or preparing an endotoxin assay agent that is a solid, liquid, or a gel as recited in claim 8. Pet. at 59; EX1003 ¶138. This would have been routine experimentation for a POSA. EX2062 ¶171. Therefore, claims 7-8 are enabled for the same reasons that claim 1 is enabled.

C. The Board Should Give Little Weight to Petitioner's Expert and Arguments

1. Petitioner's expert lacks relevant experience, undermining the weight of his opinions

Petitioner relies extensively on the Declaration of Siddhartha Jain, Ph.D. ("Jain Declaration") (EX1003). However, Dr. Jain lacks qualifications in an area that a POSA would undoubtedly have been familiar with and would need to do to carry out the Challenged Claims: isolating cDNA and constructing expression

vectors for mammalian expression. *See* EX1001 at 30:7-15, 30:57-63, 31:49-56 (describing construction of expression vectors). Thus, the Board should give little weight to his opinions on those subjects.

Dr. Jain has never cloned a gene from a cDNA library. EX1065 at 61-62. Even though RACE-PCR has been “a cornerstone of the skilled person’s common general knowledge” by at least 2016 (EX2006 ¶¶69), Dr. Jain only became aware of RACE PCR through the Patent Owner’s Preliminary Response in this matter. EX1065 at 65. He has never carried out a PCR or designed primers for PCR. EX1065 at 61. He has never created vectors for expressing recombinant proteins in any host cell. *Id.* He has not done gene sequencing. EX1065 at 72.

Dr. Jain’s lack of relevant experience significantly undermines the weight the Board should give his opinion. The lack of disclosure of the sequence information for *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme was a critical premise of his opinions. EX1003 ¶¶37, 66, 80, 133; EX1065 at 23-24. However, despite attempting a comprehensive search that included sequence databases (EX1065 at 22, 25) Dr. Jain did not discover that whole genome and amoebocyte transcriptome data was publicly available in the NCBI’s BioProject database. Dr. Jain was not familiar with this database, or others that a POSA would have been familiar with. EX1065 at 27-29; EX2062 ¶36.

Dr. Jain admitted he could only speculate as to how a POSA would have identified sequences of Factor C, Factor B, and Pro-clotting enzyme within a genomic DNA data deposit. EX1065 at 33. For example, he has never used the well-known NCBI BLAST tool to identify individual genes within genomic DNA. EX1065 at 35. Thus, Dr. Jain was not situated to provide expert opinions concerning how the *Limulus polyphemus* genetic data would have been used by a POSA even if he had found that data.

Dr. Jain's inexperience also explains his opinion that cloning the *Limulus polyphemus* Factor C gene is, standing alone, undue experimentation. EX1065 at 98. He testified that it would be "nearly impossible" for a POSA to actually obtain the cDNA sequence of *Limulus polyphemus* Factor C as of December 2013. EX1065 at 183, 187. However, he conceded that a graduate student was able to isolate the cDNA sequence of *Limulus polyphemus* Factor C at the beginning of her thesis work, which began before January 2014. EX1065 at 104, 106, 107; EX2062 ¶154.

Dr. Jain also has a mistaken understanding of what Section 112 requires. For example, he testified that that inventors must actually express a protein in every known cell line encompassed by the claims. EX1065 at 92-93. In other instances, he appeared to demand examples to show possession and enablement. EX1065 at 147-148, 157, 195.

Dr. Jain is experienced in cell culture and protein expression. EX1065 at 140. For those aspects of the Challenged Claims, Dr. Jain admitted that after obtaining the sequences of Limulus Factor C, Factor B, and Pro-clotting enzyme” it would *not* be undue experimentation “to express and put them together as per claim 1.” EX1065 at 193; *see also id.* 195. That opinion—which he is qualified to offer—should be credited.

Another reason for the Board to give little weight to Dr. Jain’s opinions is his erroneous assumption that the Challenged Claims require a functioning or functional endotoxin assay agent (EX1003 ¶¶64, 68, 73 92, 117, 137) or carrying out an assay or cascade reaction to detect endotoxin (EX1003 ¶¶65, 69, 70, 71, 74, 76, 77, 96, 97, 99, 117). He also conflated Claims 1 and 10 at times. EX1003 ¶¶65, 69, 70, 77, 123-124.

For example, in paragraph 31, Dr. Jain describes the claimed invention as “the enzymatic signaling cascade for detection of endotoxin.” EX1003 ¶65. But of course, the Challenged Claims are directed to a method for producing an endotoxin assay agent, not the cascade itself. Dr. Jain’s Declaration is rife with references to the claimed “functioning” or “functional” endotoxin assay. EX1003 ¶¶61, 65, 69, 91, 92, 99, and 101. Further, it is unclear whether this is Dr. Jain’s opinion regarding how a POSA would interpret the claim or merely his “understanding”; he also states that “[i]t is my *understanding* that due to the functional requirements of these

claims...” EX1003 ¶100 (emphasis added). As discussed above, Claim 1 does not recite the components or steps necessary to carry out the endotoxin detection assay. Dr. Jain’s opinions are therefore based on an incorrect understanding of Claim 1 and should be given little weight.

2. Petitioner’s Inconsistent Arguments to the European Patent Office Undercut its Arguments Here

In its Preliminary Response, Patent Owner presented evidence suggesting that Petitioner is the anonymous Opponent in an Opposition before the European Patent Office concerning Patent Owner’s European Patent EP 3530670¹⁴ (“Opposition”). *See* Paper 7 at 28-36. As the Board recognized, statements made in the Frohman Declaration and by the Opponent are inconsistent with positions taken by Petitioner in this proceeding. Paper 17 at 11-12 and concurrence/dissent by Judge Fitzpatrick. Patent Owner has also filed a Motion for Additional Discovery requesting the Board compel Petitioner to confirm whether it initiated the Opposition. Paper 23. That motion is pending. Patent Owner makes the arguments below on the assumption that Petitioner is the Opponent, as the evidence suggests.

The Opponent’s arguments in the Opposition contradict Petitioner’s

¹⁴ EP 3530670 claims priority to the same PCT application as the ’629 Publication. EX2006 ¶6; EX1015.

arguments here, as summarized below.

In this proceeding, Petitioner argues that “the ’109 patent inventors did not and ***could not have*** described an example of these proteins from *Limulus polyphemus* for the claimed assay because the gene and sequence information for Factor C, Factor B, and Pro-clotting enzyme for *Limulus polyphemus* horseshoe crabs was not yet disclosed as of the claimed priority date of the ’109 patent” and “the field had struggled to sequence and recombinantly produce... *Limulus polyphemus* zymogen proteins.” Pet. at 31, 42 (emphasis added). Dr. Jain testified that cloning the *Limulus polyphemus* Factor C gene, ***standing alone***, constitutes undue experimentation. EX1065 at 98 (emphasis added). The Opponent made the following arguments ***directly contradicting*** Petitioner and Dr. Jain and based largely on references published before December 2013.¹⁵

- “Regardless of which document [D1, D5 or D6] is taken as the starting point, the skilled person merely had to determine the sequence of the *Limulus* Factor C protein, or cDNA sequence encoding the same, to carry out its recombinant production... it would have merely been a matter of **routine analysis** to obtain the sequences...” EX2006 ¶31; *see also id.* ¶116. D1 is a European patent having the same

¹⁵ All emphases when quoting EX2006 are original unless otherwise indicated.

specification as the '767 Application. *Id.* ¶¶4, 39, 51.

- “The fact that the sequence determination necessary to arrive at claim 1 requires nothing more than ‘routine analysis’ has been confirmed in the expert declaration of Professor Frohman, submitted herewith as **D8**.” *Id.* ¶32.
- “As Professor Frohman explains, the sequences recited in claim 1 could have been derived in a ‘*straightforward manner*’ using techniques which were routinely used by the person skilled in the art, such as RACE or molecular cloning (see **D8**).” *Id.* ¶33.
- “Accordingly (and as demonstrated by the opposed patent), the skilled person merely had to carry out the teaching of, e.g., **D1**, **D5**, or **D6** using standard techniques to arrive at the claimed subject matter.” *Id.* ¶36.
- “[**T**he **problem** in view of **D1** may thus be formulated as the determination of the full-length *Limulus* Factor C sequence... Deriving the solution to this problem (i.e., one of the sequences recited in claim 1) does not require any inventive skill. As we have already noted above, it is a matter of routine practice for a person skilled in the art to determine the sequence of a known protein.” *Id.* ¶¶58-59.
- “By 2016 (the year of priority), it was well-established that the cDNA sequence encoding a protein of interest could be readily determined

using Rapid Amplification of cDNA Ends (RACE), if a partial sequence of the mRNA sequence encoding the protein was known (or if a partial sequence of the protein's amino acid sequence was known). In particular, it was common general knowledge that RACE (sometimes called one-sided PCR or anchored PCR)⁴ *‘provides an inexpensive and powerful tool to quickly obtain full-length cDNA when the sequence is only partially known. Starting with an mRNA mixture, gene specific primers generated from the known regions of the gene and non-specific anchors, full-length sequences can be identified in as little as 3 days. RACE can also be used to identify alternative transcripts of a gene when the partial or complete sequence of only one transcript is known’* (D11, abstract) (emphasis added).”¹⁶ *Id.* ¶¶62-63.

- RACE “can be used to obtain a full-length cDNA sequence within a matter of days... and thus effectively replaced the more time-intensive, traditional method of screening cDNA libraries in instances where partial sequence information is available. Indeed, and as may be taken from e.g., D14 (Frohman, 1994), it was common general knowledge

¹⁶ D11 is a chapter from Methods in Molecular Biology published in 2010.

EX2006 ¶4; EX2004 at 52-67.

that the use of RACE for determining full-length cDNA sequences was ‘advantageous for several reasons’.” *Id.* ¶68.

- “[T]here was ample information available regarding the sequence of *Limulus* Factor C for the skilled person to design ‘a single short’ primer for carrying out RACE in 2016. In particular, we refer to the following disclosures which provide such information, two of which constituted common general knowledge before 2016: (i) Genomic data for *Limulus polyphemus*, which was publicly available in the NCBI database as of January 3, 2014¹⁷ ... (iii) Partial amino acid sequences of the *Limulus* Factor C protein, which had been published already in **1991** (see **D22**, page 157, Figure 7S).”¹⁸ *Id.* ¶70.
- “Accordingly, the skilled person knew that: (a) partial sequence information was available for the *Limulus* Factor C protein; and (b) this information could be used to readily generate the full-length cDNA sequence within a matter of days using a commercially available RACE kit...” *Id.* ¶71.

¹⁷ In fact, genomic data for *Limulus polyphemus* was publicly available in the NCBI database as of at least October 31, 2013. EX2072; EX2073; EX2062 ¶¶35-37.

¹⁸ D22 is EX1025 in this proceeding. EX2006 ¶4.

- “In particular, and according to Professor Frohman, ‘any of the sources of information listed in (i)-(iii) above could have been used to design RACE primers... In his opinion, ‘*it would have been entirely straightforward for the skilled person to design RACE primers based on the available Limulus sequence information...and then use any one of the available RACE-PCR kits to obtain the full-length cDNA sequence*’”. *Id.* ¶72-73
- “... as Professor Frohman has confirmed, there was ‘*more than enough*’ sequence information available in 2016 to design RACE primers... Therefore, all the skilled person needed to derive the claimed sequences was a standard kit to carry out a common general knowledge technique, which had been around since 1988.” *Id.* ¶76.
- Accordingly, and as we have already noted above, the determination of the sequences [of *Limulus polyphemus* Factor C] is merely a matter of routine analysis, which does not require any inventive skill.” *Id.* ¶77.
- “Alternatively, the solution (the claimed sequences) can be derived

from **D23** or **D24** in an obvious manner.”¹⁹ *Id.* ¶79.

- “We submit that the skilled person merely had to repeat the example described in **D23** or **D24** with a cDNA library obtained from *Limulus* mRNA to arrive at the [*Limulus polyphemus* Factor C] sequences... Although this approach may have required more time than the RACE protocol described above, **it would have nevertheless merely involved routine work** and could have equally been used to determine the *Limulus* Factor C sequence.” *Id.* ¶86 (emphasis added).
- “According to Professor Frohman, the approach described in **D23** or **D24** would have been ‘*very straightforward*’ and the skilled person would have had ‘*every reason to believe that this approach would have been successful to obtain the Limulus Factor C cDNA. Given the success of obtaining the cDNA sequence of the other, closely related species, there would be no reason to expect any technical difficulties for repeating this protocol for Limulus*’...” *Id.* ¶87.
- “In view of the above, we submit that **D23** (or **D24**) provides a

¹⁹ D23 and D24 are references by Ding et al. that describe the cloning and cDNA sequence of *Carcinoscorpius rotundicauda*. EX2006 ¶¶4, 80-83. D23 is EX1009 and D24 is EX2012 in this proceeding.

straightforward roadmap that the skilled person could have followed with a reasonable expectation of success to arrive at the claimed sequences.” *Id.* ¶90.

- “In particular, **D6** already provides the skilled person with a means for determining the *Limulus* Factor C sequence in Example 11... Although **D6** does not disclose the *Limulus* Factor C amino acid sequence, it is nevertheless apparent in view of the above example that the authors had sequenced a sufficient level of coverage of this protein in order to determine that it shared ‘>90% sequence identity’ with the Asian species. It would thus be immediately apparent to the skilled person that the *Limulus* sequence could be directly obtained by performing tryptic peptide sequencing.” *Id.* ¶¶138-140.

Opponent’s characterization of D1, the ’767 Application, are particularly notable. Opponent argued that “**D1** already discloses ‘a recombinant Factor C protein derived from *Limulus polyphemus*...” EX2006 ¶155. In contrast here, Petitioner argues that “the ’109 patent inventors *did not and could not have described* an example of these proteins from *Limulus polyphemus*...” Pet. at 31. (emphasis added). Opponent also argued that by “reading the description of” the ’767 Application, a POSA “would not only be able to produce a *Limulus* Factor C without undue burden, but that they would further expect the invention of [the ’767

Application] to ‘*also work when Factor C is the Factor C of *Limulus polyphemus*.*’” EX2006 ¶50 (emphasis in original). In contrast here, Petitioner and its expert have argued that it would be nearly impossible to obtain the Factor C sequence, and that effort standing alone would constitute undue experimentation. Pet. at 31; EX2062 at 98, 183-184, 187.

Opponent’s arguments also undercut Petitioner’s reliance on the statements in the ’629 Publication and WO 2018/074498 (“PCT ’498”) (EX1015 and EX1005, respectively)²⁰ as evidence that it would have been a struggle to obtain *Limulus polyphemus* sequences. See Pet. at 31-32 and 42 (quoting and citing EX1015 and EX1005); EX1065 at 23-24. The Opponent ***criticized*** these same statements from the ’629 Publication as follows:

- “However, this statement [from par. [0012] of EX1005/EX1015] is very generic and entirely unsupported.” EX2006 ¶35.
- “Accordingly, and as we have already noted above, the mere statement in par. [0012] of the opposed patent, which alleges that the determination of this sequence ‘*could not be achieved by general techniques*’ is entirely unsupported.” *Id.* ¶75.
- “This further supports our position that the patent’s claim that the

²⁰ No U.S. patents (including the ’629 Publication) issued from PCT ’498.

sequence could not previously be determined is factually incorrect and that there were **no** unforeseen technical difficulties in determining the sequence.” EX2006 at 9, n.2. (discussing the same references as EX2048 and EX2047 in this proceeding).

Petitioner’s and Opponent’s double-talk extends to how it characterizes purported admissions from the inventors and Patent Owners. Petitioner characterized statements from the ’629 Publication as “the inventors’ own admission” of a struggle in the field to sequence and recombinantly produce *Limulus polyphemus* zymogen proteins. Pet. at 42. In contrast, Opponent asserted “it is apparent that [Patent Owner] believe[s] ‘*in view of the guidance in the specification; the high level of skill in the art; and the existing knowledge in the art a person skilled in the art would readily be able to generate and use the polypeptides that are encompassed by the claims*’, including a *Limulus* Factor C.” EX2006 ¶53. Opponent went on to argue “by the Proprietor’s own admission, the skilled person reading the disclosure of **D1** had a clear incentive and expectation of success for carrying out this specific mode of **D1**.” EX2006 ¶54.

In sum, the evidence indicates that Petitioner has advocated before the European Patent Office that the ’767 Application adequately describes and enables expression of recombinant *Limulus polyphemus* Factor C from mammalian cells as recited in the claims of the ’109 Patent. If Petitioner is indeed the Opponent, as the

evidence certainly suggests, the Board should not countenance this by granting the Petition based on arguments that Petitioner repudiated *less than two months* before filing the Petition.

Petitioner blatantly evades the critical question of its involvement in the Opposition. Answering this would unmask the gamesmanship it seeks to employ before this Panel and refute many of the positions it seeks to advance in this Petition.

VIII. CONCLUSION

Patent Owner requests the Board deny the Petition in its entirety.

Respectfully Submitted,

Dated: December 1, 2025

/s/ Alexander B. Stein
Alexander B. Stein, Reg. No. 71,397

CERTIFICATE OF COMPLIANCE

I, the undersigned, certify that the foregoing Patent Owner Response complies with the applicable type-volume limitations of 37 C.F.R. § 42.24(b)(2). Exclusive of the portions exempted by 37 C.F.R. § 42.24(a)(1), this Petition, including footnotes, contains 18,311 words, as counted by the word count function of Microsoft Word. This is less than the limit of 18,700 words as specified by 37 C.F.R. § 42.24(b)(2).

Respectfully Submitted,

Dated: December 1, 2025

/ Alexander B. Stein /
Alexander B. Stein, Reg. No. 71,397

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e)(4), lead counsel for Patent Owner hereby certifies that on December 1, 2025, a copy of this PATENT OWNER'S RESPONSE was served via the email correspondence address of record for Petitioner's counsel of record:

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