

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. IPR2025-00440

Patent No. 11,236,318

DECLARATION OF BRIAN C. SCHAEFER, PH.D.

TABLE OF CONTENTS

	Page
I. EDUCATION AND QUALIFICATIONS.....	1
II. MATERIALS REVIEWED	3
III. LEGAL PRINCIPLES.....	4
A. PRIORITY DATE	4
1. WRITTEN DESCRIPTION.....	4
2. ENABLEMENT.....	5
B. OBVIOUSNESS	6
IV. TECHNOLOGY BACKGROUND.....	8
A. HORSESHOE CRAB COAGULATION CASCADES	8
B. ISOLATION AND CHARACTERIZATION OF FACTOR C.....	10
C. LAL ASSAYS.....	18
V. OVERVIEW OF THE '318 PATENT	20
A. CHALLENGED CLAIMS	21
B. PROSECUTION HISTORY	21
C. PERSON OF ORDINARY SKILL IN THE ART.....	21
D. CLAIM INTERPRETATION.....	23
VI. CLAIM 21 IS ENTITLED TO ITS DECEMBER 10, 2013 EFFECTIVE FILING DATE	23
A. THE '767 APPLICATION PROVIDES SUFFICIENT WRITTEN DESCRIPTION SUPPORT FOR CLAIM 21 OF THE '318 PATENT.....	24
1. A POSA IN DECEMBER 2013 WOULD HAVE UNDERSTOOD THE INVENTORS POSSESSED THE SEQUENCES OF FACTOR C	27
2. A POSA IN DECEMBER 2013 WOULD HAVE UNDERSTOOD THE INVENTORS POSSESSED METHODS OF EXPRESSING FACTOR C IN HEK293 CELLS	37
3. FACTOR C PROTEINS ARE HIGHLY CONSERVED AMONG HORSESHOE CRAB SPECIES	39

4.	THE FIELD HAD NOT STRUGGLED TO SEQUENCE OR STRUCTURALLY OR FUNCTIONALLY CHARACTERIZE <i>LIMULUS POLYPHEMUS</i> FACTOR C	49
B.	CLAIM 21 IS ENABLED BY THE '767 APPLICATION AND KNOWLEDGE IN THE ART	53
1.	THE BREADTH OF THE CLAIMS (FACTOR 8).	53
2.	NATURE OF THE INVENTION, INFORMATION KNOWN IN THE PRIOR ART, AND UNPREDICTABILITY (FACTORS 4, 5, 7).	54
3.	THE REQUIRED LEVEL OF SKILL TO PRACTICE THE CHALLENGED CLAIMS (FACTOR 6)	55
4.	GUIDANCE, WORKING EXAMPLES, AND DEGREE OF EXPERIMENTATION (FACTORS 1-3).....	56
VII.	GROUND 1: CLAIM 21 IS NOT OBVIOUS OVER MIZUMURA IN VIEW OF THE '498 PCT	58
VIII.	GROUND 2: CLAIM 21 IS NOT OBVIOUS OVER MIZUMURA IN VIEW OF MCCLYMONT.....	61

I, Brian C. Schaefer, declare:

1. I have been retained by counsel for Seikagaku (“Patent Owner” or “SKK”) to provide expert testimony in the above captioned Patent Trial and Appeal Board proceeding brought by Charles River Laboratories, Inc. (“Petitioner” or “CRL”). I am being compensated at my standard hourly rate, and my compensation does not depend on the outcome of this proceeding.

I. EDUCATION AND QUALIFICATIONS

2. I received my Bachelor of Science degree in Biology in 1989 from Massachusetts Institute of Technology and my Ph.D. in Immunology in 1995 from Harvard University. I performed my postdoctoral fellowship from 1996 – 2002 at the National Jewish Center with Drs. Philippa Marrack and John Kappler, focusing on T cell biology and signal transduction.

3. I am currently Professor and Vice-Chair of the Department of Microbiology and Immunology at Uniformed Services University of the Health Sciences (USUHS) in Bethesda, MD. I have been Chair of the Faculty Advisory Committee for the USUHS Biomedical Instrumentation Center since 2009, overseeing the operations of the University core facilities that provide Genomics, Proteomics, Flow Cytometry and Microscopy services to the University Faculty. A copy of my complete curriculum vitae is included with this declaration.

4. My major research interest is in defining molecular mechanisms by which T cells successfully control pathogen infections. For the last decade, work in my lab has focused on generating novel approaches to combat infections with neurotropic viruses, a pathogen class for which no effective therapeutics currently exist. Previous research, during my training and early career, was focused on the molecular regulation of Epstein-Barr virus latency and cytoplasmic signaling mechanisms that connect activation of the T cell receptor to activation of the NF- κ B transcription factor.

5. My areas of expertise span molecular biology, immunology, virology and biochemistry. Throughout my career, I have had an interest in developing new methods and reagents and improving existing methods and reagents, publishing detailed protocols to enable other investigators to utilize cutting-edge techniques. The technical innovations that I have published include the creation of a novel family of selectable retroviral vectors; the generation of a transgenic mouse strain that ubiquitously expresses a variant of green fluorescent protein and is a go-to model for cell transplantation studies (this strain has been commercialized and the manuscript has been cited hundreds of times); a description of confocal microscopy methods to study signaling and molecular distribution events following T cell receptor activation; a patented method for immortalizing macrophages using

retroviral transduction; and a description of the use of bioluminescence imaging for longitudinal tracing of in vivo neurotropic virus infection.

6. With regard to molecular biology techniques, in 1995, I published a review in Analytical Biochemistry that provided a synthesis of the literature related to the Rapid Amplification of cDNA Ends (RACE) protocol, including a number of reported improvements to the originally-described method. This review also included a detailed protocol that incorporated the latest advances to the RACE methodology, along with an explanation of commonly encountered pitfalls, with a description of techniques to overcome such obstacles. This review has been cited hundreds of times.

7. A current version of my curriculum vitae is provided as Exhibit 2039.

II. MATERIALS REVIEWED

8. In providing my opinions, I have relied on my education and experience in the field, as well as the material cited herein. I have also reviewed the Petition; the Declaration of Tina Morris, Ph.D. provided as Exhibit 2042; the Declaration of Siddhartha Jain, Ph.D., PMP (EX1002); the deposition of Dr. Jain (EX1065); the exhibits cited within the Jain Declaration and the Petition (EXS1001, 1003-1039); Patent Owner's Preliminary Response; the exhibits cited as part of Patent Owner's Preliminary Response (EXS2001-2037); and the exhibits included as part of Patent Owner's Response.

III. LEGAL PRINCIPLES

9. In expressing my opinions in this declaration, I have relied upon certain legal principles that have been explained to me by counsel. I am not an attorney and do not offer any opinions as to the law.

A. Priority Date

10. I understand that in order to determine whether a reference qualifies as prior art to a challenged patent claim, one must first determine the priority date (or effective filing date) of the challenged patent claim. I understand that a patent claim may claim priority to earlier filed applications. I understand that in order for a patent claim to be entitled to the earlier priority claim, that earlier application must provide (i) written description support for the patent claim and (ii) enable a person of skill in the art to make and use the invention of the patent claim without undue experimentation.

1. Written Description

11. I understand that to satisfy the written description requirement, the patent's disclosure, including the specification, figures, and claims, must convey to persons of ordinary skill in the art that the inventor invented what is claimed. In other words, after an objective inquiry into the four corners of the patent document from the perspective of a person of ordinary skill, that person would recognize the inventor was in possession of the claimed subject matter as of the effective filing date of the patent.

12. I understand that a patent specification that renders the claimed invention obvious does not satisfy the written description requirement. I also understand that the disclosure needed to satisfy the written description requirement varies with the nature and scope of the invention at issue, the extent of scientific knowledge in the field, and the predictability of the aspect at issue. I also understand that the written description requirement does not demand working examples or actual reduction to practice. I also understand that the written description requirement can rely on what was known in the art such that the patent specification does not need to describe elements already well-known in the art.

2. Enablement

13. I understand the enablement requirement is separate from the written description requirement. To meet the enablement requirement, I understand that the patent's disclosure, including the specification, figures, and claims, must enable a person of ordinary skill in the art as of the effective filing date to make and use the claimed inventions without undue experimentation. I understand the question of undue experimentation is a matter of degree, i.e., that some reasonable amount of experimentation is permissible. I further understand that a considerable amount of experimentation is permissible if it is merely routine or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

14. I understand that factors to be considered and balanced in determining whether experimentation is undue, known as the *Wands* factors, include: (i) the nature of the claimed invention; (ii) the breadth of the patent claims; (iii) the relative skill of those in the art; (iv) the presence or absence of working examples in the patent specification; (v) the amount of guidance or direction presented in the patent specification; (vi) the predictability or unpredictability of the art; (vii) the state of the prior art; and (viii) the quantity of experimentation necessary to carry out the full scope of the claimed invention.

15. I understand that the specification need not teach, and preferably omits, what was well-known in the art. I further understand that enablement does not require the specification to describe how to make and use every possible embodiment of the claimed inventions because the knowledge in the art along with routine experimentation can fill perceived gaps. I understand that as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, this satisfies the enablement requirement.

B. Obviousness

16. I understand that a patent may be invalid for obviousness. I understand that this analysis must be from the perspective of a person of ordinary skill in the art (“POSA”), and whether such POSA would consider any differences between the

prior art and what is claimed to have been obvious. To make this assessment, I understand that the concept of patent obviousness involves four factual inquiries: the scope and content of the prior art; the differences between the claimed invention and the prior art; the level of ordinary skill in the art; and so-called secondary considerations of non-obviousness.

17. I further understand that one must consider what the person of ordinary skill in the art knew, and what the art taught; suggested; or motivated the person of ordinary skill in the art to further pursue; and to differentiate between steps that were routinely done (such as in response to known problems, steps, or obstacles), and those which, for example, may have represented a different way of solving existing or known problems. Further, I have been informed that one cannot use the challenged patent itself as a guide from which to select prior art elements or otherwise engage in hindsight.

18. I also understand that to demonstrate obviousness, Petitioner must show that a POSA would have been motivated to combine the teachings in the prior art and to modify the prior art to arrive at the claimed invention and must also show that there would have been a reasonable expectation of success in achieving the claimed invention.

19. I understand that before reaching any final conclusion on obviousness, the obviousness analysis requires consideration of objective indicia of non-

obviousness, if offered. These must be considered to ensure that, for example, there were not some unanticipated problems, obstacles, or hurdles that may seem easy to overcome in hindsight, but which were not readily overcome prior to the relevant invention date of the patents/claims at issue here. I understand that these objective indicia are also known as “secondary considerations of non-obviousness,” and may include unexpected results, among others.

IV. TECHNOLOGY BACKGROUND

A. Horseshoe Crab Coagulation Cascades

20. There are four identified living species of horseshoe crab: *Carcinoscorpius rotundicauda*, *Limulus polyphemus*, *Tachypleus gigas*, and *Tachypleus tridentatus*. EX2068 at 1-2. *Limulus* is primarily found along the east coast of North America while the *Carcinoscorpius* and *Tachypleus* species are found in Asia. *Id.* It was well-known that horseshoe crabs are primitive organisms that evolved slowly, hence their “living fossils” moniker. *See* EX2044 at 5 (“unusually low divergence rates in horseshoe crabs may be the relatively long generation time... [which] are expected to produce slower evolutionary rates.”); *see also* EX1038 at 6¹.

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

21. 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. EX2045 at 3. Endotoxin is a lipopolysaccharide (“LPS”) that is present in the cell envelope of Gram-negative bacteria. EX2069 at 4-5. In humans, exposure to endotoxin via injectable drugs, vaccines, or medical devices results in critical medical conditions, including sepsis, which can lead to organ failure and death. *Id.* at 5. Thus, detection of endotoxin is critical in pharmaceutical manufacturing. *Id.* The FDA has accepted the use of the LAL assay for endotoxins in lieu of the rabbit pyrogens test since 1977. EX2063 at 5.

22. The coagulation cascade utilized by the LAL assay is part of the horseshoe crab innate immune defense system and is critical for survival. EX2070 at 11. By 1992, the mechanism of the horseshoe crab coagulation cascade had largely been elucidated, and its enzyme components identified. *See* EX2046 at 2. 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 $\bar{B}$ then

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

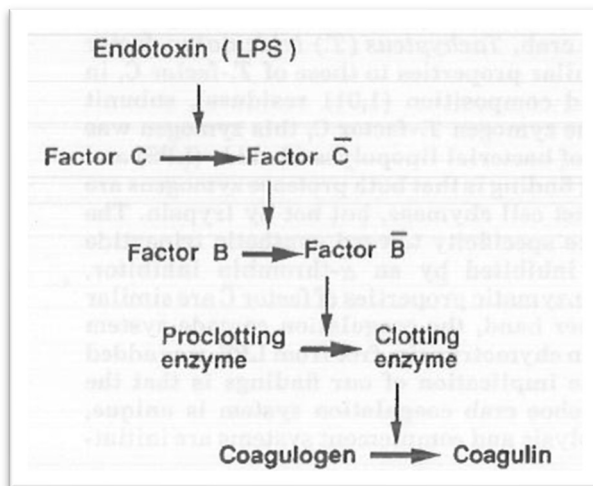


Figure 1. Mechanism for coagulation cascade of horseshoe crab hemocyte lysate. EX1022 at 151, Fig. 1.

23. Each of the four species of horseshoe crab utilizes the same coagulation cascade in response to endotoxin exposure. *See* EX1012 at 1:17-26, Fig. 1.

B. Isolation and Characterization of Factor C

24. Factor C is the first serine protease zymogen in the coagulation cascade of all known horseshoe crabs. The sequence and biochemical properties for Factor C in *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda* have been studied extensively. By December 2013, it was clear that Factor C was highly conserved and functionally identical across these species.

25. Factor C was first purified from *Tachypleus tridentatus* in 1986 by Nakamura et al. EX2047 at 1. Nakamura determined that Factor C is a glycoprotein consisting of two polypeptide chains—a heavy chain and a light chain. *Id.* at 7.

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. EX2048 at 1. Thus, Factor $\bar{C}$ consists of three chains—heavy chain, A chain, and B chain—linked via disulfide bonds.

26. In 1991, Muta et al. published the *Tachypleus tridentatus* Factor C cDNA sequence and amino acid sequence and characterized their domains. EX1007 at 6554. Muta determined that Factor C includes several domains: a signal peptide, a Cys-rich region, an EGF-like domain, five sushi domains², one lectin-like domain, one Pro-rich region which is located within the fifth sushi domain, and one serine protease domain. *Id.* at 6558. Figure 2 shows a schematic representation of these domains.

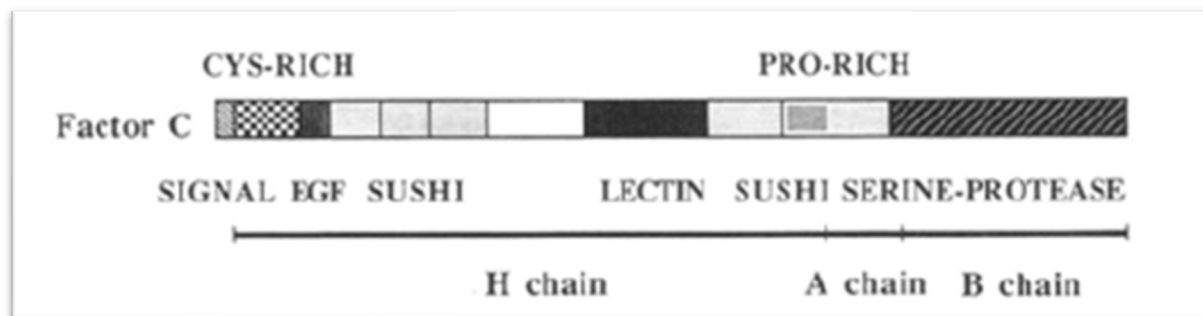


Figure 2. The domain structure of *Tachypleus tridentatus* Factor C. EX1007, Fig. 7.

² Sushi or short consensus repeat domains are found in many mammalian complement system proteins. EX1007 at Abstract.

27. Muta identified the catalytic triad of Factor C, which was that of “a typical serine protease,” reporting that Factor $\bar{C}$ “has a similar specificity toward synthetic fluorogenic peptide substrates as α -thrombin” and has a conserved substrate-binding site. EX1007 at 6557. Muta also identified that the sequence of Factor C was related to mammalian complement factors. *Id.* at 6557-6558 and Fig. 6.

28. In 1991, Tokunaga purified *Limulus polyphemus* Factor C (“LpFC”) from amoebocyte lysate. EX1022. 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.

29. Tokunaga conducted an amino acid sequence analysis comparing the sequence of *Limulus polyphemus* Factor C with *Tachypleus tridentatus* Factor C 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.

30. Importantly, the B chain of *Limulus* Factor C begins with an Ile (highlighted in yellow below), suggesting that, like *Tachypleus tridentatus* Factor C, it includes the Phe-Ile cleavage site between the A chain and B chain. Also, the A chain begins with a Ser, suggesting the same Arg-Ser cleavage site between the H chain and A chain (highlighted in green below).

7S		1	10	20
H chain	<i>T. t.</i>	R	G V D L G L C D E	T R F E C K C G D P -
	<i>L. p.</i>	X	G V D L G L C D D	T R F E X K C G D P -
		1	10	20
L and A chains	<i>T. t.</i>	S	S Q P S T V D L A	S K V K L P E G H Y -
	<i>L. p.</i>	S	A P P S T V D L S	S K V K L -
		1	10	20
B chain	<i>T. t.</i>	I	W N G N S T E I	G Q W P W Q A G I S R -
	<i>L. p.</i>	I	V N G L G L -	

Figure 3. Comparison of the N-terminal sequences of heavy, A chain, and B chain of LpFC and TtFC³. EX1022 at 157.

³ Post-priority date publications revealed that the sequences Tokunaga reported for the B chain of *Limulus polyphemus* contained discrepancies that **decreased** its apparent identity to the *Tachypleus tridentatus* sequence. The first 7 amino acids of the B chain are IVNGNST. See EX1052 at 18-24 (SEQ ID NOS: 2 and 4). Thus, the *Limulus polyphemus* Factor C includes the same glycosylation site and the exact same amino acid residues as *Tachypleus tridentatus* at these positions.

31. 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. EX2021 at Abstract.

32. In 1995, the cDNA sequence and amino acid sequence of *Carcinoscorpius rotundicauda* Factor C were published. EX2008 at 1. *Carcinoscorpius rotundicauda* Factor C, like the *Tachypleus tridentatus* Factor C, consists of a heavy chain and a light chain. *Id.* Upon activation by endotoxin, like *Tachypleus tridentatus* Factor C, it undergoes limited proteolysis of its light chain into A chain and B chain upon endotoxin activation. *Id.* at 5, Fig. 5.

33. As the 1995 Ding publication explained “an overall homology of 97.7% was observed in comparison with the *Tachypleus tridentatus* Factor C” and the *Carcinoscorpius rotundicauda* Factor C such that the “high degree of homology between Factor C from *T. tridentatus* and *C. rotundicauda* substantiates, at the molecular level, the proximity of these two species in the course of evolution.” EX2008 at 1. Both Factor C proteins include the same domains and importantly the

Tokunaga’s reported sequences for the H chain and L and A chains are a 100% match with SEQ ID NOS: 2 and 4. *Id.*

same Phe-Ile cleavage site between the A chain and B chain. EX2008 at 4; EX1012 at Example 6. Moreover, the *Carcinoscorpius rotundicauda* Factor C is, like *Tachypleus tridentatus* Factor C, catalytically similar to trypsin-type serine proteases. EX1012 at 14:66-15:62 (Example 7).

34. In 2003, the amino acid composition of *Limulus polyphemus* Factor C had been compared to the sequence of *Carcinoscorpius rotundicauda* Factor C. EX2035 at 21 ([¶[0134]) (“[t]ryptic peptide sequencing revealed that the Limulus factor C sequence closely matched the Asian species, with >90% sequence identity.”). Other publications disclosed similar information in 2006 and 2009. EX2036 at 4 (“the Factor C of *Carcinoscorpius rotundicauda* has 89.3% homology with that of *Limulus polyphemus*”);⁴ EX2049 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*.”).

35. 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

⁴ The authors defined “homology” to mean sequence identity in the preceding sentence. EX2036 at 4.

to have high identity to *Carcinoscorpius rotundicauda* Factor C, and by extension to *Tachypleus tridentatus* Factor C as well.

36. Although the full cDNA sequence of *Limulus polyphemus* Factor C was not published contemporaneously with the foregoing reports, 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 whole genome shotgun sequence of the *Limulus polyphemus* under BioProject accession number PRJNA178587.⁵ EX2051 (PRJNA178588); EX2050 (PRJNA178587).

⁵ 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.” EX2055. 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].

37. BioProject PRJNA178588 generated experimental dataset SRX202160 with run SRR610305 in the Sequence Read Archive (“SRA”). This data was published as of October 31, 2013. EX2051; EX2052 at 1; EX2053 at 1. The experimental datasets SRX202152 and SRX202154 generated by the whole genome shotgun sequencing project, PRJNA178587 was also published as of the same date. EX2050; EX2054. As of December 10, 2013, a keyword search for “limulus polyphemus” on NCBI’s BioProject or SRA databases would have found these BioProjects or their associated sequence deposits. EX2057.

38. 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.⁶ As discussed further below, a

Typically, a BioProject is registered first or during the submission of a genome assembly being submitted to WGS.” *Id.*

⁶ BLAST was first introduced in 1990. EX2056 at 5. BLAST finds regions of similarity between biological sequences, identifies members of gene families, and shows evolutionary relationships between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. BLAST can be used to compare sequences between

POSA could have assembled the large majority of the cDNA sequences of Factor C from the transcriptome deposit using these well-established methods.

C. LAL Assays

39. 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. EX2045 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. EX2070 at 12-13.

40. 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 2:14-23. There was also a desire for endotoxin specific assays and to reduce the potential for lot-to-lot variations in amoebocyte lysate-based assays. EX2036 at 2; EX2070 at 12. In

species or search for similar sequences regardless of species. BLAST allows a user to select a specific organism or deposited sequence listing and compare it with the known sequence. CD-Search searches a comprehensive collection of domain models to identify conserved domains within a queried sequence and was available by 2004. EX2058.

2012, FDA withdrew its 1987 Guidance and issued a new one. EX2063 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.

41. A commercial product employing recombinant *Carcinoscorpius rotundicauda* Factor C was commercially launched in 2004. EX2070 at 16 (discussing PyroGene). Other products using *Tachypleus tridentatus* Factor C followed. EX2062 at 5. The difference in the pathways of the amoebocyte-based LAL assay and the recombinant Factor C assay is shown below:

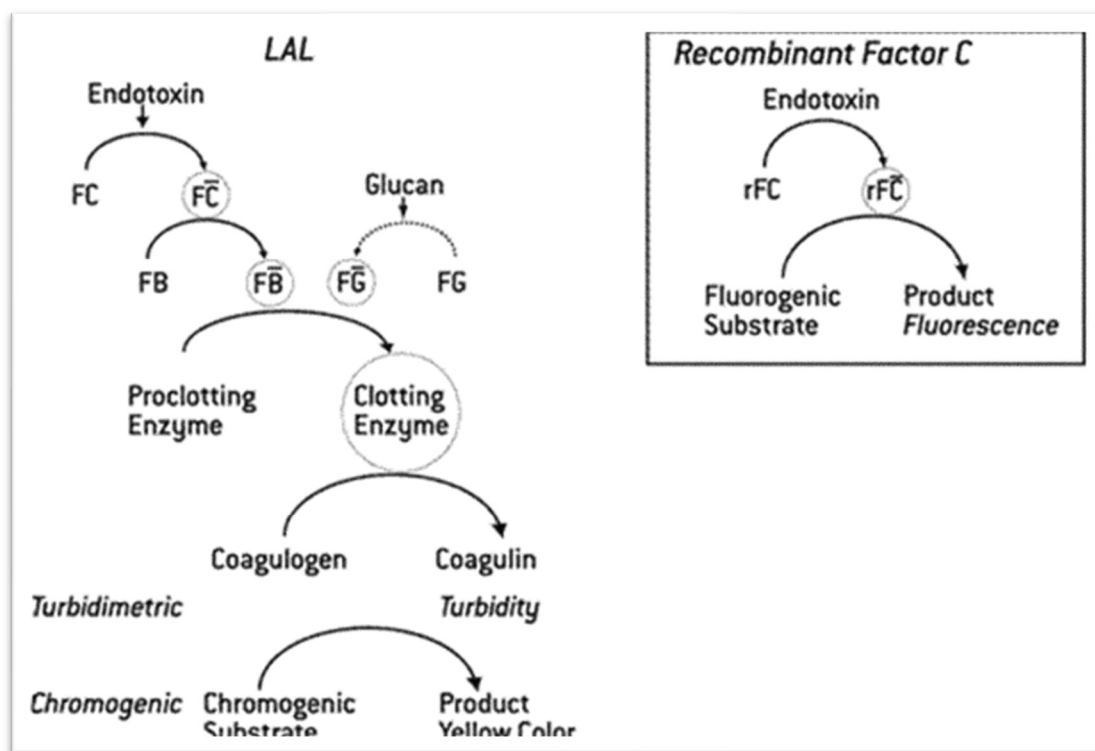


Figure 4: Reactions involved in the LAL and recombinant Factor C assays. EX2049 at 2, Fig. 1

42. 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. EX2074 at 35-38 ([0171]-[0182]); EX2072 at 4 ([0009]); EX2062 at 4. Assays using recombinant Factor C were found to yield comparable results to native lysates. *See* EX2036 at 4; EX2075 at 6; *see also* EX2062 at 5-8 (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 specific of LAL with respect to beta glucans.”).

V. OVERVIEW OF THE '318 PATENT

43. The '318 patent was filed as U.S. Patent Application No. 16/399,386 on April 30, 2019. EX1001 at 1. I have been informed that Patent Owner claims priority through a series of continuation applications to December 10, 2013, the filing date of International Patent Application No. PCT/JP2013/083082, also assigned U.S. Application No. 14/650,767 (“the '767 Application”). Dr. Jain agrees that the disclosure of these two applications is identical, but for a paragraph just before Paragraph 19 of the '767 Application. EX1002 ¶ 64. This additional paragraph described information that was already known in the prior art as of December 10, 2013. EX1065 at 154-155. Dr. Jain also states that the '767 Application is “identical” to the '318 patent specification.” EX1002 ¶67.

A. Challenged Claims

44. I understand claim 21 (“Challenged Claim”) of the ’318 Patent is at issue. Claim 21 recites:

A horseshoe crab Factor C protein having activity of Factor C,
wherein the horseshoe crab is *Limulus polyphemus*, and wherein
the horseshoe crab Factor C protein is produced through being
recombinantly expressed from a Human Embryonic Kidney (HEK) 293
cell, and

wherein the Factor C protein exhibits a residual activity of at
least one selected from the group consisting of:

- (1) 10% or higher in the presence of 21 mM sodium citrate; and
- (2) 15% or higher in the presence of 16 mM magnesium sulfate.

EX1001 at cl. 21.

B. Prosecution History

45. I have reviewed the prosecution history of U.S. application no. 16/399,386, which issued as the ’318 Patent. EX1001. I have also reviewed Dr. Jain’s summary of the prosecution history. EX1002 at ¶¶50-60.

C. Person of Ordinary Skill in the Art

46. I understand that 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.

47. The field of the '318 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. I understand inventor Shun-ichiro Kawabata has a Ph.D. in biology and experience in biochemistry. EX2065.

48. In my opinion, determining the gene and amino acid sequences of proteins, expressing them in various types of host cells, and characterizing their properties were the type of problems encountered in the art, which were solved using molecular biology techniques. Thus, experience and knowledge in biochemistry and molecular biology was essential to those working in the field. Education and experience in biological, chemical, or biochemical engineering, which to my understanding relate to developing industrial scale processes for protein production, is outside of the field and not required.

49. Accordingly, I disagree with Dr. Jain's inclusion of these disciplines in his definition of a POSA. Instead of Dr. Jain's definition, in my opinion, a POSA would have a Ph.D. in molecular biology, biochemistry, or enzymology and at least two years of experience with cloning genes, expressing cloned genes as proteins, and characterizing them. I understand a POSA may collaborate with others, including those with specific experience in conducting structure-function analyses of enzyme function, evaluating the activity of horseshoe crab zymogen proteins

individually, or in a cascade reaction for endotoxin detection and quantitation. My opinions remain the same regardless of the definition of a POSA that is adopted, and I was a POSA under both parties' definitions as of at least December 2013.

D. Claim Interpretation

50. I understand that claim terms are given their ordinary and customary meaning to a person of ordinary skill in the art, consistent with the use of the claim term in the patent disclosure, unless the specification clearly sets forth a different definition of the term. I have applied the plain and ordinary meaning of the claim terms to my analysis.

VI. CLAIM 21 IS ENTITLED TO ITS DECEMBER 10, 2013 EFFECTIVE FILING DATE

51. Dr. Jain asserts that claim 21 is entitled to an effective filing date no earlier than the April 30, 2019, which is the filing date of the '318 Patent because the earlier applications lack written description support and do not enable the full scope of the claim. EX1002 at Section VI. I disagree for the reasons discussed below. I note that Dr. Jain analyzed U.S. Patent Application No. 14/650,767 (EX1006) when assessing whether claim 21 is entitled to the priority date of December 10, 2013 and states that the '767 Application is "representative of each disclosure (including the '840 JP Priority Application) in the priority chain extending back to December 10, 2012." EX1002 ¶ 75. I therefore analyze the '767

Application for written description support and enablement of claim 21 of the '318 Patent.

52. In my opinion, claim 21 of the '318 Patent is entitled to a December 10, 2013 priority date because the disclosures in the '767 Application coupled with what was well understood in the art to a POSA provides written description and enablement support for the full scope of claim 21.

A. The '767 Application Provides Sufficient Written Description Support for Claim 21 of the '318 Patent

53. Dr. Jain asserts that the '767 Application lacks written description support for claim 21. EX1002 ¶ 76. I disagree.

54. Looking first at the disclosure in the '767 Application itself, I note that there is discussion within the specification about *Limulus polyphemus* Factor C. For example, the “Background Art” section states “it has been known that the American horseshoe crab (*Limulus polyphemus*) undergoes blood clotting when it is infected with a Gram-negative bacterium.” EX1006 at 12 [0003]. 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. EX1006 at 98 ([0156]).

55. In the '767 Application, there is a section titled “***Modes for Carrying Out the Invention***” that states the “Factor C of the present invention is a ***Factor C derived from a horseshoe crab***” and specifically identifies *Limulus polyphemus* as

a known species. EX1006 at 40. In my opinion, a POSA would have understood that the inventors considered the *Limulus polyphemus* Factor C to be a part of their invention.

56. The '767 Application further includes an example of “Expression of Factor C in mammalian cells (HEK 293).” EX1006 at 87. This section describes steps to produce Factor C using HEK293 cells as the host cell, which is the same host cell recited in claim 21. *Id.* In my opinion, a POSA would have understood that the inventors considered expressing Factor C in HEK293 cells to be part of the invention.

57. The '767 Application also includes an example titled “Inhibition of activity by injection” that details measuring the residual activity of Factor C in the salt solutions, including the claimed 21 mM sodium citrate and 16 mM magnesium sulfate. EX1006 at 96-99. The '767 Application further details the procedure used for this measurement in Figure 4 and provides results in Figure 7 that show that expression of Factor C in HEK293 cells resulted in the claimed residual activity of 10% or higher in the presence of 21 mM sodium citrate and 15% or higher in the presence of 16 mM magnesium sulfate. *Id.* at 96-99, 119. As seen in Figure 7, the expression of Factor C in HEK293 cells also exhibited higher residual activity as compared to Factor C expressed in Sf9 cells and native Factor C (TAL) in the claimed salt solutions.

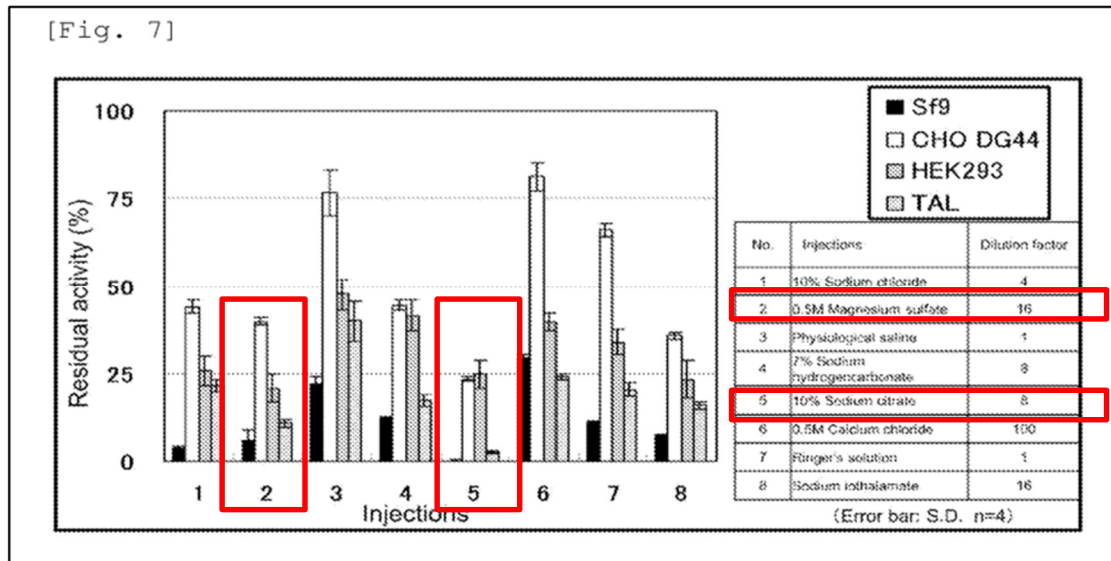


Figure 5. EX 1006 at 122 (annotated)

58. The '767 Application further notes that the “present inventors have found that a recombinant horseshoe crab Factor C can be produced by use of human cells . . . and that endotoxin can be measured by use of the thus-produced recombinant Factor C while reaction inhibition in the presence of a salt (ion) is mitigated.” EX1006 at 16 [0016]. The '767 Application continues, stating the “present invention encompasses the following modes” and then lists a number of modes, including recombinantly produced in HEK293 cells (i) Factor C exhibiting a residual activity of 10% or higher in the presence of 21 mM sodium citrate; (ii) Factor C exhibiting a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate; (iii) Factor C exhibiting a higher residual activity in the presence of 21 mM sodium citrate as compared to Factor C expressed in Sf9 cells and/or a native Factor C; and (iv) Factor C exhibiting a higher residual activity in the presence

of 16 mM magnesium sulfate as compared to Factor C expressed in Sf9 cells and/or a native Factor C. *Id.* at 16-38 [0017].

59. In my opinion, this demonstrates to a POSA that the inventors considered Factor C from *Limulus polyphemus* recombinantly expressed in HEK293 cells and exhibiting the claimed residual activities as part of their invention. In my opinion, the inventors also considered as part of their invention that the recombinantly produced Factor C would exhibit a higher residual activity in the presence of the claimed salt solutions as compared to the residual activity of Factor C expressed in Sf9 cells and native Factor C.

60. While Dr. Jain points to the lack of specific examples of recombinant Factor C from *Limulus polyphemus* in the '767 Application, I understand from counsel that specific examples are not required to show written description support. I also understand that when assessing written description support, what was known in the art must be considered because the specification must be assessed from the perspective of a POSA.

1. A POSA in December 2013 would have understood the inventors possessed the sequences of Factor C

61. The '767 Application discloses the sequence of Factor C of *Tachypleus tridentatus*. As known in the art before 2013, “the individual amino acid compositions of the two proteins [*Tachypleus tridentatus* and *Limulus polyphemus*] and of their chain subunits were indistinguishable.” EX1022 at 151. As discussed

above, the NCBI BioProject database contains two BioProjects registered by the University of Connecticut for *Limulus polyphemus*. Number PRJNA178588 is for a cDNA amoebocyte transcriptome, which generated experimental dataset SRX202160 based on run SRR610305. EX2051; EX2052; EX2053. The other BioProject, number PRJNA178587 was a whole genome shotgun sequencing project, which generated experimental datasets SRX202152 and SRX202154. EX2050; EX2054. The sequence data deposited in connection with these two BioProjects was published by October 31, 2013. EX2052; EX2053; EX 2054; EX2057.

62. I understand there is a presumption that a POSA would have been aware of and had access to these data deposits prior to December 10, 2013.

63. The 2013 deposits contain short and intermediate sequence fragments or reads generated from *Limulus polyphemus* amoebocyte RNA or whole genome DNA. To confirm one or both of these 2013 deposits contained the sequence of Factor C, a POSA would have used the *Tachypleus tridentatus* sequence disclosed in the '767 Application as an input for the BLAST tool to find similar sequences in the deposited data. Alternatively, or in combination, a POSA would have used the CD-Search tool and publications to identify conserved domains in *Tachypleus tridentatus* Factor C and then searched the deposit data for the same conserved domains using BLAST. Either of these approaches would find the corresponding

sequences within the transcriptome for Factor C, from which a nearly complete open reading frame of the gene in question would be assembled.

64. Given the knowledge of the slow evolutionary rate of horseshoe crabs and the consequent assumption that the clotting cascade proteins would be highly conserved, I used the TBLASTN function to query the SRX202160 library of cDNA reads, using the full protein sequences from *Tachypleus tridentatus* Factor C. I confirmed that 97.2% of the full Factor C coding sequence could be assembled from the transcriptome deposit (a segment of 29 amino acids from residues 516-543 was not covered by any sequence reads in the BLAST alignment). However, this sequence could be easily recovered by standard PCR of *Limulus polyphemus* cDNA using primers complementary to sequences from the reads on each side of this gap; specifically, using reads SRR610305.117350.2 at the 5' end of the gap and SRR610305.219525.2 at the 3' end of the gap. Also, final validation of the assembled complete sequences for Factor C would be performed by PCR and sequencing of the entire ORF for Factor C. Design of the required primers would be readily enabled by analysis of the BioProject sequencing data, as described above.

65. A list of the top BLAST hits in the SRR610305 transcriptome data when queried with the *Tachypleus tridentatus* amino acid sequence of Factor C is provided in Exhibit 2098. Graphical depictions of how reads within the transcriptome data align with the query sequence are shown below. Notably, most

reads yield high scoring matches to the query sequence, indicating high similarity between the *Tachyplesus tridentatus* protein sequence and the translated sequences from the *Limulus polyphemus* clones. Also, nearly the entire Factor C coding region is confirmed by multiple clones, yielding high confidence in the resulting assembled sequences of *Limulus polyphemus* Factor C.

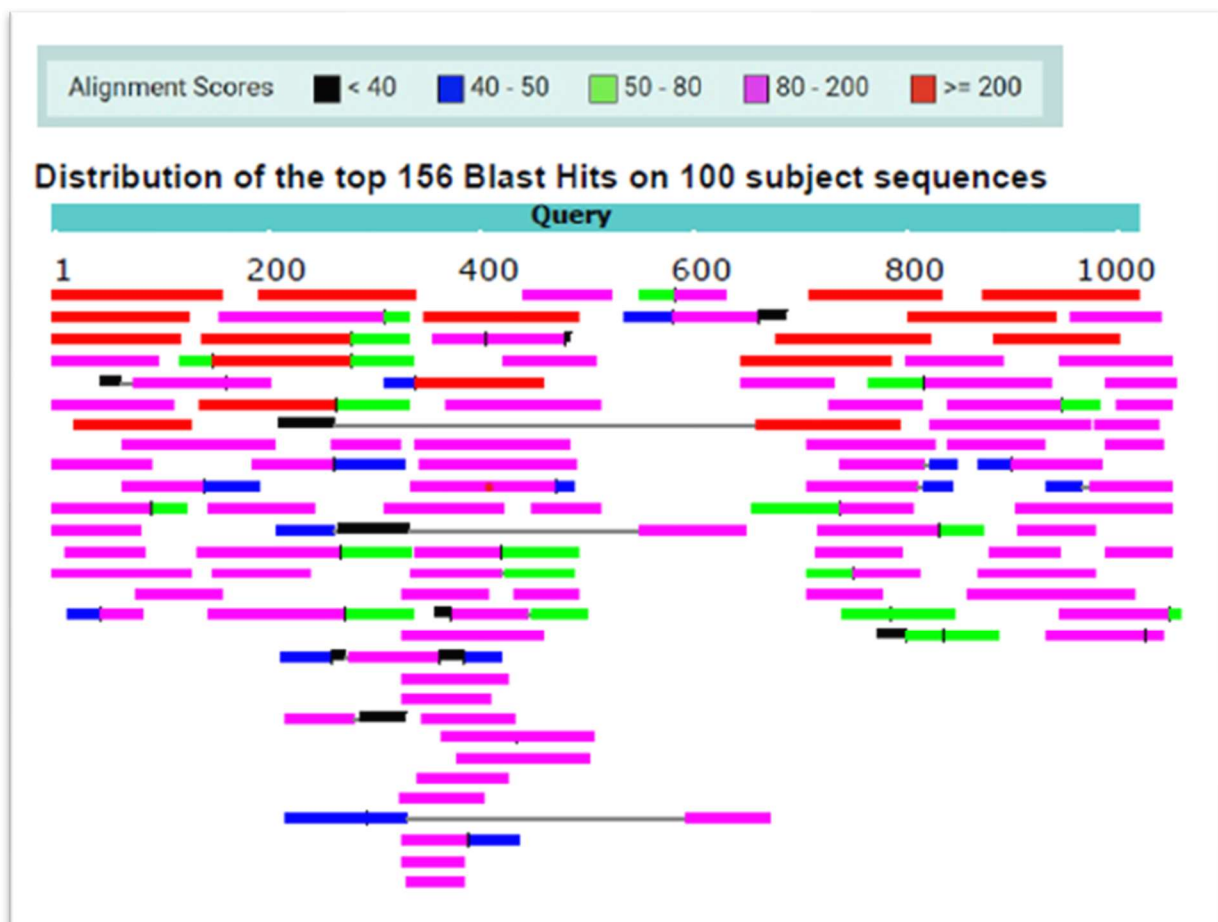


Figure 6: Distribution of the top 156 BLAST hits (line segments below numbers) for *T. tridentatus* Factor C (the query, in teal, above numbers).

66. A POSA in December 2013 would have understood that the inventors would have been able to do the same analysis to identify the cDNA sequences of *Limulus polyphemus* Factor C.

67. Alternatively, a POSA in December 2013 would have been able to isolate the sequences for *Limulus polyphemus* Factor C by using a technique called RACE-PCR (for rapid amplification of cDNA ends) or a similar technique. As I wrote in 1995, these methods may be used to “rapidly generate full-length cDNA clones after a partial cDNA has been obtained from a cDNA library.” EX2066. The RACE-PCR method was first described by Frohman *et al.* in 1988. *Id.* at 256; EX2004 ¶8. I described how to modify and optimize this method in 1995. EX2066. RACE-PCR was so commonplace that kits were available for purchase. EX2004 at 103-179.

68. I reviewed a November 24, 2024 declaration by Michael A. Frohman, MD, PhD, the author of the first publication describing the RACE-PCR technique. EX2004. I understand Professor Frohman was offering opinions as of October 18, 2016 concerning one of SKK’s European patents. EX2004 ¶3. I understand that the claim of that European patent involves expressing recombinant Factor C from *Limulus polyphemus*, thus it overlaps in subject matter with the claims of the ’318 Patent. EX2004 ¶4; EX1065 at 67.

69. Other than the opinions expressly based on the predicted Factor C sequence published on September 9, 2015 (EX2004 ¶6 (citing D18 and D20) and 180-184 (D18), 206-212 (D20)), Prof. Frohman's opinions apply equally to the capabilities of a POSA as of December 10, 2013. I generally agree with Prof. Frohman's opinions. For example, I agree with Prof. Frohman's statements in paragraph 6 of his declaration, except that in addition to the NCBI entry he references (EX2004 at 180-184), amoebocyte transcriptome as well as whole genome data for *Limulus polyphemus* was publicly available in the NCBI database as of October 31, 2013 (see discussion of NCBI BioProjects above).

70. Prof. Frohman's opinion that the genomic data "provided more than enough information to obtain the single, short gene-specific primer minimally needed to carry out RACE" (EX2004 ¶17) holds true when applied to the sequence deposits associated with BioProjects PRJNA178587 and PRJNA178588 published on October 31, 2013. Moreover, the combination of available protein sequences from *Limulus polyphemus* in combination with alignment with the complete sequences of Factor C from *Carcinoscorpius rotundicauda* and *Tachyplesus tridentatus* would have provided adequate information to successfully use a combination of degenerate primers and the RACE-PCR method to isolate the complete *Limulus polyphemus* Factor C cDNA.

71. Prof. Frohman states that “RACE can be used to obtain a full-length cDNA sequence in a matter of days.” EX2004 ¶8. I agree. Prof. Frohman opines that “[i]n 2016, the skilled person would have most likely used [RACE-PCR] to obtain the sequence of the cDNA encoding Factor C from *Limulus polyphemus*”; “[i]t would have been entirely straightforward for the skilled person to design RACE primers based on the available *Limulus* sequence information,” including EX1022 (cited as D22 by Prof. Frohman) “and then use any of the available RACE-PCR kits to obtain the full-length cDNA sequence.” EX2004 ¶¶ 6, 7, 10, 30-33. I agree. Prof. Frohman’s description of how to analyze the genomic DNA and design primers based on the January 3, 2014 deposit (EX2004 ¶¶18-26) applies equally to the 2013 deposits by the University of Connecticut. That being said, the general completeness of the assembled clones using the University of Connecticut cDNA deposit would mean that standard PCR, using these sequence reads as the basis for primer design, would suffice for amplification of the complete open reading frame of *Limulus polyphemus* Factor C.

72. It is further my opinion that carrying out a RACE protocol to obtain the cDNA sequence of *Limulus polyphemus* Factor C would have been equally likely, straightforward, and rapid for a POSA as of December 10, 2013 using the cDNA amoebocyte transcriptome data deposited by the University of Connecticut. I have not queried the whole genome data deposited by that institution, but based on Prof.

Frohman's analysis of the January 2014 deposit by Washington University of St. Louis, I expect that it would allow me to assemble the complete or nearly complete coding sequence for Factor C. EX2004 ¶¶18-19. The analysis required to re-assemble the sequence of Factor C from amoebocyte transcriptome data, which contains cDNA sequences only, would be significantly less complex than starting from whole genome data, which contains introns and other non-coding sequences.

73. It is further my opinion that the sequence of *Limulus polyphemus* Factor C could also have been obtained by at least December 10, 2013 using a RACE method based on the sequence information in the '767 Application or other publications describing the *Tachypleus tridentatus* sequences, in combination with the amino acid sequences from *Limulus polyphemus*. EX1022; EX2077; EX2078. I agree with Prof. Frohman that success does not depend on the specific protocol or kit used. EX2004 ¶¶34-37.

74. Using only the identified amino acid sequences of *Limulus polyphemus* Factor C, a POSA electing to use the RACE method could have followed any of a number of published protocols and/or purchased any of a number of commercially available kits. The initial step would have been the design of one or two gene-specific primers. One primer is generally adequate for RACE-cloning of the cDNAs of high-abundance transcripts. However, for low-abundance transcripts, competition from spurious, non-specific PCR products often represents a significant

problem. In the case of low abundance transcripts, such as the Factor C mRNAs in horseshoe crab species, the use of two gene-specific primers would be recommended. Thus, a POSA would design two nested primers to account for this possibility.

75. The peptide sequences reported by Tokunaga (EX1022) suggest that two nested gene-specific primers could have been designed for the region of both the N-terminus of the heavy chain (identical to the N-terminus of the full Factor C zymogen) and the N-terminus of the light chain. More specifically, 14 contiguous amino acids (=42 nucleotide bases) were identified for the N-terminus of the heavy chain and 15 contiguous amino acids (=45 nucleotide bases) were identified for the light chain. Highly specific PCR primers require only 17-18 nucleotides of known sequence, thus meaning that two nested gene-specific primers could have been accommodated at each of these regions of the *L. polyphemus* sequence.

76. A POSA could elect either to make the gene-specific primers degenerate to account for each of the possible nucleotide positions in the “wobble” (3rd) codon position for each amino acid, or a POSA could elect to use the known nucleotide sequences from *Tachypleus tridentatus* and/or *Carcinoscorpius rotundicauda*; or a POSA could use a hybrid of these approaches. For the sake of reducing the overall primer degeneracy, a POSA would most likely choose the hybrid approach, introducing degeneracy only (i) where the amino acid sequence of

Limulus polyphemus and *Tachypleus tridentatus* differ, and (ii) at wobble positions in the five most 3' bases of each primer sequence, where sequence identity is most important for sequence-specific annealing of PCR primers.

77. Moreover, prior to initiating the RACE protocol, a POSA would likely attempt to clone the majority of the *Limulus polyphemus* Factor C by simply using nested primers complementary to the identified N-terminus of the heavy chain and the identified N-terminus of the light chain from Fig 7S of Tokunaga et al (EX1022). If successful, the resulting clone would represent the sequence of the entire heavy chain, except for the most N-terminal portion of the coding sequence (i.e., the few amino acids not successfully identified at the N-terminus of the heavy chain in Tokunaga et al.). 5' RACE would then be used to identify the remaining portion of the N-terminus of the heavy chain. 3' RACE would be used to identify the C-terminus of Factor C, including the light chain and B chain sequences.

78. In the unlikely event that the nested primer-based PCR cloning of the heavy chain was not successful, the RACE approach could be used for the entire Factor C sequence. A POSA would most likely use both of the above peptide sequences for 5' RACE and 3' RACE. More specifically, nested forward and reverse primers would be designed to perform 5' RACE and 3' RACE with gene specific primers located in each region, in order to maximize the probability of success.

79. Upon identification of the full open reading frame (ORF) of Factor C via one of the above strategies, a POSA would then assemble a clone containing the full Factor C ORF using standard PCR and/or restriction enzyme-based cloning methods. The complete ORF would be cloned into an appropriate vector for expression in insect or mammalian cells.

80. I agree with Prof. Frohman, that as an alternative to relying on the sequence information in Tokunaga 1991 (EX1022), those working in the field in October 2016 could have followed the “very straightforward” protocol for screening a λ phage library that was used to determine the cDNA sequence of *Carcinoscorpius rotundicauda* Factor C to successfully obtain the *Limulus polyphemus* Factor C cDNA. EX2004 ¶¶11-12, 14-16 (citing D23 and D24); *id.* at 221-296 (D23), 227-310 (D24), and this was true as for POSAs as of December 10, 2013.

81. I agree with Dr. Frohman that “there would be no reason to expect any technical difficulties for repeating” the library screening “protocol for *Limulus*” in October 2016 (EX2004 ¶14), and this was true as for POSAs as of December 10, 2013 as well.

2. A POSA in December 2013 would have understood the inventors possessed methods of expressing Factor C in HEK293 cells

82. With respect to expression of Factor C in HEK293 cells, the '767 Application discloses expression of functional Factor C protein in HEK293 cells.

EX1006 at 85-88 ([0107]-[115]). Prof. Frohman noted these disclosures (EX2004 ¶45⁷) and concluded that “[g]iven that these cells were able to incorporate vectors containing cDNA for *T. tridentatus* and/or *C. rotundicauda* and express the Factor C-encoding mRNAs and active Factor C proteins, there’s no reason to think that they would not be equally capable of handling the *Limulus* sequence.” EX2004 ¶46. Prof. Frohman continued, “If I were to follow the example of [the ’767 Application] using the *Limulus* sequence, I would start with the cells exemplified in this disclosure and would not anticipate experiencing any difficulties.” *Id.* ¶47. Thus, Prof. Frohman’s opinion is that those working in the field would have expected to be able to recombinantly express *Limulus* Factor C in at least CHO DG44 and HEK cells in view of the ’767 Application.

83. I agree with Prof. Frohman. Further, it is my opinion that POSAs as of December 10, 2013 would have had the same expectation and would have understood from the specification that the inventors possessed methods of recombinantly expressing *Limulus polyphemus* Factor C in HEK293 cells.

84. Thus, it is my opinion that a POSA in December 2013 would have understood from the ’767 Application that the inventors possessed methods of

⁷ Prof. Frohman’s reference D1 is EP 2930241A1, which has the same disclosure as the ’767 Application. *See* EX2006 ¶51.

recombinantly expressing Factor C in mammalian cells, including the claimed HEK293 cells in claim 21 of the '318 Patent.

3. Factor C Proteins Are Highly Conserved Among Horseshoe Crab Species

85. Dr. Jain opines that a “POSA would not expect as default that [the '767 Application's] protein expression protocols would yield recombinant proteins in soluble and functional form from a *different* species of crab. EX1002 ¶82. I disagree. As discussed above, it was well-known that horseshoe crabs evolve slowly. *See* EX2044 at 5 (“unusually low divergence rates in horseshoe crabs may be the relatively long generation time... [which] are expected to produce slower evolutionary rates.”). The coagulation cascade is a key component of the horseshoe crab innate immune defense system, which is critical for survival. EX2070 at 11. It is widely understood that genes that are critical for survival are generally protected from functionally detrimental mutations by “purifying selection.” *See, e.g.,* EX2078 at 6. In other words, the effect of purifying selection is to slow the rate of mutation of essential genes and functional regions, such as the genes of the horseshoe crab clotting cascade. The expectation would thus be that key regions encoding functional units of each enzyme and motifs for interactions between partner proteins would be highly conserved across species, due to their required function for survival of individuals and the species.

86. Further, despite the geographical and general physical or genetic differences across the species, it was known as of December 2013 that the cascade reaction in each species involved the same set of three zymogen proteins (Factor C, Factor B, and Pro-clotting enzyme), and that these proteins were structurally similar and functionally indistinguishable across species. *See, e.g.*, EX1022 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.”); EX2069 at 2 (stating that LAL [*Limulus* amebocyte lysate] is commercially manufactured from *Limulus polyphemus* and *Tachypleus tridentatus* amebocytes and “can also be obtained from the other two species”) and 4 (Fig. 2 describing “Consensus biochemistry of the LAL reaction”).

87. Further, it was known that all three zymogen proteins are serine proteases, and their cleavage/activation site and catalytic triad and substrate binding sites had been characterized in the *Tachypleus tridentatus* Factor C and *Limulus polyphemus* Factor C. EX1007 at 6556 (Fig. 2), 6557; EX1022 at 150-151; EX1016 at 112-115 and Fig. 3 (reproduced below).

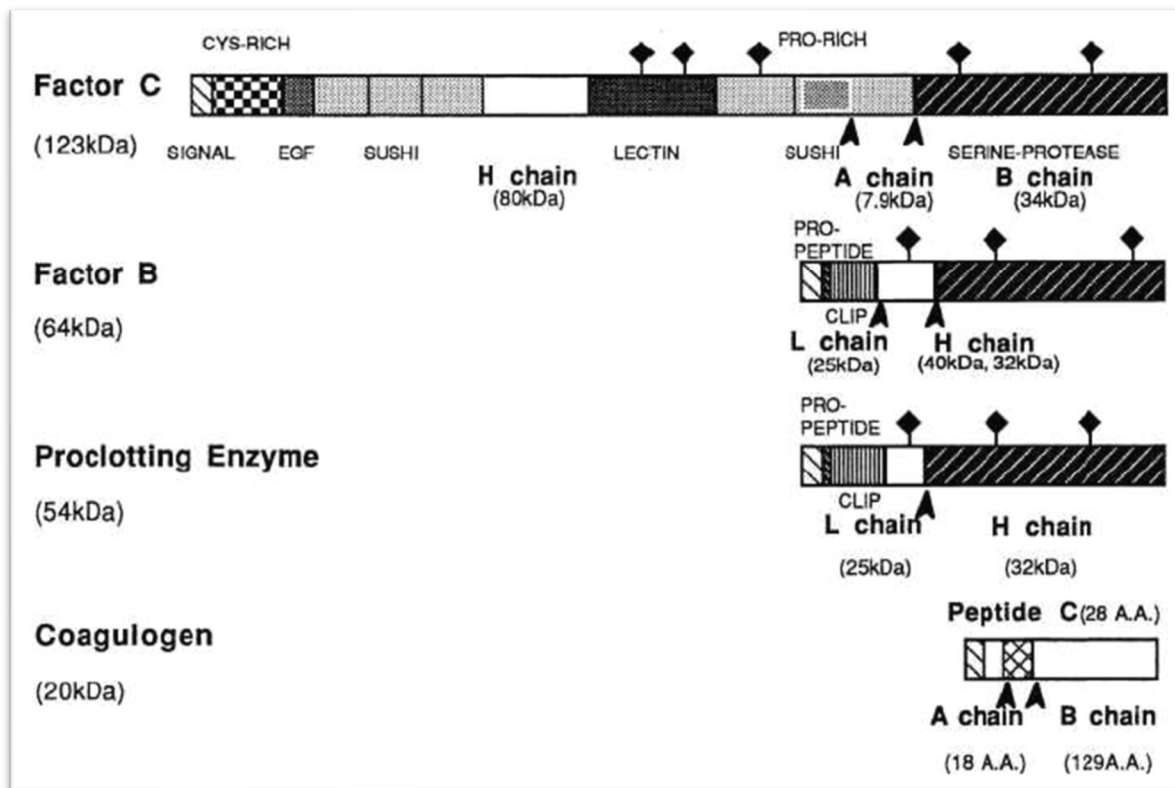


Figure 7: Domain structure of *limulus* clotting factors. EX1016, Fig. 3. Arrowheads indicate cleavage sites for zymogen activation; diamonds indicate potential glycosylation sites.

88. A POSA in December 2013 would have expected that, because the structure and biochemistry of Factor C was highly similar across horseshoe crab species, it would be similar in sequence across species, even if their sequences had not been published in full. A POSA would have expected with a high degree of certainty that any sequence differences would not affect their function within the cascade. Further, a POSA would have expected the protease function of these proteins to be robust, like other members of the trypsin family. *See* EX1007 at 6558 (Fig. 6); EX2070 at 11 (Factor C C-terminal serine protease domain has substantial

similarities with human alpha-thrombin); EX2080 at 2 (Fig. 1 – showing Factor C in chymotrypsin family); EX2046 at 17-18.

89. I further note that Dr. Jain fails to actually compare the sequences of *Carcinoscorpius rotundicauda*, *Tachypleus Tridentatus*, and *Limulus polyphemus* Factor C to determine whether a POSA would expect *Limulus polyphemus* Factor C to have the same functional properties, including maintaining the desired residual activity in salt solutions, across the various species. Finally, Dr. Jain ignores the vast research conducted by December 2013 using *Limulus polyphemus* Factor C in LAL assays and demonstrating its functional equivalence to *Tachypleus tridentatus* Factor C.

90. A POSA in December 2013 would have known that Factor C proteins from *Carcinoscorpius rotundicauda*, *Tachypleus tridentatus* and *Limulus polyphemus* were highly conserved across species. The '767 Application provides the alignment of *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* Factor C, reflecting their overall identity of around 97%. EX1006 at 123-124 (Figs. 8-9) (alignment of *Tachypleus tridentatus* and *Carcinoscorpius. rotundicauda* Factor C); EX2012 at 1 (“overall [identity] of 97.7% was observed”). It was also known before December 10, 2013 that *Carcinoscorpius rotundicauda* Factor C had approximately 90% identity with *Limulus polyphemus* Factor C. EX2035 ¶ [0134]; EX2036 at 4;

91. Although a direct comparison of the sequences had not been published, a POSA would have understood that the Factor C genes and proteins from *Tachypleus tridentatus* and *Limulus polyphemus* would have high sequence identity and similarity. The reported 90.5% identity in amino acid sequence between the phylogenetically diverse (within the family of 4 species) *Carcinoscorpius rotundicauda* and *Limulus polyphemus* and the even higher 97.7% identity between *Carcinoscorpius rotundicauda* and *Tachypleus tridentatus* would have reinforced this belief of the high sequence conservation between the latter and *Limulus polyphemus* Factor C. EX2044 at 4; EX2049 at 2; EX2012 at 1.

92. As a POSA in December 2013 could have and would have done, I compared the amino acid sequences of *Limulus polyphemus* Factor C with the *Carcinoscorpius rotundicauda* sequence and the *Tachypleus tridentatus* sequence disclosed in the '767 Application. My goal was to identify residues in the *Limulus polyphemus* sequence that are non-conserved with regard to both other species. I used the Clustal algorithm to identify fully conserved residues, conservation between groups of strongly similar properties, conservation between groups of weakly similar properties, and non-conserved residues.⁸

⁸ Clustal is a widely used program for sequence alignment. The Omega version was introduced in 2011. EX2082 at 1.

93. My analysis is found in EX2081. It indicates that out of 1020 amino acids, there were only **11 non-conservative substitutions** found in neither *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C. In other words, the *Limulus* Factor C is 98.9% similar to the other two Factor C proteins, when considered together. My analysis shows that these proteins are highly similar overall.

94. A POSA in December 2013 would have known that, in addition to being highly similar in terms of gene and amino acid sequence, the Factor C proteins from these three species had a high degree of functional similarity as well. EX1022 at 153 (reporting that the Factor C proteins from *Limulus polyphemus* and *Tachypleus tridentatus* were “indistinguishable” in terms of “immunological and biochemical properties”); EX2036 at 4 (reporting that recombinant *Carcinoscorpius rotundicauda* Factor C and native *Limulus polyphemus* Factor C “are highly comparable” in terms of detecting endotoxin).

95. I generated sequence similarity and structure comparisons (Predictive 3D structures via the Phyre2 web server. Visualizations of structures were via YASARA, and comparisons between structure overlays were via the MUSTANG algorithm in the YASARA software package).⁹ Ex. 2100.

⁹ Phyre2 was launched in January 2011 and YASARA was first released in 1993.

96. In comparing the Factor C protein of *Limulus polyphemus* with that of both *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, the Clustal alignment shows that there are relatively few amino acids that are non-conserved between *Limulus polyphemus* and BOTH *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (i.e., in this analysis amino acids have been marked as non-conserved only when the substitution differs between *Limulus polyphemus* and both other species of horseshoe crab), and these are relatively evenly dispersed throughout the Factor C molecule.

97. Of the 11 non-conservative substitutions, only five are within identified conserved domains (1/159= 0.6% in the PHA02927/secreted complement-binding protein domain; 2/129= 1.6% in the CLECT domain; 2/255= 0.78% in the serine protease domain), meaning that the **functional domains are very highly conserved between species**. A graphical depiction of the Clustal alignment for the three Factor C proteins, with conserved domains and non-conservative substitutions is provided below and in EX2103.

CLUSTAL O(1.2.4) Factor C (3 horseshoe crab species)

L=Limulus polyphemus, C=Carcinoscorpius rotundicauda, T=Tachyplesus tridentatus



Figure 8: Clustal alignment of *L. polyphemus*, *C. rotundicauda* and *T. tridentatus* Factor C amino acid sequences, with functional domains marked. Non-conservative substitutions where the *Limulus* sequence differed from either one or both of the other species in green and red, respectively.

98. Based on the above analysis, a POSA would have expected that functional properties, such as maintaining residual activity in the claimed salt solutions, would be highly conserved between each of the known horseshoe crab species. Comparing the predicted 3-dimensional structures of *Limulus polyphemus* and *Tachypleus tridentatus* (using Phyre2, and selecting the model based on template c9ekeD, which was the highest scoring model for both *Tachypleus tridentatus* and *Limulus polyphemus* Factor C proteins), the resulting models included the Factor C region encompassing both CCP/SUSHI domains and the serine protease domain. The structural prediction shows a very high degree of overlap between these regions of the Factor C molecules from the two species, with the two predicted structures being essentially indistinguishable. Thus, considering the above data collectively, a POSA would conclude that the key functional domains of Factor C are highly conserved between *Limulus polyphemus* and *Tachypleus tridentatus*, and the enzymes from each species would most likely behave in an indistinguishable manner in the clotting cascade. A POSA in December 2013 would have also understood that the *Limulus polyphemus* and *Tachypleus tridentatus* Factor C proteins in particular were functionally identical or interchangeable even without doing the sequence analysis described above.

99. 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.” EX1022 at 151, 152, 153. Tokunaga’s data showed that both Factor C zymogens were rapidly activated by LPS and lipid A analogue over a similar time course. EX1022 at 151, 153, Fig. 2, Suppl. Figs. 6-8. 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. EX1022 at 152.

100. Moreover, the procedures for testing pharmaceutical products for bacterial endotoxins was fully harmonized across the U.S., European, and Japanese Pharmacopoeias by December 2013, and allowed use of amoebocyte lysate from either *Limulus polyphemus* or *Tachypleus tridentatus*. EX2104 at 1; EX2105 at 1; EX2106 at 2. I understand that this harmonization was accomplished by extensive efforts to demonstrate equivalence in terms of interchangeability of procedures, specifications, and results. EX2061.

101. Researchers had also established comparable function between commercially available recombinant *Carcinoscorpius rotundicauda* Factor C and the native lysate-based endotoxin assays by December 2013. *See, e.g.*, EX2036 at 5; EX2070 at 15-17; EX1028; EX2075 at 7; EX2070 at 14-16; EX2049 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”).

102. I note that Petitioner itself stated that recombinant Factor C assays “rely on the underlying assumption that the Factor C enzymes from all horseshoe crab genera (*limulus*, *carcinoscoripus* [sic] and *tachypleus*) will behave similarly to all of the proteins that are found within an aqueous amoebocyte extract.” EX2083 at 3. Petitioner characterized data from “initial studies” as “confirm[ing] that both versions of rFC [*Tachypleus* species and *Carcinoscorpius rotundicauda*] 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.” *Id.* (citing a publication from 2000)).

4. The field had not struggled to sequence or structurally or functionally characterize *Limulus polyphemus* Factor C

103. Dr. Jain relies on statements made in a post-priority date publication from Patent Owner, PCT Publication WO 2018/074498 (“the ’498 PCT”) ’498 PCT (EX1005), as evidence of the alleged “struggle the field encountered in accomplishing the recombinant production of *Limulus polyphemus* proteins.” EX1002 ¶¶112, 136. 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.

104. 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 (C-reactive protein and agglutination-aggregation factor) and coagulation (coagulogen and anti-lipopolysaccharide), refute Dr. Jain's assertion. 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. EX2084 at 3 (citing papers from 1984 and 1986).
- The amino acid sequence and structure of the *Limulus* C-reactive protein was published in 1986. EX2085.
- The complete amino acid sequence of anti-lipopolysaccharide factor from *Limulus polyphemus* was determined in 1987. EX2086.
- A *Limulus polyphemus* endotoxin-binding protein with protease inhibitory activity was purified and its cDNA cloned in 1991. EX2087.
- A protein called limunectin was isolated from *Limulus polyphemus* amoebocytes and its cDNA sequence isolated from a lambda phage cDNA library in 1991. EX2088.

- A hemagglutinin and amebocyte aggregation factor from *L. polyphemus* was isolated, characterized, and its cDNA cloned in 1992. EX2089.
- The amino acid, DNA sequence, and recombinant expression of an endotoxin binding and neutralizing protein from *Limulus polyphemus* was reported in 1992. EX2090 at 11 (Figs. 15-16).
- The complete cDNA sequence of a *Limulus polyphemus* arginine kinase was determined and analyzed in 1995. EX2091.
- A GTP binding protein subunit of *Limulus polyphemus* was cloned in 1996. EX2092.
- A cyclic nucleotide-gated ion channel cDNA from *Limulus polyphemus* was cloned in 1999. EX2093.
- A protein kinase C from *Limulus polyphemus* was cloned in 2003 (using RACE-PCR). EX2094.
- A protein regulating eye development was cloned in 2008 (using RACE-PCR). EX2095.
- A search of the NCBI GenBank database for *Limulus polyphemus* sequences released before December 10, 2013 yielded 8825 entries. EX2096.

105. Dr. Jain also relies on statements in the '498 PCT to argue the inventors did not possess recombinant *Limulus polyphemus* Factor C proteins having the claimed functional property. I have reviewed the process described in the '498 PCT for how those inventors cloned the *Limulus polyphemus* Factor C genes. EX1005 at [0149]-[0177]. Based on the description, the inventors used standard RACE primers and an established protocol. Specifically, primer SK15-dT20 (SEQ ID NO: 24) includes a lengthy (20 nucleotide) 3' poly T tract. EX1005 at [0151]. This is a typical design for a 3' RACE primer (to prime from a poly A tail to make 1st strand cDNA). This same primer would typically also be used for 2nd strand cDNA synthesis in 5' RACE, assuming poly A-tailing of the first strand cDNA with terminal deoxynucleotidyl transferase (TdT). The primer LFC-5race (identical to primer SK15-dT20, without the poly T tract), in this same primer list would be the appropriate PCR primer to use for the 5' RACE protocol employing SK15-dT20 as the 2nd strand cDNA primer. EX1005 at [0151]. The additional steps describe the sequential execution of an initial 3' RACE followed by a 5' RACE and then final PCR amplification of the full *L. polyphemus* Factor C. EX1005 at [0152]-[0177]; *see also* EX2004 ¶¶40-43. This process would have succeeded in December 2013 in the same way it succeeded at some point before the October 2016 filing date of the Japanese priority application.

B. Claim 21 is Enabled by the '767 Application and Knowledge in the Art

106. Dr. Jain argues that claim 21 of the '318 Patent is not enabled by, and therefore not entitled to the priority date of the '767 Application. EX1002 ¶¶117-146. I disagree with Dr. Jain and respond to his arguments based on his parsing of the *Wands* factors below.

1. The breadth of the claims (factor 8).

107. Dr. Jain asserts that “Claim 21 broadly encompasses Factor C proteins satisfying a wide range of values of residual activity in the presence of certain ions.” To the contrary, claim 1 is relatively narrow, and none of the procedures involved in practicing claim 1—generating expression vectors, expressing proteins in mammalian cells, and testing their activities using well-established techniques—require undue experimentation.

108. Dr. Jain argues that “the '767 Application links the type of host cell employed to differences in post-translational modifications . . . as evidenced by the differing weights of the resulting protein.” Yet, Claim 21 does not invite a POSA to determine *which* cell to use—claim 21 requires that the *Limulus polyphemus* Factor C protein is recombinantly expressed in an HEK 293 cell. EX1001, cl. 21.

109. I disagree that with Dr. Jain’s conclusion that the “scope of claim 21 therefore requires an extensive amount of experimentation across multiple fronts.” EX1002, ¶135. Dr. Jain argues that undue experimentation would have been required

to “discover and characterize the gene and amino acid sequence for *Limulus polyphemus* Factor C itself.” *Id.* As I described above, it would not require undue experimentation to determine the *Limulus polyphemus* Factor C cDNA and amino acid sequences.

110. Dr. Jain also argues that that undue experimentation would have been required to “use this as-yet undiscovered genetic information to recombinantly generate Factor C protein across the full scope of residual activity function characteristic.” EX1002 ¶135. Dr. Jain ignores that Claim 21 and the ’767 Application describes *how* to achieve the claimed residual activity—expression of the *Limulus polyphemus* Factor C protein in HEK 293 cells. EX1001, cl. 21. He also ignores that, as I have discussed above, the ’767 Application provides a description of recombinantly producing Factor C in HEK293 cells, determining the residual activity in various salt solutions (Fig. 4), and provides data showing recombinantly produced Factor C in HEK293 cells demonstrates the claimed residual activity and residual activity that is higher as compared to that seen in Factor C expressed in Sf9 cells and native Factor C. EX1006 at Fig. 7.

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

111. Dr. Jain asserts that “the field had struggled to sequence *Limulus polyphemus* Factor C protein and could not make accurate predictions as to its structural and functional properties in view of the biodiversity between horseshoe

crab species.” EX1002 ¶136. However, he does not cite any peer-reviewed publications to this effect. Further, as discussed above, the numerous publications relating to *Limulus polyphemus* and the University of Connecticut sequence deposits rebut Dr. Jain’s conclusion, as does my analysis (described above) regarding the minimal non-conservative substitutions between the species and its impact on maintaining functional properties, such as meeting the claimed residual activity in the claimed salt solutions and having a residual activity in HEK293 cells that is higher than in Sf9 cells or native *Limulus polyphemus* Factor C. Additionally, Professor Frohman’s declaration discussed above also supports that sequencing *Limulus polyphemus* Factor C and recombinantly producing *Limulus polyphemus* Factor C in HEK293 cells would not have required undue experimentation for a POSA.

112. In my opinion, recombinant production in HEK293 cells of the recited Factor C would not have required undue experimentation as of December 10, 2013 in view of the information provided in the ’767 Application.

3. The required level of skill to practice the Challenged Claims (factor 6)

113. Dr. Jain agrees that the level of ordinary skill in the art is high. Dr. Jain nevertheless argues that “making a recombinant Factor C protein that actually has any desired activity . . . was known to be difficult in view of challenges, for example, with getting such proteins to be secreted by the host cells themselves.” EX1002,

¶140. Dr. Jain makes no attempt to analyze these “difficulties” in view the teachings in the Specification. For example, Dr. Jain sweeping conclusion that it is difficult to “get[] proteins to be secreted by the host cells themselves” is not tied to the claimed expression HEK 293 cell expression system or even to horseshoe crab Factor C. As explained above, the ’767 Application describes the process for making recombinant Factor C using HEK293 cells and provides a procedure for assessing whether the recombinantly produced Factor C meets the claimed residual activity minimum thresholds in the claimed salt solution concentrations.

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

114. Dr. Jain analyzes *Wands* factors 1-3 together. Yet, he focuses only on whether there is the presence of a working example of Factor C protein within the scope of Claim 21. EX1002 ¶¶142-145. I disagree with Dr. Jain’s conclusion that “nothing in the application suggests any steps the inventors envisioned for ensuring that such *Limulus polyphemus* Factor C proteins . . . would satisfy the claimed residual activity requirement” or that the ’767 application “provided no guidance whatsoever” as to how to generate the claimed *Limulus polyphemus* Factor C proteins. *Id.* ¶¶142-143.

115. The ’767 Application describes producing Factor C in mammalian cells, including HEK293 cells, and discusses a specific example detailing the production of recombinant Factor C in HEK293 cells. EX1006 at 53-54, 87-88. It

also provides a specific procedure in Figure 4 for measuring Factor C activity and discloses a specific example where the residual activity of recombinantly produced Factor C, including Factor C expressed in HEK293 cells, was measured in the claimed salt solution concentrations and determined to meet the minimum residual activity threshold required by Claim 21. EX1006 at 96-99, Figs. 4, 7.

116. In my opinion, a POSA would have understood how to make and use HEK293 cells to recombinantly express Factor C and how to measure the residual activity of the Factor C in the claimed salt solution concentrations to achieve the minimum residual activity threshold claimed based on the methods described in the '767 Application and working examples using *Tachypleus tridentatus* Factor C. It is immaterial that a working example of *Limulus polyphemus* Factor C is not included because the '767 Application specification describes all of the steps to make and use the claimed *Limulus polyphemus* Factor C protein.

117. I note that in a patent claiming priority to an application filed June 25, 2010, Petitioner's patent claims methods of preparing *Limulus* amebocyte lysates, which the specification defines as including recombinantly produced cascade proteins, including Factor G. EX2101 at 5:19-24 and cl. 1. Factor G comprises two subunits, alpha and beta. See EX2084 at 4-5, Fig. 4. Petitioner's patent does not disclose nucleic acid or amino acid sequences for Factor G and, as reflected in EX2102, the sequence of Factor G-subunit alpha was not published in an article until

2011.¹⁰ I also understand from my review of Dr. Morris' declaration that data exists showing that the *Limulus polyphemus* Factor C recombinantly produced in HEK293 cells had near 100% to over 100% residual activity in the claimed salt solutions of 16 mM magnesium sulfate and 21 mM sodium citrate. Ex. 2033. This further supports my opinion that a POSA would have understood how to make and use the full scope of the claimed invention as of December 10, 2013.

118. Thus, in my opinion it would not have required undue experimentation to make and use the *Limulus polyphemus* Factor C protein with the claimed residual activity of claim 21.

VII. GROUND 1: CLAIM 21 IS NOT OBVIOUS OVER MIZUMURA IN VIEW OF THE '498 PCT

119. As Dr. Jain explains, Mizumura is the United States publication of the '767 Application and has an "identical disclosure to the '318 patent." EX1002 ¶ 148. Mizumura published on October 29, 2015. EX1002 ¶ 148. The '498 PCT (Ex. 1005) published on April 26, 2018. If claim 21 is entitled to its December 10, 2013 priority date, then I understand the '498 PCT is not prior art

¹⁰ The authors of EX2102 submitted the sequence to GenBank on July 2, 2010. See <https://www.ncbi.nlm.nih.gov/nuccore/AB547712.1> (last accessed Nov. 26, 2025).

120. Thus, I understand if the '318 Patent is entitled to its December 10, 2013 priority date, Mizumura and the '498 PCT are not prior art and Ground 1 fails for this reason alone.

121. To the extent the December 10, 2013 priority date is not adopted and Mizumura and the '498 PCT are considered prior art, I disagree that they render claim 21 of the '318 Patent obvious because Dr. Jain's assertions as to obviousness directly contradict his assertions as to the lack of written description support and enablement in Mizumura.

122. First, Dr. Jain argues that "Mizumura and the '498 PCT together disclose the various characteristics of recombinantly produced Factor C proteins recited in claim 21." EX1002 ¶ 161. But neither Mizumura nor the '498 PCT discloses the claimed residual activity for *Limulus polyphemus* Factor C recombinantly produced in HEK293 cells. Indeed, the only citation to this disclosure that Dr. Jain provides in his chart refers to the data in Mizumura relating to residual activity for *Tachypleus tridentatus* Factor C. To overcome this deficiency, Dr. Jain asserts that a POSA "would have reasonably expected such resulting protein [*Limulus polyphemus* Factor C in HEK293 cells] to have the residual activity characteristics claimed." EX1002 ¶ 161. This assertion, however, directly contradicts Dr. Jain's arguments throughout his priority date analysis where he repeatedly stated that residual activity data in *Tachypleus tridentatus* Factor C is

insufficient to show that the claimed residual activity would be maintained for *Limulus polyphemus* Factor C. Thus, Dr. Jain fails to show where the prior art teaches the claimed residual activity values.

123. Second, Dr. Jain has also failed to demonstrate a POSA would have had a reasonable expectation of success in combining Mizumura and the '498 PCT to arrive at the claimed invention.

124. As Dr. Jain admits, Mizumura does not disclose residual activity for the claimed *Limulus polyphemus* Factor C recombinantly produced in HEK293 cells but instead only provides residual activity data for *Tachypleus tridentatus*. EX1002 ¶

161. Dr. Jain opines that “by April 30, 2019 it would have been obvious for a POSA to have replaced the *Tachypleus tridentatus* Factor C proteins of Mizumura using the by-then-known *Limulus polyphemus* gene and/or amino acid sequences for recombinant protein production.” EX1002 ¶158.

125. Dr. Jain argues that by April 2019, “POSAs would have reasonably expected to successfully generate recombinant Factor C of the *Limulus polyphemus* species via HEK293 cells where that recombinant Factor C has a residual activity failing within the range defined in claim 21.” EX1002 ¶ 178. In my opinion, this opinion cannot be reconciled with Dr. Jain’s opinion relating to the lack of written description and enablement support in the '767 Application, such as his statement that “a POSA would have expected that differences in gene or amino acid sequence

(as would be expected across different horseshoe crab species) lead to differences in protein structure, and, in turn, differences in protein function.” EX1002 ¶89.

VIII. GROUND 2: CLAIM 21 IS NOT OBVIOUS OVER MIZUMURA IN VIEW OF MCCLYMONT

126. Dr. Jain also asserts that claim 21 is obvious over Mizumura in view of McClymont (Ex. 1010). EX1002 at Section IX. I disagree. As I discuss with respect to Ground 1, if the ’318 Patent is entitled to its December 10, 2013 priority date, then Mizumura is not prior art. McClymont, which Dr. Jain asserts is dated March 2017, would also not be considered prior art. For this reason alone, Dr. Jain’s Ground 2 fails to render claim 21 obvious.

127. To the extent the claim is not entitled to its December 10, 2013 priority date such that Mizumura and McClymont are considered prior art, I disagree that the combination of these references renders claim 21 obvious. Dr. Jain relies on McClymont for teaching the “full cDNA and amino acid sequence for *Limulus polyphemus* Factor C protein.” EX1002 ¶ 192. Dr. Jain states “for the same reasons discussed above in Ground 1, POSAs would have had reason to utilize the sequence information from McClymont to generate a recombinant Factor C protein of the *Limulus polyphemus* species in HEK293 cells as disclosed for *Tachypleus tridentatus* in Mizumura, and would have reasonably expected the resulting proteins to have residual activity described in Mizumura and falling within the claimed range.” EX1002 ¶ 192

128. For the same reasons as I discuss in Ground 1, incorporated here, I disagree. Thus, in my opinion, claim 21 would not have been obvious in view of this combination.

129. I further note that I understand secondary considerations of non-obviousness must be considered when assessing whether a claim is unpatentable as obvious. I understand that one such secondary consideration of non-obviousness that can be considered is unexpected results. I understand from the declaration of Dr. Morris that the claimed invention exhibits unexpected results over the closest prior art, which further supports my opinion that the claim is not unpatentable as obvious. I do not offer an opinion of my own as to the unexpected results of the claimed invention but instead rely on the analysis performed by Dr. Morris.

130. As such, in my opinion, assuming the art cited in Grounds 1 and 2 is considered prior art, claim 21 is still not unpatentable as obvious under either ground.

I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: 12/01/2025



Brian C. Schaefer, Ph.D.