

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

DECLARATION OF BRIAN C. SCHAEFER, PH.D.

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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. 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 copy of my curriculum vitae is found at EX2063.

II. MATERIALS REVIEWED

8. A list of the materials I have considered in preparing this declaration is provided below.

Exhibit	Description
1001	U.S. Patent No. 11,959,109
1002	Prosecution History of U.S. Patent No. 11,959,109 (Application No. 18/346,420)
1003	Declaration of Siddhartha Jain, Ph.D., PMP (“Jain”)
1004	Curriculum Vitae of Dr. Siddhartha Jain, Ph.D., PMP
1005	International Patent Publication No. WO 2018/074498 A1
1006	Higdon et al., “Modeling sequence and function similarity between proteins for protein functional annotation,” <i>Proc Int Symp High Perform Distrib Comput.</i> 2010: 499–502.
1007	Kobayashi et al., “Factor B Is the Second Lipopolysaccharide-binding Protease Zymogen in the Horseshoe Crab Coagulation Cascade,” <i>J Biol Chem.</i> 2015 Jun 24; 290(31):19379–19386.
1008	Japanese Priority Application No. JP2012-269840 (certified translation)
1009	U.S. Patent No. 5,716,834

1010	Muta et al., "Limulus Factor C. An endotoxin-sensitive serine protease zymogen with a mosaic structure of complement-like, epidermal growth factor-like, and lectin-like domains," <i>J Biol Chem.</i> 1991 Apr 5; 266(10):6554-61.
1011	Karen McClymont, Ph.D. Thesis, submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy from the University of Glasgow, Institute of Molecular, Cell and Systems Biology College of Medical, Veterinary and Life Sciences, March, 2017
1012	U.S. Patent Application Publication No. 2021/0363564
1015	U.S. Patent Application Publication No. 2019/0241629
1016	U.S. Patent Application Publication No. 2015/0307864
1017	U.S. Patent Application No. 14/650,767, as filed June 9, 2015
1019	Shi et al., "Protein N-Glycosylation in the Baculovirus-Insect Cell System," <i>Curr Drug Targets.</i> 2007 Oct; 8(10): 1116–1125.
1025	Tokunaga, F., et al. "Further studies on lipopolysaccharide-sensitive serine protease zymogen (Factor C): Its isolation from <i>Limulus polyphemus</i> hemocytes and identification as an intracellular zymogen activated by α -chymotrypsin, not by trypsin," <i>J. Biochem.</i> 1991 Jan;109(1):150-7.
1026	Dwarakanath, R.S. et al., "Recombinant COS-1 cells express <i>Carcinoscorpius rotundicuada</i> Factor C," <i>Biotechnology Letters.</i> 1997 April; 19(4):357-61.
1027	Wang, J. et al., "Functional expression of full length <i>Limulus</i> Factor C in stably transformed Sf9 cells," <i>Biotechnology Letters.</i> 2001; 23:71-76.
1030	Barrabes, S. et al., "Effect of sialic acid content on glycoprotein p/ analyzed by two-dimensional electrophoresis," <i>Electrophoresis.</i> 2010 Sep; 31(17):2903-12.
1031	Palomares, L.A., "Production of Recombinant Proteins: Challenges and Solutions," <i>From: Methods in Molecular Biology</i> , vol. 267: Recombinant Gene Expression: Reviews and Protocols, Second Edition Edited by: P. Balbás and A. Lorence © 2004 Humana Press Inc., Totowa, NJ, pp. 15-51.
1038	Sekiguchi, K. and Shuster, C.N., "Limits on the Global Distribution of Horseshoe Crabs (<i>Limulacea</i>): Lessons Learned from Two Lifetimes of Observations: Asia and America," <i>Biology and Conservation of Horseshoe Crabs</i> , 2009.
1039	Hossler et al., "Optimal and consistent protein glycosylation in mammalian cell culture," <i>Glycobiology.</i> 2009 Sep; 19(9):936-4.

1040	File History of United States Application No. 16/399,386
1041	ExpiCHO™ Expression System - USER GUIDE – For transfection of ExpiCHO-STM in a defined, serum-free medium. Catalog Number A29133, Publication Number MAN0014337 Revision D.0 (May 25, 2018).
1065	Deposition transcript of Siddhartha Jain, Ph.D. dated November 4, 2025
2001	Disclaimer of Claim 10 in U.S. Patent 11,959,109
2002	Muta, et al., “Horseshoe Crab Coagulation Factor B, A Unique Serine Protease Zymogen Activated by Cleavage of an Ile-Ile Bond,” (1993) J. Biol Chem., Vol. 268, No. 28, 21384-88
2003	Muta, et al., “Proclotting Enzyme from Horseshoe Crab Hemocytes, cDNA Cloning, Disulfide Locations, and Subcellular Localization,” (1990) J. Biol Chem., Vol. 265, No. 36, 22426-33
2004	Declaration of Michael A. Frohman, M.D., Ph.D. and exhibits from Opposition to European Patent No. 3530670
2005	Novitsky, T.J., “Biomedical Applications of Limulus Amebocyte Lysate,” in , J. Tancredi et al (eds.), Biology and Conservation of Horseshoe Crabs, Springer Science+Business Media, LLC (2010) pp. 315-329
2006	Notice of Opposition to European Patent No. 3530670
2008	Ding, et al., “Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, Carinoscorpius rotundicauda,” Molecular Marine Biology and Biotechnology (1995) 4(1), 90-103, submitted in Opposition to European Patent No. 3441466
2013	Wang, et al., “Modular Arrangement and Secretion of a Multidomain Serine Protease,” J. Biological Chemistry (2002), 277(39):36363-36372, submitted in Opposition to European Patent No. 3441466
2022	Thorne, et al., “Evaluation of the Limulus Amebocyte Lysate and Recombinant Factor C Assays for Assessment of Airborne Endotoxin,”

	Appl. Environ. Microbiol. 76(15):4988-95 (2010)
2023	International Publication Number WO 2012/118226
2024	Ding, J.L. and Ho, B., "Endotoxin detection – from Limulus Amebocyte Lysate to Recombinant Factor C," pp. 187-208 (2010)
2027	Tamura, H., "EC Bacteriol. Virol. Rsch. 2016," available at https://ecronicon.net/assets/ecbvr/pdf/ECBVR-01-ECO-03.pdf
2028	U.S. Patent No. 6,391,570
2036	D19, "A comparison of Limulus Factor C expressed with and without terminal sialic acid glycosylation"
2037	Iwanaga, S., "The limulus clotting reaction" (1993) Curr. Op. Immunol., 5(1):74-82
2038	Iwanaga, S., "Primitive Coagulation Systems and their Message to Modern Biology" (1993) Thromb. Haemost., 70(01):48-55
2039	Iwanaga, S. et al., "New Types of Clotting Factors and Defense Molecules Found in Horseshoe Crab Hemolymph: Their Structures and Functions" (1998) J. Biochem., 123:1-15
2040	Koshiba, et al., "A Structural Perspective on the Interaction between Lipopolysaccharide and Factor C, a Receptor Involved in Recognition of Gram-negative Bacteria," (2007) J. Biological Chemistry, 282(6): 3962-3967, submitted in Opposition to European Patent No. 3441466
2047	U.S. Patent Application Publication No. 2003/0054432 A1
2048	Alwis, K.U. & Milton, D.K., "Recombinant Factor C Assay for Measuring Endotoxin in House Dust: Comparison with LAL, and (1→3)-β-D-Glucans," (2006) Am. J. Indus. Med. 49:296-300
2049	Mustopa, A.Z. et al., "Characterization, protein modeling, and molecular docking of factor C from Indonesian horseshoe crab (Tachypleus gigas)," J. Genetic Eng'g and Biotechnology, (2023) 21:44

2050	Certified English translation of International Publication No. WO 2008/004674 A1
2051	U.S. Pharmacopoeia Ch. <85> “Bacterial Endotoxins Test” (Dec. 1, 2012)
2052	European Pharmacopoeia 5.0, “2.6.14. Bacterial Endotoxins,” 01/2005:20614
2053	Excerpts from Japanese Pharmacopoeia-XV, “4.01 Bacterial Endotoxins Test” (March 31, 2006)
2054	Dubczak, J., “Standing Guard,” Charles River Laboratories, PDA Letter (Sept. 26, 2018)
2055	U.S. Patent Application Publication No. 2009/0208995 A1
2057	Iwanaga, S., “Biochemical principle of Limulus test for detecting bacterial endotoxins,” Proc. Jpn. Acad., Ser. B (2007), 83:110-119
2058	Kawabata, S., et al., “The lipopolysaccharide-activated innate immune response network of the horseshoe crab,” Invertebrate Survival J. (2009), 6(1):59-77
2059	Iwanaga, S. and Lee, B.K., “Recent Advances in the Innate Immunity of Invertebrate Animals,” J Biochem. and Mol. Biol. (2005), 38(2):128-150
2060	D’Amato A., et al., “In Depth Exploration of the Hemolymph of Limulus polyphemus via Combinatorial Peptide Ligand Libraries,” J. Proteome Rsch. (2010), 9:3260-3269
2061	RESERVED
2062	Declaration of Brian C. Schaefer, Ph.D.
2063	Curriculum vitae of Brian C. Schaefer, Ph.D.

2064	Declaration of Wan-Shon Lo
2065	Obst M, et al., Molecular phylogeny of extant horseshoe crabs (Xiphosura, Limulidae) indicates Paleogene diversification of Asian species. Molecular Phylogenetics and Evolution (2012) 62:21-26
2066	TJ Novitsky and H Donald Hochstein, Limulus Endotoxin Test, in Rapid Microbial Methods in the Pharmaceutical Industry, Martin C. Easter, ed. (2005)
2067	Iwanaga S et al., Molecular Mechanism of Hemolymph Clotting System in Limulus, Thromb Res (1992) 68:1-32
2068	T Nakamura and S Iwanaga, Lipopolysaccharide-sensitive serine-protease zymogen (factor C) found in Limulus hemocytes: isolation and characterization, Eur J Biochem (1986) 154:511-521
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2070	Loverock, B et al., A Recombinant Factor C Procedure for the Detection of Gram-negative Bacterial Endotoxin, Pharmacopoeial Forum [Nov.-Dec. 2009] 35(6): 1613-1621.
2071	NCBI BioProject PRJNA178587 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA178587
2072	NCBI BioProject PRJNA178588 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA178588/
2073	NCBI Sequence Read Archive SRX202160 from BioProject PRJNA178588 https://www.ncbi.nlm.nih.gov/sra?LinkName=bioproject_sra_all&from_uid=178588
2074	NCBI Sequence Read Archive Run SRR610305 for Horseshoe Crab Transcriptome (metadata and data access tabs) https://trace.ncbi.nlm.nih.gov/Traces/?view=run_browser&acc=SRR61

	0305&display=metadata https://trace.ncbi.nlm.nih.gov/Traces/?view=run_browser&acc=SRR610305&display=data-access
2075	NCBI SRA link from BioProject 178587 and entries for SRX202152 and SRX202154 (combined) https://www.ncbi.nlm.nih.gov/sra/?term=srx202152 https://www.ncbi.nlm.nih.gov/sra/?term=srx202154
2076	NCBI BioProjects FAQ https://www.ncbi.nlm.nih.gov/bioproject/docs/faq/
2077	A Brief History of NCBI's Formation and Growth https://www.ncbi.nlm.nih.gov/books/NBK148949/
2078	NLM GenBank and SRA Data Processing https://www.ncbi.nlm.nih.gov/sra/docs/sequence-data-processing/
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2081	Cheng, SM et al., Characterization of a complementary deoxyribonucleic acid for the coagulogen of Limulus polyphemus, Biochimica et Biophysica Acta (1986) 868:1-8.
2082	Pharmacopoeial Discussion Group https://www.usp.org/harmonized-standards/pdg
2083	Bolden J et al., Currently Available Recombinant Alternatives to Horsehoe Crab blood Lysates: Are they Comparable for the Detection of Environmental Bacterial Endotoxins? A Review. PDA J Pharm Sci

	and Tech (2020) 74:602-611.
2084	FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers, June 2012.
2085	U.S. Patent Application Publication No. 2024/0125789
2086	ORCID entry for Shun-ichiro Kawabata https://orcid.org/0000-0002-8772-7522
2087	B. Schaefer, Revolutions in Rapid Amplification of cDNA Ends: New Strategies for Polymerase Chain Reaction Cloning of Full-Length cDNA Ends, Anal Biochem (1995) 227(2):255-273.
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2089	pCI-neo brochure
2090	AC Dalton and WA Barton, Over-expression of secreted proteins from mammalian cell lines, Prot Sci (2014) 23:517-525
2091	BE Shakhnovich and EV Koonin, Origins and impact of constraints in evolution of gene families, Genome Res (2006) 16:1529-1536
2092	Rose, T and Di Cera, E, Substrate Recognition Drives the Evolution of Serine Proteases. J Biol Chem (2002). 277(22):19243-19246.
2093	BLAST hits and CD-Search results for Factor C, Factor B, and Pro-clotting enzyme against SRR610305 (figure/diagrams)
2094	Clustal alignments of Tachyplesus tridentatus, Limulus polyphemus and Carinoscorpius rotundicauda Factor C and Tachyplesus tridentatus and Limulus polyphemus Factor B and Pro-clotting enzyme
2095	Sievers F et al., Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega, Molecular Systems Biology 7:539
2096	JL Ding and B Ho, A new era in pyrogen testing, TRENDS Biotech

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2098	Nguyen et al., The amino acid sequence of Limulus C-reactive protein. Evidence of polymorphism, J. Biol Chem (1986) 261(22): 10456-10465
2099	Muta et al., Primary structure of anti-lipopolysaccharide factor from American horseshoe crab, <i>Limulus polyphemus</i> , J Biochem (1987) 101(6): 1321-1330
2100	Minetti CA et al., Purification and characterization of an endotoxin-binding protein with protease inhibitory activity from <i>Limulus</i> amebocytes, J Biol Chem (1991) 266(31): 20773-20780
2101	Liu T et al., Limunectin: A phosphocholine-binding protein from <i>Limulus</i> amebocytes with adhesion-promoting properties, J Biol Chem (1991) 266:14813-13821
2102	Fujii N et al., Isolation, cDNA cloning, and characterization of an 18-kDa hemagglutinin and amebocyte aggregation factor from <i>Limulus polyphemus</i> , J Biol Chem. (1992) 267(31): 22452-22459
2103	WO92020715A1
2104	SJ Strong and WR Ellington, Isolation and sequence analysis of the gene for arginine kinase from the chelicerate arthropod, <i>Limulus polyphemus</i> : insights into catalytically important residues, Biochim Biophys Acta (1995) 1246(2): 197-200
2105	Munger SD et al., Molecular and immunological characterization of a Gq protein from ventral and lateral eye of the horseshoe crab <i>Limulus polyphemus</i> , Invert Neurosci. (1996) 2(3):1 75-182
2106	Chen FH et al., Molecular cloning of a putative cyclic nucleotide-gated ion channel cDNA from <i>Limulus polyphemus</i> , J Neurochem. (1999) 72(2): 461-471 (1999)
2107	Reid EA et al., Molecular cloning and identification of a putative PKC epsilon cDNA from <i>Limulus polyphemus</i> brain, Life Sci (2003) 72(8):

	961-976 (Abstract)
2108	Blackburn DC et al., Isolation and Expression of Pax6 and atonal Homologues in the American Horseshoe Crab, <i>Limulus polyphemus</i> , Dev Dyn (2008) 237(8): 2209-19
2109	NCBI Nucleotide search results for <i>limulus polyphemus</i> released prior to December 10, 2013
2110	AL Demain and P Vaishnav, Production of recombinant proteins by microbes and higher organisms, Biotech Adv (2009) 27:297-306
2112	Predicted 3-D structure comparisons for <i>Limulus polyphemus</i> and <i>Tachypleus tridentatus</i> Factor C, Factor B and Pro-clotting enzyme
2113	A Yoneda and T Kurokawa, A sensitive sandwich ELISA to measure (1→3)-β-D-glucan levels in blood, J Immun Meth (2011) 365:158-165
2114	Graphical depiction of Clustal alignment of <i>Limulus polyphemus</i> , <i>Carcinoscorpius rotundicauda</i> , and <i>Tachypleus tridentatus</i> Factor C sequences

III. LEGAL PRINCIPLES

A. Written Description

9. 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.

10. 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.

11. I understand that disclosure of structural features common to the members of the genus such that a person of ordinary skill in the art can visualize or recognize the members of the genus may provide sufficient written description.

12. I understand that disclosure of a representative number of species within the genus also may provide sufficient written description. However, I understand that a patent applicant is not required to disclose every species encompassed by their claims even in an unpredictable art.

B. 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 invention 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 invention 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.

C. Anticipation

16. I understand that a patent claim is “anticipated” and thus invalid if a single prior art reference discloses every claim limitation as arranged in the claim, either expressly or inherently.

D. Priority Date

17. I understand that a later-filed patent application may claim the benefit of, or priority to, a prior application filed in the United States or a foreign country. I understand that to receive the benefit of the earlier filing date, the specification must provide written description and enablement support for the later-filed claims.

IV. TECHNOLOGY BACKGROUND

A. Horseshoe Crab Coagulation Cascade

18. There are four identified living species of horseshoe crab: *Carcinoscorpius rotundicauda*, *Limulus polyphemus*, *Tachypleus gigas*, and *Tachypleus tridentatus*. EX2058 at 1-2. *Limulus* is primarily found in 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* EX2065 at 1, 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.

19. In 1964, Levin and Bang identified that the amoebocytes 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. Endotoxin is a lipopolysaccharide (“LPS”) that is present in the outer cell envelope of Gram-negative bacteria. EX2005 at 4-5. In humans, exposure to endotoxin via injectable drugs, vaccines, or medical devices results in serious medical conditions, including sepsis, which can lead to organ failure and death. *Id.* at 5. Thus, detection of endotoxin is a critically important quality control step 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. EX2084 at 5.

20. The coagulation cascade utilized by the LAL assay is part of the horseshoe crab innate immune defense system, and this immune mechanism is critical for survival. EX2024 at 11. By 1992, the mechanism of the horseshoe crab coagulation cascade had largely been elucidated, and its enzyme components identified. *See* EX2067 2. A sequential activation of three serine protease

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

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.

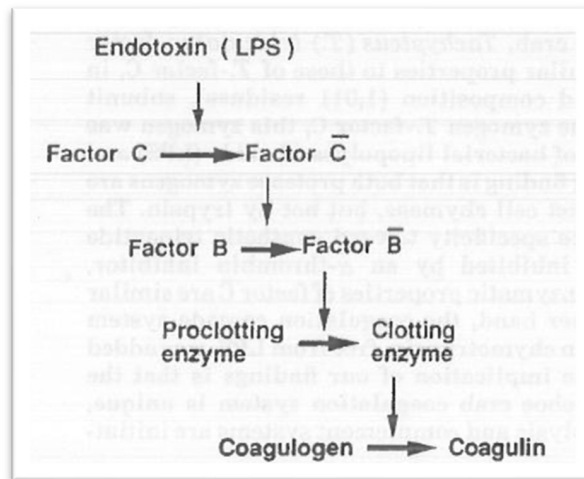


Figure 1. Mechanism for coagulation cascade of horseshoe crab hemocyte lysate. EX1025 at 151, Fig. 1.

21. 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.

B. Isolation and Characterization of the Coagulation Cascade Proteins

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

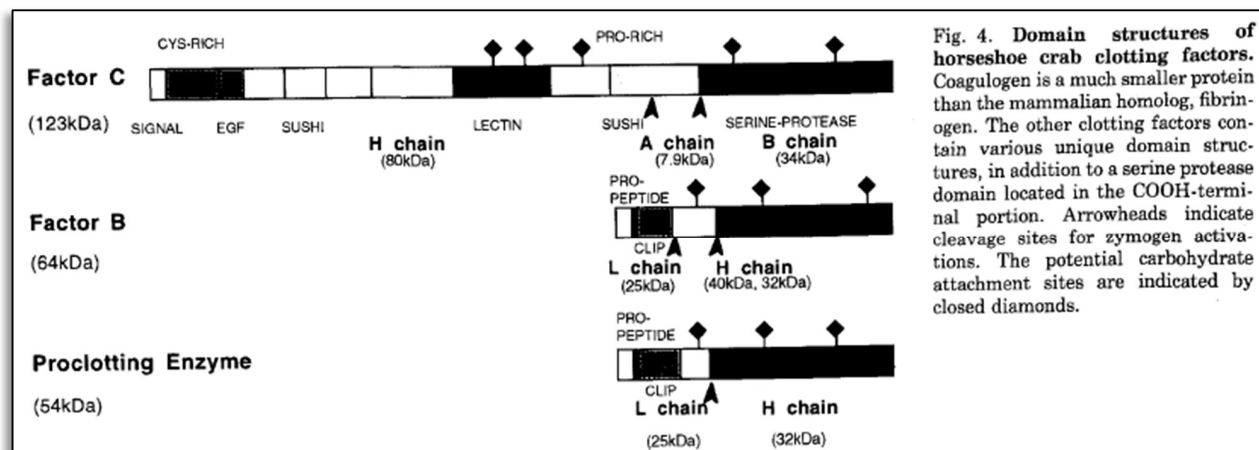


Figure 2. Domain structures of horseshoe crab clotting factors. EX2039 at 6, Fig. 4. Arrowheads indicate cleavage sites and diamonds indicate potential glycosylation sites.

1. Factor C

23. 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.

24. Factor C was first purified from *Tachypleus tridentatus* in 1986 by Nakamura et al. EX2068 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. EX2069 at 1. Thus, Factor $\bar{C}$ consists of three chains—heavy chain, A chain, and B chain—linked via disulfide bonds.

25. In 1991, Muta et al. published the *Tachypleus tridentatus* Factor C cDNA and amino acid sequence and characterized its domains. EX1010 at 6554. Muta determined that Factor C includes several domains: a signal peptide, a Cys-rich region, an EGF-like domain, five sushi domains,² 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.

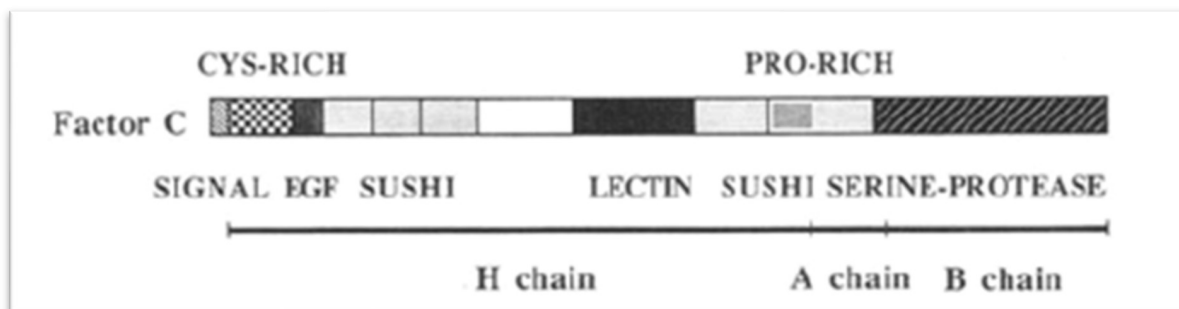


Figure 2. The domain structure of *Tachypleus tridentatus* Factor C. EX1010, Fig. 7.

² Sushi or short consensus repeat domains are found in many mammalian complement system proteins. EX1010 at Abstract.

26. Muta identified the catalytic triad of Factor C, which was that of “a typical serine protease,” reporting that Factor 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.

27. In 1995, the cDNA sequence and amino acid sequence of *Carcinoscorpius rotundicauda* Factor C were published. EX2012 at 1; *see also* EX1009 at 13:25-50 (Example 4). *Carcinoscorpius rotundicauda* Factor C, like the *Tachypleus tridentatus* Factor C, consists of a heavy chain and a light chain. EX2012 at 1-2. Upon activation by endotoxin, like *Tachypleus tridentatus* Factor C, it undergoes limited proteolysis of its light chain into an A chain and a B chain upon endotoxin activation. *Id.*, at 5, Fig. 5.

28. 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 *Tachypleus tridentatus* and *Carcinoscorpius 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 same Phe-Ile cleavage site between the A chain and B chain.

EX2008 at 4; EX1009 at Example 6. Moreover, the *Carcinoscorpius rotundicauda* Factor C is, like *Tachypleus tridentatus* Factor C, catalytically similar to trypsin-type serine proteases. EX1009 at 14:66-15:62 (Example 7).

29. 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.

30. 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 C chains showed a “close resemblance” to the corresponding *Tachypleus tridentatus* sequence. *Id.* at 151-152.

31. 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 beings with a Ser, suggesting that the same Arg-Ser cleavage site between the H chain and A chain (highlighted in green below).

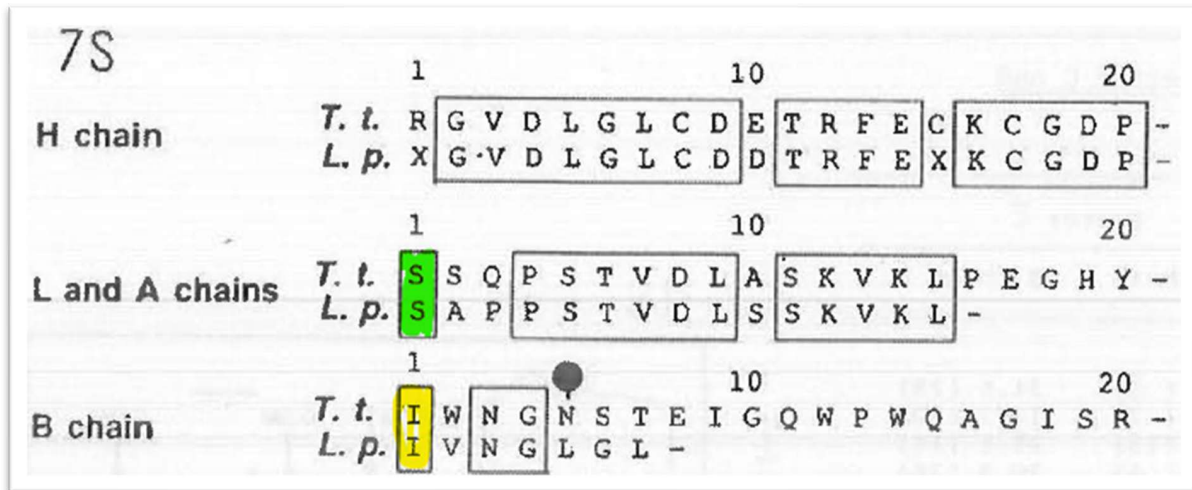


Figure 3. Comparison of the N-terminal sequences of heavy, A chain, and B chain of LpFC and TtFC.³ EX1025 at 157 (annotated).

32. Tokunaga concluded that “the gross structure of [LpFC] must resemble that of known [TtFC]” and indicates that they “are identical protein entities, both

³ Post-priority date publications revealed that the sequences Tokunaga reported for the B chain contained discrepancies that *decreased* its apparent identity to the *Tachypleus 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, the *Limulus polyphemus* Factor C includes the same glycosylation site as *Tachypleus tridentatus* at these positions. 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.*

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.

33. As early as 2001, the amino acid composition of *Limulus polyphemus* Factor C had been 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.”). Other publications disclosed similar information in 2006 and 2009. EX2048 at 4 (“the Factor C of *Carcinoscorpius rotundicauda* has 89.3% homology with that of *Limulus polyphemus*”);⁴ 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*.”).

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.

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

35. The full cDNA sequence of *Limulus polyphemus* Factor C was not published contemporaneously with the foregoing reports. However, researchers at the 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 whole genome shotgun sequence of the *Limulus polyphemus* under BioProject accession number PRJNA178587.⁵ EX2071 (PRJNA178587); EX2072 (PRJNA178588).

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.” EX2076. 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.” *Id.*

36. BioProject PRJNA178588 generated experimental dataset SRX202160 with run SRR610305 in the Sequence Read Archive (“SRA”). This data was published as of October 31, 2013. EX2072 PRJNA178588; EX2073 SRX202160 at 1; EX2074 SRR610305 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. EX2071 PRJNA178587; 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. EX2078.

37. 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

⁶ BLAST was first introduced in 1990. EX2077 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 species or search for similar sequences regardless of species. BLAST allows a user to select a specific

POSA could have assembled the large majority of the cDNA sequences of Factor C and Factor B, and the entirety of Pro-clotting enzyme from the transcriptome deposit using these well-established methods.

2. Factor B and Pro-Clotting Enzyme

38. 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.*

39. 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

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. EX2079.

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.*

40. 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, Fig. 4 (reproduced in part below as Figure 4). 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.

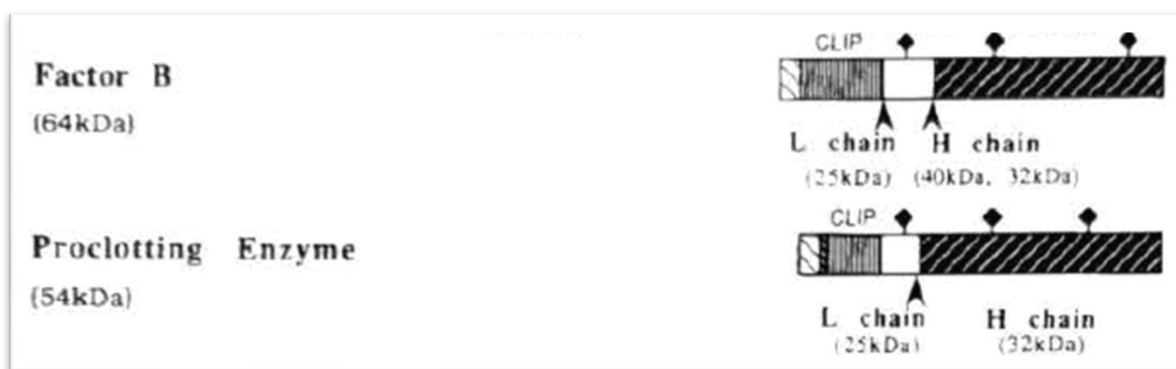


Figure 4. Domain structure of *Tachypleus tridentatus* Factor B and Pro-clotting Enzyme. EX2038, Fig. 4. The arrowheads indicate cleavage sites for zymogen activation and diamonds indicate potential glycosylation sites.

41. 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. EX2038, Fig. 3 (reproduced with annotations below as Figure 5):

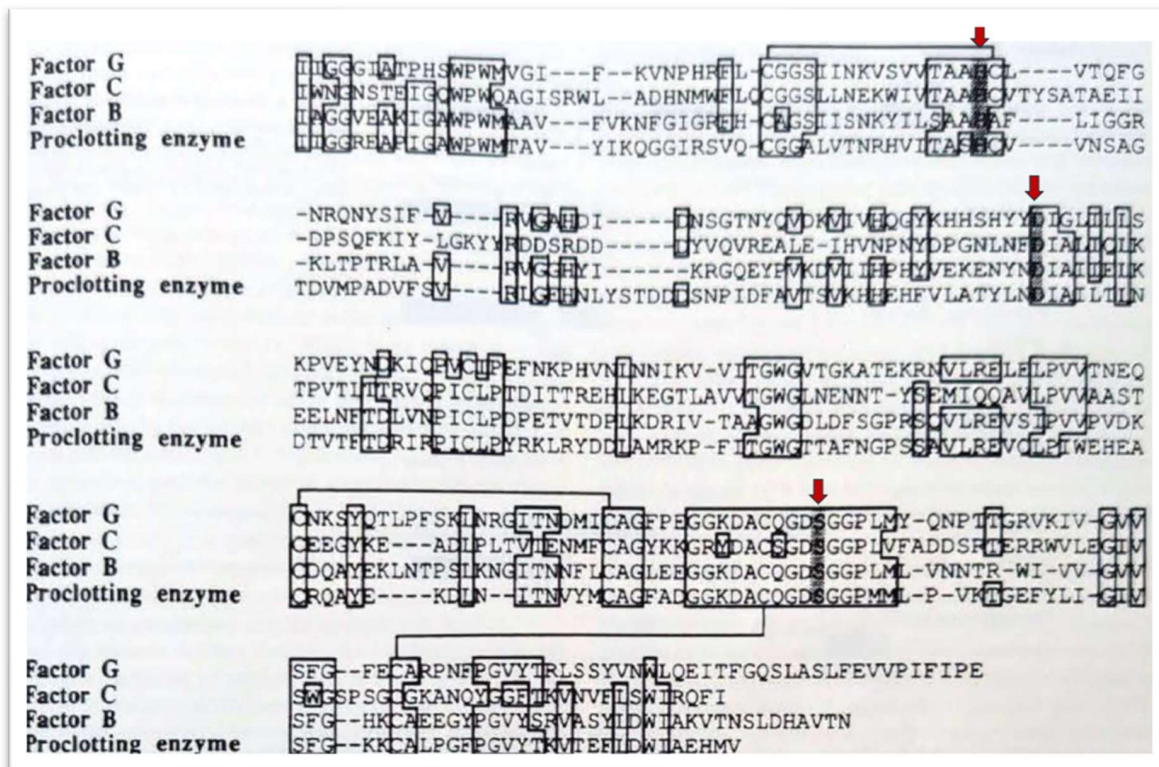


Figure 5. Sequence Alignment of serine protease domains of *Tachypleus tridentatus* coagulation cascade proteins with the residues of the active triad indicated with red arrows.⁷ EX2038, Fig. 3 (annotated).

3. Coagulogen

42. 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 the chromogenic

⁷ 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.

version of the LAL assay; its place at the end of the cascade is replaced by a detection substrate. EX2070 at 1.

43. The amino acid and cDNA sequences of coagulogen from *Limulus polyphemus* was determined in 1984 and 1986, respectively. EX2080 at 4, Fig. 15 (reproduced below as Figure 6); EX2081. The cleavage sites in coagulogen, *i.e.*, the substrate sites of activated Pro-clotting enzyme, were known. EX2080 at Fig. 15.

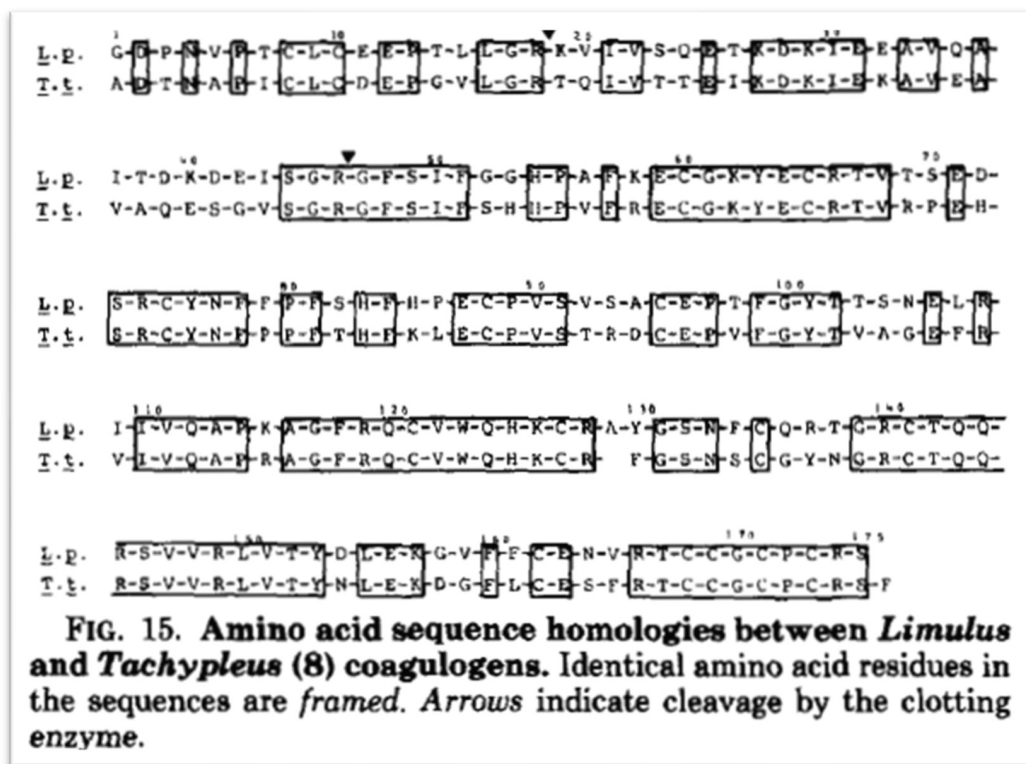


Figure 6. Amino acid sequence homologies between *Limulus polyphemus* and *Tachypleus tridentatus* coagulogens, with cleavage sites indicated by arrows. EX2080, Fig. 15.

44. Miyata characterized the amino acid sequence 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 12-13 ([0034]), 26 ([0214]) (providing examples of peptides used in synthetic chromogenic substrates, and stating Boc-LGR-pNA is an “optimum substrate in conventional LAL reaction”).

C. Functional Interchangeability

45. 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. *See, e.g.*, EX1025 at 153 (stating in 1991 that “[t]hese findings also lead to the idea that the hemocytes of *Limulus polyphemus* probably contain a coagulation system similar to that established for the *Tachypleus tridentatus* hemocytes.”); *see also* EX2005 at 2 (stating LAL 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”).

46. The U.S. Pharmacopeia created a chapter on bacterial endotoxin testing using a *Limulus polyphemus* lysate-based reagent and validated an endotoxin reference standard. 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.

47. 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 *Carcinoscorpius rotundicauda* and recombinant Factor B and Pro-clotting enzyme from *Tachypleus 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. A post-priority date publication confirmed that native *Tachypleus tridentatus* Factor C could activate recombinant *Limulus polyphemus* Factor B and that native *Limulus polyphemus* Factor C could activate recombinant *Tachypleus tridentatus* Factor B. EX2085 at 28-29 ([0177]-[0186]).

D. LAL Assays

48. 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. EX2024 at 12-13.

49. 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. 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.

50. A commercial product employing recombinant *Carcinoscorpius rotundicauda* Factor C was commercially launched in 2004. EX2024 at 16 (discussing PyroGene); *see also* EX2023 at 4-5 ([0010]). Other products using *Tachypleus tridentatus* Factor C followed. EX2027; EX2083 at 5. The difference

in the pathways of the amoebocyte-based LAL assay and the recombinant Factor C assay is shown below:

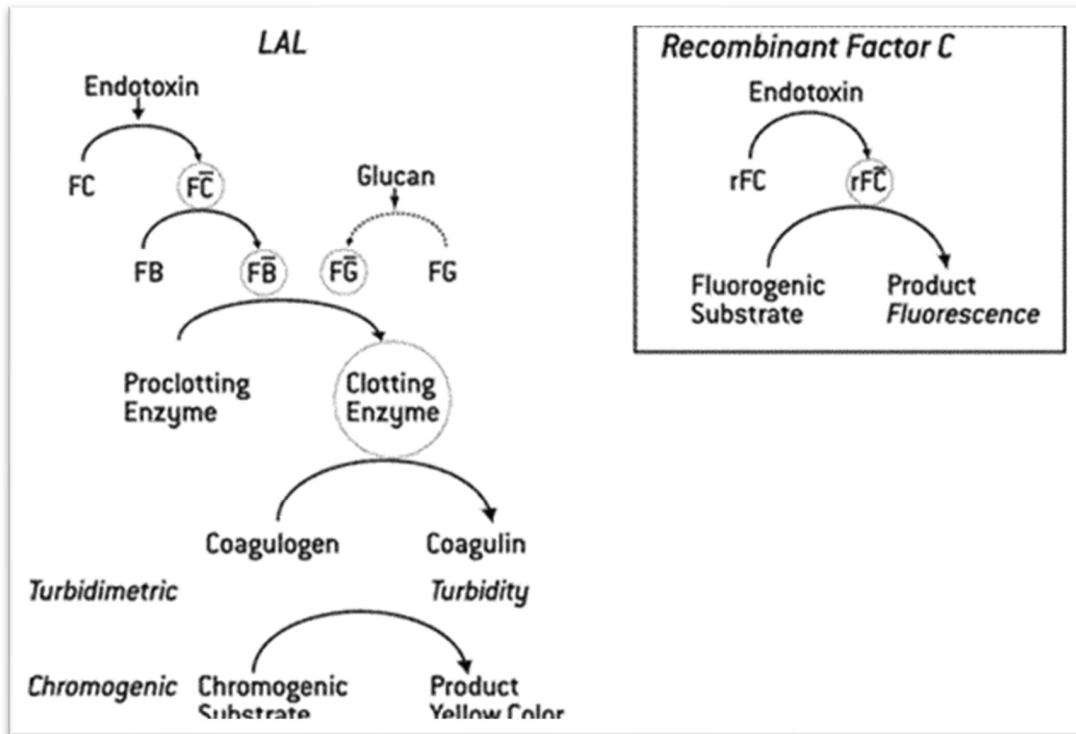


Figure 7: Reactions involved in the LAL and recombinant Factor C assays. EX2070 at 2, Fig. 1.

51. 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; *see also* EX2083 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 specificity of LAL with respect to beta glucans.”).

V. OVERVIEW OF THE '109 PATENT

52. As Dr. Jain has explained, the '109 patent was filed as U.S. Patent Application No. 18,346,420 on July 3, 2023. EX1003 ¶33. 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. EX1003 ¶ 51. 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 '109 patent specification.” EX1003 ¶142.

A. Challenged Claims

53. I understand claims 1-9 (the “Challenged Claims”) of the '109 Patent are at issue. I have reproduced the table from Dr. Jain’s declaration (EX1003 ¶38) for claims 1-9 below:

Claim 1
[1PRE.A] A method for producing an endotoxin assay agent
[1PRE.B] comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

[1A] expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell,
[1B] secreting the horseshoe crab Factor C into an extracellular culture supernatant,
[1C] and recovering the horseshoe crab Factor C in the culture supernatant,
[1D] wherein the recovered horseshoe Factor C has activity of Factor C, and
[1E] wherein the horseshoe crab Factor C is a <i>Tachypleus tridentatus</i> Factor C or a <i>Limulus polyphemus</i> Factor C,
[1F] expressing the horseshoe crab Factor B having activity of Factor B in a mammalian cell as a host cell,
[1G] expressing the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme in a mammalian cell as a host cell, and
[1H] producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme,
[1I] 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.
Claim 2
The method according to claim 1, wherein the mammalian cell expressing the Factor B is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.
Claim 3
The method according to claim 1, wherein the mammalian cell expressing the Pro-clotting enzyme is a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell.
Claim 4
The method according to claim 1, wherein the horseshoe crab for the Factor B and/or the horseshoe crab for the Pro-clotting enzyme is selected from the group consisting of <i>Tachypleus tridentatus</i> and <i>Limulus polyphemus</i> .
Claim 5
The method according to claim 1, wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.
Claim 6
The method according to claim 1, wherein the horseshoe crab Factor C has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a nonreducing condition.
Claim 7
The method according to claim 1, further comprising filtering the culture supernatant comprising the horseshoe crab Factor C.

Claim 8
The method according to claim 1, wherein the endotoxin assay agent is formulated as a solid, a liquid, or a gel.
Claim 9
The method according to claim 1, wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are separately expressed in individually established host cells.

B. Prosecution History

54. I have reviewed the prosecution history of U.S. application no. 18/346,420, which issued as the '109 Patent. EX1002. I have also reviewed Dr. Jain's summary of the prosecution history. EX1003 at ¶¶39-47.

C. Person of Ordinary Skill in the Art

55. 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.

56. 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. I understand inventor Shun-ichiro Kawabata has a Ph.D. in biology and experience in biochemistry. EX2086.

57. 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.

58. 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.

D. Claim Interpretation

59. 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.

60. Dr. Jain has not offered any claim construction opinions. However, his opinions that the Challenged Claims are invalid for lack of written description and enablement are based on a mistaken assumption regarding the scope of the Challenged Claims. Although Dr. Jain did not opine that a POSA would conclude the Challenged Claims have a functional requirement, his opinions proceeded from that premise. In my opinion, a POSA would not view the Challenged Claims as requiring use of the agent to detect endotoxin. That would require mixing the agent with a sample and measuring the progress of a cascade reaction, as recited in claim 10. A comparison of claims 1 and 10 is provided below, emphasizing the differences in their scope.

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 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	A method for <u>measuring endotoxin in a test specimen</u> , the method 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 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 producing an endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme, 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.	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 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, <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 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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61. Although the Challenged Claims require each protein to have activity, that activity can be determined individually. For example, Factor C activity can be detected by adding LPS or a synthetic lipid A analogue to the recombinant Factor C,

along with a suitable substrate for detection. EX1025 at 153; EX2067 at 11 (discussing substrates suitable for detecting activity of each of Pro-clotting enzyme, Factor B, and Factor C). Factor B activity can be detected by adding recombinant or purified native Factor C and a suitable detection substrate. EX2002. Pro-clotting enzyme activity can be detected by adding recombinant or purified native Factor B or trypsin, and a suitable detection substrate. EX2003 at 4. Thus, there is nothing in the Challenged Claims that requires a cascade reaction or detection of endotoxin via such a reaction.

62. Dr. Jain's 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) pervades both his written description and enablement analysis. He also appears to conflate Claim 1 and Claim 10 at times. *See, e.g.*, EX1003 ¶¶65, 69, 70, 77, 123-124. It is not possible to parse his opinions to identify those directed to the Challenged Claims, i.e., claims 1-9, that were not affected by his misunderstanding of the claims.

VI. GROUND 1: WRITTEN DESCRIPTION

63. I understand that written description is assessed as of the filing date of the '109 patent, which is July 3, 2023. However, Dr. Jain's opinions are based on a POSA as of December 10, 2013, the effective filing date of the '767 Application.

EX1003 ¶51. Dr. Jain has not offered any opinions from the perspective of a POSA in July 2023. My substantive response to Dr. Jain's opinions from the perspective of a December 10, 2013 POSA is set forth in Section VIII, below.

VII. GROUND 2: ENABLEMENT

64. I understand that enablement is assessed as of the filing date of the '109 patent, which is July 3, 2023. However, Dr. Jain's opinions are based on a POSA as of December 10, 2013, the effective filing date of the '767 Application. EX1003 ¶51. Dr. Jain has not offered any opinions from the perspective of a POSA in July 2023. My substantive response to Dr. Jain's opinions from the perspective of a December 10, 2013 POSA is set forth in Section VIII, below.

VIII. GROUND 3: THE CLAIMS ARE NOT ANTICIPATED

65. In my opinion, the Challenged Claims are entitled to the December 10, 2013 filing date of the '767 Application because the disclosures in that application provide written description and enablement support for the Challenged Claims. Accordingly, the '629 Publication is not prior art because its earliest possible priority date is October 18, 2016 and the '629 Publication cannot anticipate the Challenged Claims. Even if I were to make the same assumption concerning function as Dr. Jain, it is my opinion that the '767 Application provides adequate written description support and enables the Challenged Claims as of December 10, 2013.

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

66. There is express support in the '767 Application for the Challenged Claims. The specification refers to proteins from *Limulus polyphemus* multiple times. *See* EX1017 at 30-31, 56 ([0020], [0046]). It also describes use of an assay that is fully recombinant, *i.e.*, employing recombinant Factor C, Factor B, and Pro-clotting enzyme. *Id.* at 94-95 ([0129]-[0130]). In my opinion, a POSA would have understood that the inventors considered the *Limulus polyphemus* zymogens to be a part of their invention. 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 have allowed a POSA to arrive at the cDNA and amino acid sequences of *Limulus polyphemus* Factor C through routine experimentation.

67. As discussed below, the Challenged Claims require only ten proteins to practice their full scope. The '767 Application discloses three of those proteins—one each for Factor C, Factor B, and Pro-clotting enzyme, and those proteins are representative of the corresponding proteins from other horseshoe crabs. Thus, in my opinion the '767 Application discloses a representative number of species. Further, the relationship between the structure of the three zymogen proteins and their function was well-known in the art as of December 10, 2013. I understand that

a showing of either a representative number of species or a structure-function relationship is sufficient for written description.

1. A POSA in December 2013 would have understood the inventors possessed the sequences of Factor C, and Factor B and Pro-clotting enzyme

a. Limulus polyphemus sequences

68. The '767 Application discloses the sequences of Factor C, Factor B, and Pro-clotting enzyme of *Tachypleus tridentatus*. Although the '767 Application does not expressly disclose the *Limulus polyphemus* sequences of these three proteins, their cDNA sequences could be obtained from public sources and through routine experimentation as of December 10, 2013.

69. As discussed above, the NCBI BioProject database contains two BioProjects registered by the University of Connecticut for *Limulus polyphemus*. The first, accession number PRJNA178588, is an amoebocyte cDNA transcriptome from a horseshoe crab sampled from coastal waters near Cape Cod. EX2072 (PRJNA178588). This BioProject generated experimental dataset SRX202160 with one sequencing run, number SRR610305. EX2072 (PRJNA178588); EX2073 (SRX202160); EX2074 (NCBI SRR610305). The other BioProject, accession number PRJNA178587 was a whole genome shotgun sequence, which generated two experimental datasets SRX202152 and SRX202154. EX2071 (PRJNA178587); EX2075 (SRX202152 and SRX202154). The sequence data deposited in connection

with these two BioProjects was published by October 31, 2013. EX2072; EX2073; EX2074; EX 2075; EX2078. 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. See EX1003 ¶49.

70. 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 sequences of Factor C, Factor B, and Pro-clotting enzyme, a POSA would have used the *Tachypleus tridentatus* sequences disclosed in the '767 Application as an input for the BLAST tool to find similar sequences in the deposited data. See, e.g., EX1011 at 39-43. Alternatively, or in combination, A POSA would have used the CD-Search tool and publications to identify conserved domains in each *Tachypleus tridentatus* protein 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 each protein, from which the open reading frame (ORF) of the gene in question would be assembled.

71. Given the knowledge of the slow evolutionary rate of horseshoe crabs and the high conservation across species of known clotting cascade proteins, I used the TBLASTN function to query the SRX202160 library of cDNA reads, using the full protein sequences from *Tachypleus tridentatus* Pro-clotting enzyme, Factor C

and Factor B. I confirmed that the complete or nearly complete coding sequence of *Limulus polyphemus* Pro-clotting enzyme and Factor B could be assembled from the transcriptome deposit with a high degree of accuracy, and that 97.2% of the full Factor C coding sequences 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 each protein would be performed by PCR and sequencing of the entire ORF for Factor C, Factor B and Pro-clotting factor. Design of the required primers would be readily enabled by analysis of the BioProject sequencing data, as described above.

72. Lists of top BLAST hits in the SRR610305 transcriptome data when queried with the *Tachypleus tridentatus* sequences of Factor C, Factor B, and Pro-clotting enzyme and CD-Search results for each protein are attached as Exhibit 2093. 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 *Tachypleus tridentatus* protein sequence and the translated sequences from the *Limulus polyphemus* clones.

Also, most or all of each protein is confirmed by multiple clones, yielding high confidence in the resulting assembled sequences of *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme:

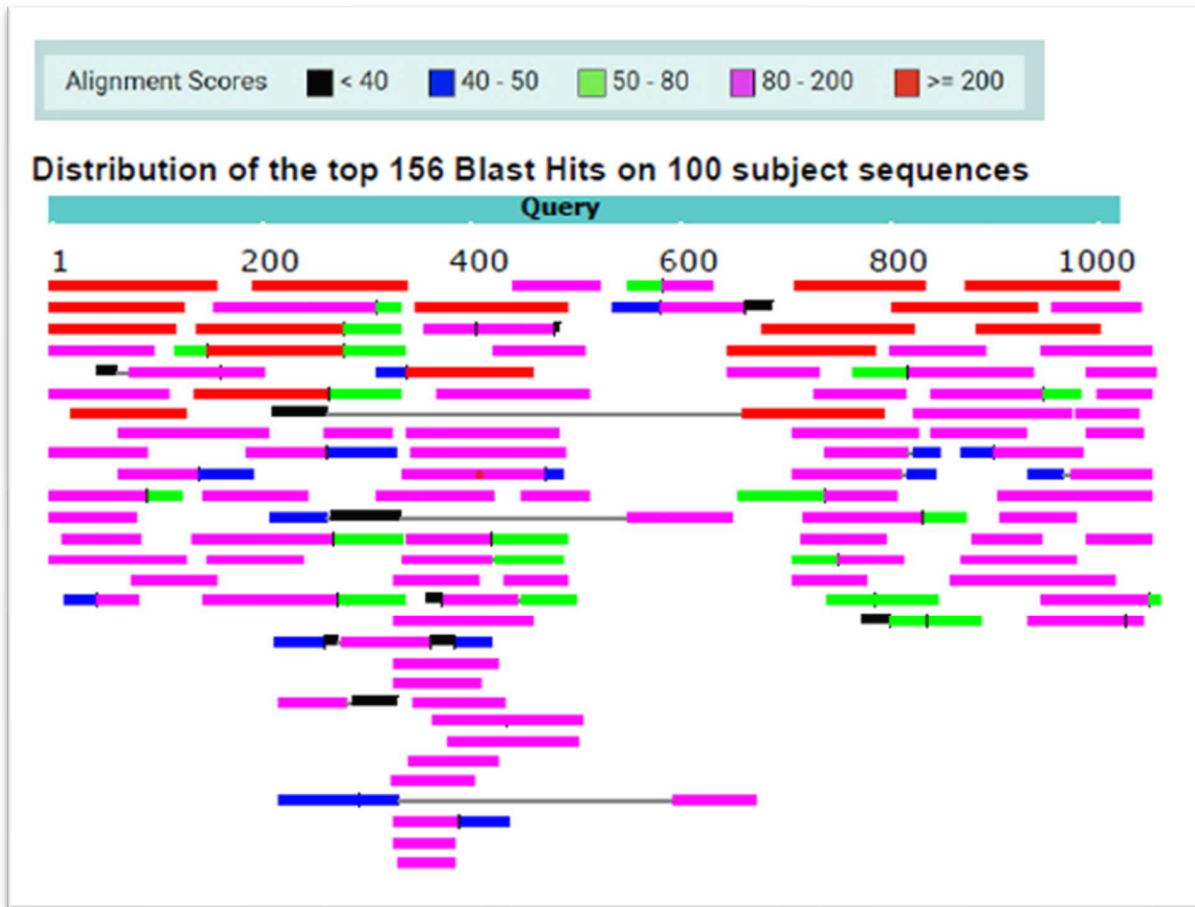


Figure 8A: Distribution of the top 156 BLAST hits (line segments below numbers) for *Tachypleus tridentatus* Factor C (the query, in teal, above numbers).



Figure 8B: Distribution of the top 114 BLAST hits (line segments below numbers) for *Tachypleus tridentatus* Factor B (the query, in teal, above numbers).



Figure 8C: distribution of the top 126 BLAST hits (line segments below numbers) on *Tachypleus tridentatus* Pro-clotting enzyme (query, in teal, above numbers)

73. A POSA in December 2013 would have understood the inventors did or would have been able to perform the above steps to identify the cDNA sequences encoding the *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme. Thus, based on information known in the art, a POSA would have understood that the inventors possessed the claimed invention.

74. Alternatively, a POSA in December 2013 would have been able to isolate the sequences for *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme by using PCR-based methodologies, including the RACE method or a variation thereof. 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.” EX2087 at 1-2. The RACE protocol was first described by Frohman *et al.* in 1988. *Id.* at 2; EX2004 ¶8. I described how to modify and optimize this method in 1995. EX2087. RACE was so commonplace that kits from multiple manufacturers were available for purchase by December 2013. EX2004 at 103-179.

75. I reviewed a November 24, 2024 declaration by Michael A. Frohman, MD, PhD, the author of the first publication describing the RACE 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 '109 Patent. EX2004 ¶4; EX1065 at 67.

76. 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 data 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).

77. 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* (Fig. 4, and EX1025 at 157), in combination with alignment with the complete sequences of Factor C from *Carcinoscorpius rotundicauda* and *Tachypleus 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.

78. 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] 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 EX1025 (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 Jan. 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 ORFs of *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme.

79. It is further my opinion that carrying out a RACE protocol to obtain the cDNA sequences of *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme 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 cDNA sequence for Factor C. EX2004 ¶¶18-19. The analysis required to re-assemble the sequences of Factor C, Factor B and Pro-clotting enzyme from amoebocyte transcriptome data, which contains cDNA sequences only, would be significantly less than starting from whole genome data, which contains introns and other non-coding sequences.

80. It is further my opinion that the sequences of *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme, could also have been obtained in December 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*. EX1025; EX2002; EX2003; EX1025 at 157). I agree with Prof. Frohman that success does not depend on the specific protocol or kit used. EX2004 ¶¶34-37.

81. 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.

82. The peptide sequences reported by Tokunaga (EX1025) 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 *Limulus polyphemus* sequence.

83. 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. EX2087.

84. 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 (EX1025). 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.

85. In the unlikely event that the nested primer 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. Once the 5' and 3' ends had been successfully cloned by RACE PCR, the remainder ("middle") of Factor C could be cloned by designing gene-specific primers within the newly cloned 5'- and 3'-end sequences of *Limulus polyphemus* Factor C.

86. 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 mammalian cells.

87. The available sequences from *Tachypleus tridentatus* would have provided adequate information for the same approach to isolate the complete *Limulus polyphemus* Factor B and Pro-clotting enzyme cDNAs (e.g., 5'- and 3'-RACE using nested primers, with the same hybrid structure as described above, followed by PCR to amplify the middle portion of each cDNA, using gene specific primers based on the cloned 5'- and 3'-ends of the *Limulus polyphemus* Factor B and Pro-clotting enzyme cDNAs. Assembly of full ORF clones and cloning into appropriate vectors would be as described for Factor C).

88. I agree with Prof. Frohman, that as an alternative to relying on the sequence information in Tokunaga 1991 (EX1025), 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), 297-310 (D24).

89. 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. This library screening protocol could have been used to obtain the cDNA sequences of *Limulus polyphemus* Factor B and Pro-clotting enzyme also, using the known *Tachypleus tridentatus* sequences to generate probes for screening each library.

b. Carcinoscopus rotundicauda and Tachypleus gigas sequences

90. The above-described PCR-based methods, including RACE-PCR could be used to obtain the cDNA sequences for Factor B and Pro-clotting enzyme from *Carcinoscorpius rotundicauda* and *Tachypleus gigas* by designing primers based on the *Tachypleus tridentatus* sequences disclosed in the '767 Application, as outlined above in paragraph 87.

91. Factor B and Pro-clotting enzyme were both known to include conserved CLIP and serine protease domains that would give a POSA a high degree

of confidence that RACE could be used to clone these genes. For example, researchers used primers based on the serine protease domain of *Tachypleus tridentatus* Factor B to clone a novel serine protease from a scallop using 5'-RACE and 3'-RACE. EX2088.

92. Alternatively, the *Tachypleus tridentatus* sequences could be used to identify these sequences in a standard phage library screening protocol.

93. 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. EX2046 at 5:19-24 and cl. 1. Factor G comprises two subunits, alpha and beta. See EX2038 at 4-5, Fig. 4. Petitioner's patent does not disclose nucleic acid or amino acid sequences for Factor G, and, as reflected in EX2113, the sequence of Factor G-subunit alpha was not published in an article until 2011.⁸

⁸ The authors of EX2113 submitted the sequence to GenBank on July 2, 2010. See <https://www.ncbi.nlm.nih.gov/nuccore/AB547712.1> (last accessed Nov. 26, 2025).

2. A POSA in December 2013 would have understood the inventors possessed methods of expressing Factor C, Factor B, and Pro-clotting enzyme in mammalian cells

94. With respect to expression of the proteins in mammalian cells, the '767 Application discloses expression of functional Factor C protein in CHO DG44 and HEK293 cells. EX1017 at 85-88 ([0107]-[0115]). Prof. Frohman noted these disclosures (EX2004 ¶45⁹) and concluded that “[g]iven that these cells were able to incorporate vectors containing cDNA for *Tachypleus tridentatus* and/or *Carcinoscorpius rotundicauda* and express the Factor C-encoding mRNAs and 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.

⁹ Prof. Frohman’s reference D1 is EP 2930241A1, which has the same disclosure as the '767 Application. *See* EX2006 ¶51.

95. 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 mammalian cells.

96. With respect to Factor B and Pro-clotting enzyme, the '767 Application teaches that procedures for expressing Factor C in mammalian cells may be applied “mutatis mutandis” to Factor B and Pro-clotting enzyme, meaning that essentially the same procedure may be used to express all three proteins in mammalian cells. EX1017 at 63 ([0057]). Dr. Jain has not cited any publications supporting that those procedures would be unsuccessful for Factor B and Pro-clotting enzyme. 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. Indeed, the '629 Publication shows that they would have been successful. EX1015 at [0350]-[0351]; *see also* Section VIII.B.4 below.

97. Thus, it is my opinion that a POSA in December 2013 would have understood from the '767 Application that the inventors possessed methods of recombinantly expressing Factor B and Pro-clotting enzyme, as well as Factor C, in mammalian cells.

3. The Challenged Claims are not broad

98. Dr. Jain asserts that claim 1 is broad and encompasses “thousands of possible methods for producing the claimed endotoxin assay agent.” *See, e.g.*, EX1003 ¶70. I disagree with Dr. Jain’s counting and calculations.

99. First, I disagree with Dr. Jain’s counting of the number of proteins encompassed by claim 1. A recombinant protein is defined by its amino acid sequence. Claim 1 requires only ten different amino acid sequences: two Factor C sequences (from each of two species of horseshoe crab), four Factor B sequences, and four Pro-clotting enzyme sequences from each of the four species of horseshoe crab. Dr. Jain made two unjustified decisions that inflated these 10 sequences into “at least 54 proteins” and “at least 3,456 possible combinations.” EX1003 ¶70.

100. Dr. Jain’s first unjustified decision was to count different mammalian expression systems as distinct proteins or “options.” Claim 1 does not specify a particular mammalian host cell for Factor B or Pro-Clotting enzyme. But regardless of the host cell, 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. *See* EX2089 at 1. 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 cDNA sequence – a total of 10 vectors – to practice claim 1 across many mammalian cell lines. Therefore, I disagree with Dr. Jain’s decision to count the same protein expressed in different mammalian cell lines as distinct proteins or “options.”

101. Correcting for this decision reduces Dr. Jain’s calculation of the number of proteins encompassed by claim 1 to a total of ten (two Factor C proteins and four each of Factor B and Pro-clotting enzyme proteins). Claim 4 further limits the options for Factor B and Pro-clotting enzyme to *Limulus polyphemus* or *Tachypleus tridentatus*—a total of six or eight proteins.

102. Dr. Jain’s second unjustified decision was to include two methods of recovery of each protein as separate “options.” 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]). Secreted or not, it is still the same the same sequence of amino acids and the same protein, not two different “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 1. 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.

103. Correcting for these two decisions reduces Dr. Jain's calculation of 3,456 possible combinations to 32 for claim 1, as shown below in Figure 9, which is an annotated version of Dr. Jain'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~~	~~<i>Tachypleus tridentatus</i>~~ ~~<i>Tachypleus gigas</i>~~ ~~<i>Limulus polyphemus</i>~~ ~~<i>Carcinoscorpius rotundicauda</i>~~	~~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~~	~~<i>Tachypleus tridentatus</i>~~ ~~<i>Limulus polyphemus</i>~~	~~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~~	~~<i>Tachypleus tridentatus</i>~~ ~~<i>Tachypleus gigas</i>~~ ~~<i>Limulus polyphemus</i>~~ ~~<i>Carcinoscorpius rotundicauda</i>~~	~~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

Figure 9: Annotated version of Dr. Jain's table (EX1003 at 28).

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

4. The specification discloses a representative number of species

105. As discussed, claim 1 requires only ten proteins: two Factor C’s, and four each of Factor B and Pro-clotting enzyme. The specification discloses one of the two Factor C proteins, one of the four Factor B proteins, and one of the four Pro-clotting enzyme sequences required to make these ten vectors.

106. The *Tachypleus tridentatus* sequences disclosed in the specification 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 identity and similarity of these proteins across species and their known functional similarity. Thus, in my opinion, the ’767 Application discloses a representative number of the ten proteins required by claim 1.

5. A structure-function correlation was known in the art as of December 2013

107. Dr. Jain argues that known differences in taxonomy, physiology, genetic features and geography would have foreclosed a POSA from drawing any conclusions about the interactions of Factor C and Factor B proteins from different species of horseshoe crab. EX1003 ¶¶71-72. I disagree.

108. As discussed above, it was well-known that horseshoe crabs evolved slowly. *See* EX2065 at 1, 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. EX2024 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.*, EX2091 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.

109. 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.*, EX1025 at 153 (stating in 1991 that “[t]hese findings also lead to the idea that the hemocytes of probably contain a coagulation system similar to that established for the *T. tridentatus* hemocytes.”); EX2005 at 2 (stating in 2010 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”).

110. 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* proteins, and *Limulus polyphemus* Factor C. EX1010 at 6556 (Fig. 2), 6557; EX2002 at 3-4 (Figs. 3, 4); EX2003 at 4; EX1025 at 150-151; EX2057 at 3-6 and Fig. 3 (reproduced below).

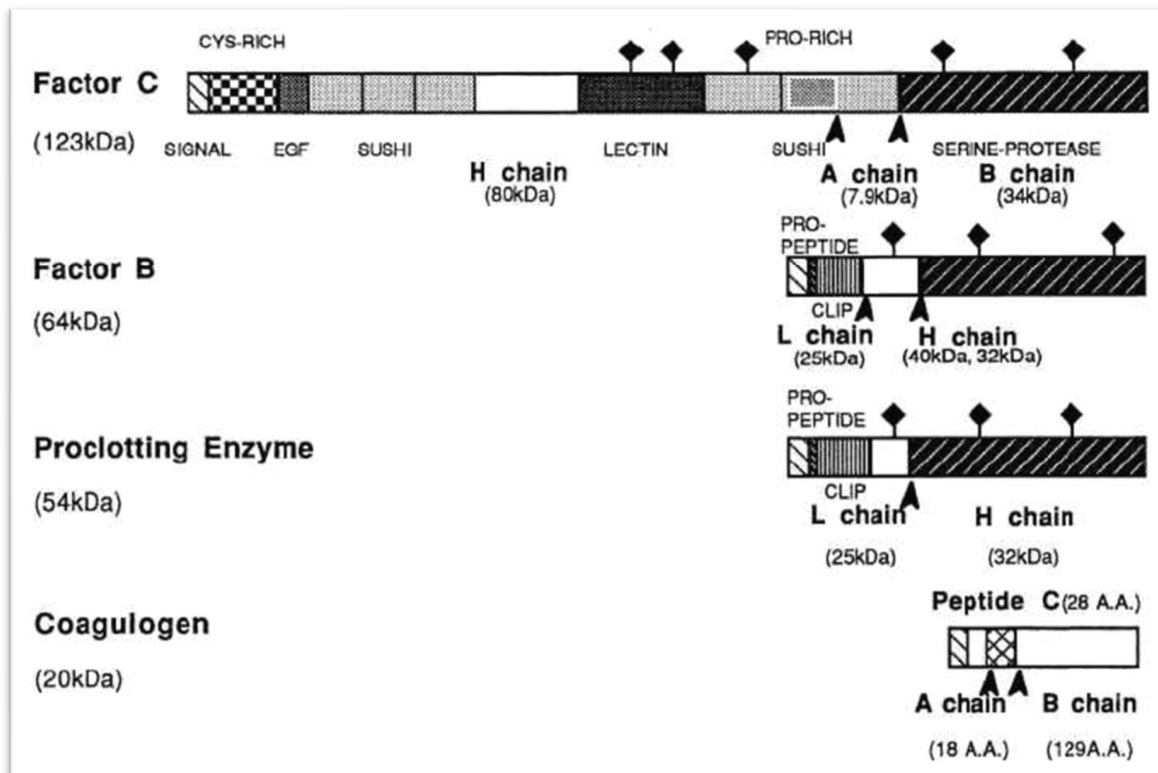


Figure 10: Domain structure of *limulus* clotting factors. EX2057, Fig. 3. Arrowheads indicate cleavage sites for zymogen activation; diamonds indicate potential glycosylation sites.

111. A POSA in December 2013 would have expected that, because the structure and biochemistry of these three proteins were highly similar across horseshoe crab species each 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 EX1010 at 6558 (Fig. 6); EX2024 at 11 (Factor C C-terminal serine protease domain

has substantial similarities with human alpha-thrombin); EX2092 at 2 (Fig. 1 – showing Factor C in chymotrypsin family); EX2067 at 17-18.

112. Finally, the '767 Application's description 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, is significant. EX1017 at 92-95 [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.”). This disclosure 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.

a. Factor C

113. 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 97.7%. EX1017 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 that *Carcinoscorpius rotundicauda* Factor C had approximately 90% identity with *Limulus polyphemus* Factor C. EX2047 ¶ [0134]; EX2048 at 4; EX2070 at 2 (“The rFC protein derived from shares a 90.5% identical amino acid sequence with the native Factor C protein derived from *L. polyphemus* (unpublished data).”); 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*).

114. 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. EX2065 at 4.

115. 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 known *Carcinoscorpius rotundicauda* sequence and the *Tachypleus tridentatus* sequence

disclosed in the '767 Application. My goal was to identify residues in the *Limulus* 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.¹⁰ My analysis is found in EX2094 at 1-2. 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.

116. A POSA in December 2013 knew 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. 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*

¹⁰ Clustal is a widely used program for sequence alignment. The Omega version was introduced in 2011. EX2095 at 1.

rotundicauda Factor C and native *Limulus polyphemus* Factor C “are highly comparable” in terms of detecting endotoxin).

117. I generated sequence similarity and structure comparisons and predictive 3D structures via the Phyre2 web server. EX2094; EX2112. Visualizations of structures were via YASARA, and comparisons between structure overlays were via the MUSTANG algorithm in the YASARA software package).¹¹

118. 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. EX2094.

119. However, 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. *Id.* A graphical depiction of the Clustal alignment for

¹¹ Phyre2 was launched in January 2011 and YASARA was first released in 1993.

the three Factor C proteins, with conserved domains and non-conservative substitutions is provided below and in EX2114.

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

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

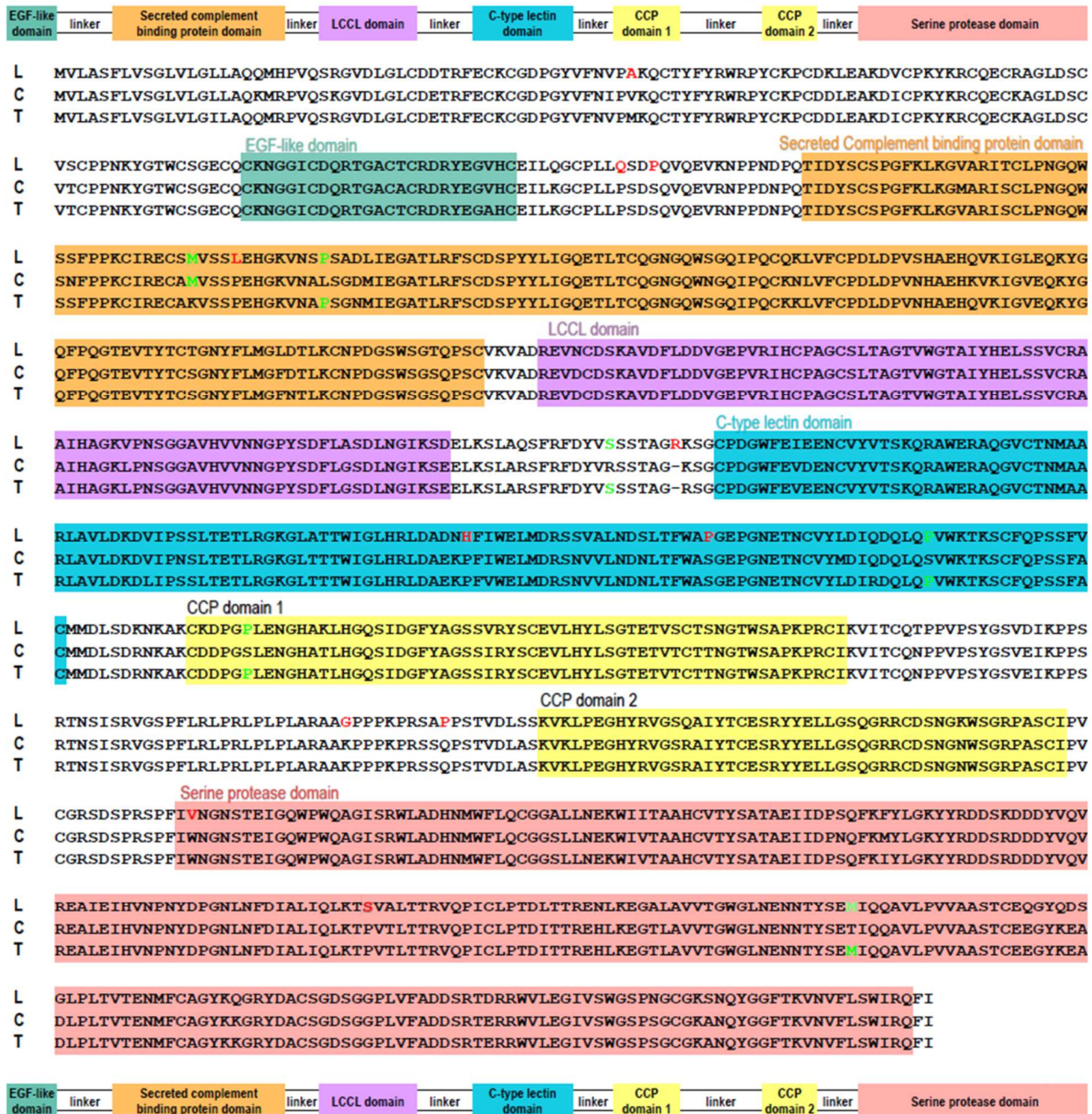


Figure 11: Clustal alignment of *Limulus polyphemus*, *Carcinoscorpius rotundicauda* and *Tachypleus tridentatus* Factor C amino acid sequences, with functional domains marked. Non-conservative substitutions where the *Limulus* sequence differed from either one or both of the other species in green and red, respectively.

120. 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. EX2112. 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. *Id.* 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.

121. However, a POSA in December 2013 would have 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.

122. 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. 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. 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.

123. 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*. EX2051 at 1; EX2052 at 1; EX2053 at 2. I understand that this harmonization was accomplished by extensive efforts to demonstrate equivalence in terms of interchangeability of procedures, specifications, and results. EX2082.

124. 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., EX2048 at

4; 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”).

125. 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*, *carinoscoripus* [sic] and *tachypleus*) will behave similarly to all of the proteins that are found within an aqueous amoebocyte extract.” EX2054 at 3. Petitioner characterized data from “initial studies” as “confirm[ing] 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.” *Id.* (citing a publication from 2000)).

126. Finally, a POSA in December 2013 would have been aware that recombinant Factor C from *Carcinoscorpius rotundicauda* and recombinant Factor B and Pro-clotting enzyme from *Tachypleus tridentatus* had been used to successfully reconstruct an endotoxin detection cascade in 2008. EX2023 ¶9 (citing EX2050); EX2050 at 35-38 ([0110], [0171]-[0182]). Thus, it was known in the art as of December 2013 that Factor C from one species would activate Factor B from

another species. Given the high sequence similarity between Factor B and Pro-clotting enzyme discussed further below, a POSA would similarly expect interchangeability across species for these components of the cascade reaction as well. EX2002 at 4 (Fig. 2).

b. Factor B and Pro-Clotting Enzyme

127. As discussed above, Factor B and Pro-clotting enzyme from *Tachypleus tridentatus* were cloned and sequenced in 1993 and 1990, respectively. 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. EX2038 at 3-4; EX2039 at 2-3 and Fig. 4. These two domains are conserved across arthropods, including other crustaceans and insects. See, e.g., EX2088 2; EX2097 at 7-9.

128. 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.

129. Dr. Jain cites post-priority date evidence that the *Tachypleus tridentatus* and *Limulus polyphemus* Factor B and Pro-clotting enzyme proteins have “a low degree of sequence homology.” EX1003 ¶72. He does not provide the analysis upon which this conclusion was based, but this appears to be derived from a simple BLAST search using *Tachypleus tridentatus* Factor B to find the *Limulus*

polyphemus Factor B protein sequence, which yields a reported “percent identity” of 84.53%.

130. Regardless, I disagree with his characterization of this reported 84% number as a measure of sequence *similarity* (indeed, “homology” is not used in the proper context by Dr. Jain), as the number 84% is only a measure of sequence *identity*, which is a more strict (and less useful) metric as compare to a measure of sequence conservation across species, which should also include consideration of conserved substitutions. When a comparison is performed that also takes similarity into account, using the Clustal algorithm, the percentage of non-conservative substitutions is only 4.3%, meaning that the actual percent similarity (i.e., including similar amino acids in addition to identical amino acids) is 95.7%. EX2094. The Pro-clotting enzymes of *Limulus polyphemus* and *Tachypleus tridentatus* exhibit a similar degree of similarity, with 5.3% non-conservative substitutions and 94.7% similarity. A POSA would consider these *high* sequence similarities. Unsurprisingly, in view of their sequence similarity, these enzymes were known to have the same function.

131. Further, with respect to Pro-clotting enzyme from each species, it was known that their target—coagulogen—was remarkably similar across species, and are cleaved at the same sites. EX2080 at 4-5, Fig. 15. Thus, any differences in sequence between the two Pro-clotting enzyme proteins does not affect their ability

to bind to and activate coagulogen. This emphasizes that any differences in their sequences are not relevant to their function.

132. I generated sequence similarity and structure comparisons for Factor B and Pro-clotting enzyme across *Limulus polyphemus* and *Tachypleus tridentatus*. As for Factor C, above, I utilized the Phyre2 web server, structural visualizations via YASARA, and comparisons between structure overlays via the MUSTANG algorithm in the YASARA software package.

133. In comparing the Factor B protein of *Tachypleus tridentatus* with that of *Limulus polyphemus*, the Clustal alignment shows that the most dense region of non-conserved amino acids lies between residues 85 and 110 ($6/26 = 23\%$), which lies between the CLIP domain (residues 38-82; $1/45 = 2.2\%$ non-conserved residues) and the serine protease domain (residues 148-390; $7/243 = 2.9\%$ non-conserved residues), a region which is of no known functional importance. In performing modeling of the 3-dimensional structures (using Phyre2, and selecting the model based on template c2anyA, which was the common model with the highest score for *Tachypleus tridentatus* and *Limulus polyphemus*), for high scoring models shared between both species, only the serine protease domain was modeled, and the region of low sequence conservation could thus not be compared by this methodology. In comparing the two serine protease domains, the two resulting predicted structures were highly similar, with a very high degree of overlap. Thus, considering these

data together, a POSA would conclude that the key functional domains of Factor B 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.

134. In comparing the Pro-clotting enzymes of *Tachypleus tridentatus* with that of *Limulus polyphemus*, the Clustal alignment shows that the most dense region of non-conserved amino acids lies between residues 89 and 117 ($7/29 = 24\%$). Comparing the predicted 3-dimensional structures (using Phyre2, and selecting the model based on template c2xx1A, which was the highest scoring model for both *Tachypleus tridentatus* and *Limulus polyphemus* Pro-clotting enzymes), this same region includes the only clear difference in the structures (approximately residues 94-110). Importantly, this region of low sequence conservation lies between the two predicted conserved domains (CLIP domain, residues 40-85; protease domain, residues 128-370), suggesting that it is simply a connecting region for which 3D structure is of minimal importance. The Clustal alignment shows only two non-conserved substitutions ($2/46 = 4.3\%$) in the CLIP domain and seven non conserved substitutions ($7/243 = 2.9\%$) in the serine protease domain. Additionally, the structural prediction shows a very high degree of overlap between these two conserved domains, with the two predicted structures being essentially indistinguishable in these regions. Thus, considering the above data collectively, a

POSA would conclude that the key functional domains of Pro-clotting enzyme 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.

135. In sum, the structures of Factor C, Factor B and Pro-clotting enzyme and their functions had been established and characterized well before December 2013. For Factor C, significant similarity and functional identity across three out of four horseshoe crab species was known, including the two species recited in the Challenged Claims. A POSA would have expected the same to be true for Factor B and Pro-clotting enzyme based on the similarity to Factor C and to each other established for *Tachypleus tridentatus* proteins.

6. The field had not struggled to sequence or structurally or functionally characterize *Limulus polyphemus* genes or proteins

136. Dr. Jain uncritically relies on statements made in a post-priority date publication from Patent Owners, the '498 PCT (EX1005), which is substantially identical to the '629 Publication (EX1015), as evidence that “the field had struggled to sequence and structurally and functionally characterize the *Limulus polyphemus* species of horseshoe crab” and “*Limulus polyphemus* zymogen proteins.” EX1003 ¶¶67, 88. 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.

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 (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 of *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.
- A *Limulus polyphemus* 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 sequence isolated 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 WO 92/20715 at 11 (Figs. 15-16).
- The complete cDNA sequence of a *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-PCR). EX2107.
- A protein from *Limulus polyphemus* regulating eye development was cloned in 2008 (using RACE-PCR). EX2108.
- The cDNA sequence of *Limulus 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 8825 entries. EX2109.

138. Dr. Jain also relies on the statement 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, this does 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 EX1010 (1991 for Factor C); EX2002 (1993 for Factor B); EX2003 (1990 for Pro-clotting enzyme). Further, publications reflect that amino acid or cDNA sequences had been obtained for *Limulus polyphemus* Factor C well before 2013. EX1025; EX2047; EX2048; EX2070. Dr. Jain does not address these publications in the context of his opinions concerning a purported struggle in the field.

139. Finally, I have reviewed the process described in the ’498 PCT/’629 Publication for how those inventors cloned the *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme genes. EX1005 at [0149]-[0177]; EX1015 at [0321]-[0349]. 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 *Limulus 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.

140. Thus, I disagree with Dr. Jain that the vague and unverified statements from the '629 Publication establish that determining the cDNA sequences of *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme would have been a struggle as of December 10, 2013, or that the inventors "could not have" described those proteins. *See* EX1003 ¶66. Instead, I agree with Prof. Frohman that it would have been a matter of routine experimentation to determine those sequences using

information available as of December 10, 2013, either by analyzing the amoebocyte transcriptome data or by RACE.

7. Claims 2-9

141. In my opinion, based on the '767 Application's disclosure, a POSA would have understood the inventors to have possessed the invention of claim 1. Because claims 2-4 further narrow the scope of the elements recited in claim 1, a POSA would also have understood the inventors to be in possession of the invention of claims 2-4.

142. Claims 5-6 recite characteristics of the recombinant Factor C. The '767 Application describes how to determine these properties. EX1017 at 100-107 ([0141]-[0156]). A POSA would have understood that being in possession of the recited Factor C proteins, the inventors would also have possessed the properties recited in claims 5-6.

143. Claim 7 recites the step of filtering the culture supernatant into which the Factor C is secreted. This is expressly described in the '767 Application. EX1017 at 66, 87, 88 ([0066], [0112], [0115]). Claim 8 recites formulating the endotoxin assay agent into a particular form, i.e., a solid, liquid, or gel. This is expressly described in the '767 Application. EX1017 at 70, 94 ([0074], [0129]).

144. Finally, claim 9 describes an embodiment in which the three zymogen proteins are "separately expressed in individually established host cells." This is also

described in the '767 Application. EX1017 at 67, 93-94 ([0068], [0128]-[0130]). Thus, a POSA would have understood the inventors possessed the invention of claims 5-9.

B. The Challenged Claims are Enabled by the '767 Application and Knowledge in the Art

145. Dr. Jain argues that the Challenged Claims are not enabled by, and therefore not entitled to the priority date of the '767 Application. EX1003 ¶¶86, 142. I disagree with Dr. Jain and respond to his arguments based his parsing of the *Wands* factors below.

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

146. Dr. Jain asserts that “the method of claim 1 broadly encompasses a genus of at least 3,456 possible options for producing the claimed assay agent.” EX1003 ¶85. As discussed in Section VIII.A.1 above, Dr. Jain’s calculation of the number of combinations encompassed by the Challenged Claims is substantially overstated. Accordingly, I disagree that the Challenged Claims are broad. 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. Indeed, these would all be considered “boilerplate” techniques in the repertoire of a POSA during that period of time.

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

147. Dr. Jain asserts that “the field had struggled to sequence and recombinantly produce... *Limulus polyphemus* zymogen proteins.” EX1003 ¶88. However, he does not cite any peer-reviewed publications to this effect. Further, as discussed in Section VIII.A.6, above, the numerous publications on and sequence deposits of *Limulus polyphemus* genes rebut Dr. Jain’s conclusion. Those publications included 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); EX2100 (endotoxin-binding protein); EX2101 (limunectin); EX2102 1992 (hemagglutinin and amebocyte aggregation factor). Professor Frohman’s declaration discussed in Section VIII.A above, supports that sequencing and recombinantly producing *Limulus polyphemus* Factor C would have been routine and straightforward for a POSA.

148. The references Dr. Jain cites to support his opinion that “a significant amount of research and experimentation was still required to generate a particular protein, in a functional state, across a number of different expression systems” (EX1003 ¶89) do not reflect the state of the art as of December 2013, given that they were published in 2004 (EX1031), 2010 (EX1030), and 2009 (EX1039). Further, the two more recent references concern how to analyze two-dimensional

electrophoresis and optimizing protein glycosylation in manufacturing processes, which bears little relation to the Challenged Claims. EX1030; EX1039.

149. Dr. Jain points to another group's difficulties with expressing *Carcinoscorpius rotundicauda* Factor C in COS-1 cells, EX1003 ¶¶90, but this ignores that: 1) *Carcinoscorpius rotundicauda* Factor C is outside the scope of the Challenged Claims; and 2) the '767 Application describes in detail how to express functional recombinant Factor C from two exemplary mammalian cell lines. As the largest 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.

150. Dr. Jain's other opinions concerning these factors are based on his erroneous assumption that claim 1 requires a functioning endotoxin assay and functioning endotoxin assay agent and are therefore inapplicable to the correct scope of claim 1. EX1003 ¶¶91-92.

151. Dr. Jain cites EX1031, which again was outdated as of 2013, and EX1006 to support his opinions that "designing, expression, and testing recombinant proteins is laborious, time-consuming, complicated, and highly unpredictable." EX1003 ¶¶96. EX1006 concerns 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”). EX1006 deals with a different issue entirely. There is no need to attempt to predict function here because Factor C, Factor B and Pro-clotting enzyme were well characterized in terms of structure and function. EX2038 at 3-6; EX2057 at 3-4, 5-6; EX2058 at 2-6

152. In my opinion, recombinant production of the recited Factor C, Factor B, and Pro-clotting enzyme proteins would have been routine 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)

153. Dr. Jain’s opinions concerning *Wands* factor 6 is again based on his erroneous assumption that the Challenged Claims require a working cascade that detects endotoxin. EX1003 ¶96. Even if the Challenged Claims required a working cascade, I disagree that practicing them would be beyond the abilities of a POSA.

154. As Dr. Jain agrees, the level of ordinary skill is high under his definition. EX1003 ¶98. It is high under my proposed definition as well. Illustrating that practicing the Challenged Claims would not be beyond the skill of a POSA, Karen McClymont, a graduate student and therefore someone with less than ordinary skill, was able to use the genomic data for *Limulus polyphemus* to assemble the cDNA sequence of Factor C, engineer the sequence to suit her needs, express it in

multiple expression systems, and conduct structural studies on the mechanism of LPS binding for her thesis work. EX1011 at 48, 145-146; EX1003 ¶¶37, 66 (citing EX1011). That work was likely begun before December 2013. EX1065 at 106-107. Assembling the Factor C cDNA sequence from the raw genomic data was, as a matter of logic, the first step of her thesis work. EX1011 at 36. In my opinion, Ms. McClymont's thesis work shows that practicing the Challenged Claims was within the level of skill of a POSA as of December 2013.

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

155. As with other factors, Dr. Jain's analysis of factors 1-3 is based on his erroneous assumption that the Challenged Claims require a "functioning assay for detecting endotoxin." EX1003 ¶¶99-100, 102, 111. Even if the Challenged Claims required a functioning assay, I disagree with Dr. Jain. In my opinion, the '767 Application provides sufficient guidance to make an endotoxin assay agent having the three recited components and determining whether those assays were functional such that practicing the Challenged Claims would not require undue experimentation.

a. Production of Factor B and Pro-clotting enzyme

156. Dr. Jain focuses on the lack of examples of expressing Factor B and Pro-clotting enzyme in mammalian cells. EX1003 ¶¶103-118. However, as discussed in Section VIII.A.5 above, the '767 Application provides guidance and

working examples of expression of Factor C in mammalian cells and teaches that those methods could be used “mutatis mutandis” to express Factor B and Pro-clotting enzyme. Dr. Jain made an assumption that the ’767 Application’s teachings that the methods used to successfully express Factor C in mammalian cells could be applied to Factor B and Pro-clotting enzyme was not based on actual experiments. EX1003 ¶107; EX1065 at 157:8-21. This assumption is at odds with his argument that a POSA would not assume the translatability of the expression process across proteins. EX1003 ¶107. If so, then a POSA would expect the “mutatis mutandis” statement to be based on laboratory work.

157. Also as discussed above, Dr. Jain has not provided any citations showing that Factor B and Pro-clotting enzyme would have been difficult to express in mammalian cells via the methods disclosed in the ’767 Application. Indeed, Dr. Jain’s opinions regarding the ’629 Publication (as well as the reference itself) shows that those methods *were* enabling for such expression.

158. Dr. Jain opines that that the ’629 Publication has “detailed directives” such that assembling an endotoxin assay within the scope of the Challenged Claims would not require undue experimentation. EX1003 ¶¶192-193. 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 specification. *Compare* EX1017 at 87-88 ([0108]-[0115]) *with* EX1015 at [0350]-[0351].

'767 Application [0108]-[0115]	'629 Publication [0350]-[0351]
(2) Expression of Factor C in mammalian cells (CHO DG44) In this section, Factor C was prepared by use of CHO DG44, which is a Chinese hamster ovary cell line, as a host through the following procedure. A DNA encoding Factor C of Japanese horseshoe crab, <i>Tachypleus tridentatus</i>, was inserted between the <i>EcoRI</i> recognition site and the <i>XbaI</i> recognition site of pCI-neo (product of Promega), which is a vector for expression in mammalian cells, to thereby construct a Factor C expression plasmid. A kozak sequence (GCCACC) was added just before the start codon of Factor C. The Factor C expression plasmid and a dhfr expression plasmid were incorporated into cultured CHO DG44 cells through simultaneous transfection using a lipofection reagent (Lipofectamine LTX) (product of Life Technologies Corporation). The CHO DG44 cells were statically cultured at 37°C under feeding of 5% CO₂ in an RPMI 1640 medium (5% dialyzed serum, containing 1X penicillin/streptomycin) (product of Sigma) added with 1 mg/mL Geneticin (product of Life Technologies Corporation), whereby CHO DG44 strains into which the genome the	The cDNA [of pro-clotting enzyme] (SEQ ID NO: 13) obtained in the above (5) was ligated to a vector pCA7 for protein expression in mammalian cells, hereby an expression plasmid was obtained. The [Factor B] cDNA (SEQ ID NO: 5) obtained in the above (4) was ligated to the vector pCA7 for protein expression according to a literature (Kobayashi et al., 2015, J Biol Chem, 290: 19379-19386), whereby an expression plasmid was obtained. By using these expression plasmids and the expression plasmid (in which the [Factor C] cDNA of SEQ ID NO: 1 was integrated) obtained in the above (3-7), and ExpiCHO™ Expression System (Thermo Fisher Scientific), recombinant proteins thereof were obtained as culture supernatant instructions. The transfection of the expression plasmids into cells and collection of the recombinant proteins were carried out according to the accompanying instructions.

expression plasmids had been incorporated were selected.

Fifteen days after the simultaneous transfection, MTX (methotrexate) (product of Waka Pure Chemical Industries, Ltd.) was added to the culture medium. The MTX concentration was elevated stepwise from 50 nM to 5,000 nM over three months, and CHO DG44 cell lines highly expressing Factor C were selected as polyclones. Effective amplification of the incorporated Factor C gene by virtue of MTX was confirmed by detecting Factor C in culture supernatant through western blotting using a 2C12 antibody, and quantitating the transcriptional amount of mRNA of Factor C through real-time PCR.

The group of polyclonal CHO DG44 cell lines highly expressing Factor C was diluted by the medium to obtain a cell concentration of 0.5 cells/well, and the dilute was inoculated to a 96-well plate. From about 10 to 20 days after inoculation, the formed cell colonies were successively subjected to expansion culture, whereby CHO DG44 cell lines highly expressing Factor C were cloned. The transcriptional amount of mRNA of Factor C was confirmed through real-time PCR. The amount of Factor C protein secreted to the medium was confirmed through western blotting using a 2C12 antibody.

The Factor C activity (property of being activated by endotoxin) in the culture supernatant was confirmed through

activity measurement using a synthetic substrate Boc-LGR-pNA. Based on these results, a monoclonal CHO DG44 cell line highly expressing Factor C was selected.

The CHO DG44 cell line highly expressing Factor C (monoclonal) was conditioned in a serum-free complete synthetic medium containing 5 μ M MTX and thus floated. The cell line was grown at 37°C under feeding of 5% CO₂ to a later phase of the logarithmic growth phase. The thus-obtained floating culture broth was centrifuged (3,000xg, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22- μ m filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (CHO).

(3) Expression of Factor C in mammalian cells (HEK293)

In this section, Factor C was prepared by use of HEK293, which is a human embryo kidney cell line, as a host through the following procedure.

A DNA encoding Factor C of a Japanese horseshoe crab, *Tachypleus tridentatus*, was inserted between the *Eco*RI recognition site and the *Xho*I recognition site of pCA7 (Makoto Takeda, et al., J. Virol. 79 (22): 14346-54 (2005)), which is a vector for expression in mammalian cells, to thereby construct a Factor C expression plasmid. A kozak sequence (GCCACC) was added just before the start codon of Factor C.

The Factor C expression plasmid was incorporated into cultured HEK293 cells through transfection using polyethylenimine (product of Sigma) (Hashiguti et al., Expression of Recombinant Protein Using Cultured Human Cells-Standard Protocol by 293-type cells-, PSSJ Archives, 1, e017 (2008) [URL omitted]. HEK293 cells were statically cultured in a DMEM medium (containing 10% fetal bovine serum and 2mM L-glutamine) (product of Sigma) at 37°C under feeding of 5% CO2 without replacement of the medium. Four days after transfection, the culture broth was recovered, and was centrifuged (3,300xg, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22-μm filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (HEK).	
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159. And the '629 Publication's instructions for assembling a recombinant cascade reaction *refer back to* the disclosures of the '767 Application. EX1015 at [0361]-[0362] (WO 2014/092079 is the international publication of the '767 Application). Given that he considers the '629 Publication to have "detailed directives" Dr. Jain should consider the '767 Application to have highly detailed directives.

160. Dr. Jain dismisses the examples of recombinant production of Factor B and Pro-clotting enzyme in insect cells found in the '767 Application. EX1003

¶120. However, these examples show that the proteins were secreted and functional. The '767 Application also informs a POSA that similar expression techniques could be used for each of the three proteins, reinforcing the “mutatis mutandis” instructions for mammalian cell expression. EX1017 at 89-90 ([0118]-[0120]).

161. Thus, in my opinion, the '767 Application enables the recombinant expression of Factor B and Pro-clotting enzyme in mammalian cells.

b. The experimentation required was routine

162. Dr. Jain seems to believe that because sequence information had not been expressly disclosed, it was either unobtainable or required undue experimentation to obtain. EX1003 ¶¶66, 133; EX1065 at 98. As discussed in Section VIII.A.4 above, unpublished sequence information would have been readily obtained by searching the available genomic data, by utilizing the RACE-PCR method, or by probing a phage library. As discussed above, the '767 Application instructs how to construct expression vectors for mammalian expression, but this was also part of a POSA's general skill and knowledge as of December 2013.

163. It was routine as of December 2013 for those 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 36, 84-96. Insect cells are widely used for recombinant protein production, including glycoproteins. EX1019 at 8.

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. Baculovirus-insect cell expression systems often have lower yields of properly folded and secreted protein than mammalian cells. *See, e.g.,* EX2090 at 2. Therefore, a POSA would expect (absent information to the contrary) fewer difficulties expressing a protein in mammalian cells than insect cells.

164. Dr. Jain seems to believe that a POSA would need to test expression in many mammalian cell lines before drawing a general conclusion that the '767 Application is enabled. I disagree. In view of the '767 Application's disclosure of successful expression of Factor C in two commonly used mammalian cell lines—CHO DG44, and HEK293—a POSA would conclude that these zymogens generally may be successfully expressed in those mammalian cell lines most commonly used for expression of recombinant proteins.

165. 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 92-95 [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.”). As discussed with respect to written description above, 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. 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.

166. After recombinant proteins were obtained from one or more mammalian cell lines, testing for Factor C, Factor B, and Pro-clotting enzyme activity could be carried out on multiple samples in as few as three experiments, one for each zymogen. The '767 Application discloses a method for measuring endotoxin detecting activity. EX1017 at 93-95 ([0128]-[0131]). However, ascertaining the function of each individual zymogen need not involve the full cascade, but rather known detection substrates, as discussed in Section VIII.A.2, above.

167. As of December 2013, both LAL and recombinant Factor C assays were carried out on multiple samples in parallel, so testing ten proteins for activity would not have been undue experimentation. See EX2070 at 2 (describing analytical

procedure for recombinant Factor C); EX2066 at 7-8 (discussing automated methods), 14-15 (discussing methodology).

168. Even if the Challenged Claims required testing 3,456 combinations of proteins, such experimentation would not have been undue in this field where endotoxin assays could be carried out by automated machines, e.g., in 96-well, 384-well or 1536-well plates, in parallel, and completed in less than an hour. EX2066 at 7-8, 14-15.

5. Claims 2-9

169. Claims 2-9 narrow the scope of claim 1, reducing the extent of experimentation needed to practice the full scope of the claims. Therefore, it is my opinion that they are enabled by the '767 Application for the same reasons that claim 1 is enabled. Further, 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 or eight, further reducing the amount of routine experimentation needed to generate, test individually for function, and combine these proteins into an endotoxin assay agent.

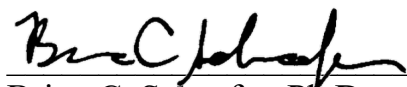
170. Claims 5-6 recite features of Factor C. The '767 Application includes extensive description of how to determine the sialic acid content and molecular weight of Factor C. EX1017 at 100-108 ([0141]-[0156]). Such experiments would

have been routine for a POSA in view of the '767 Application. Thus, in my opinion, claims 5-6 are enabled.

171. With respect claims 7-8, Dr. Jain does not opine 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. EX1003 ¶138. This would have been routine experimentation for a POSA. Therefore, in my opinion, claims 7-8 are enabled for the same reasons that claim 1 is enabled.

172. Finally, Dr. Jain's opinion concerning claim 9 are based on his erroneous assumption that claim 1 requires a functional endotoxin detection agent. EX1003 ¶137. Dr. Jain does not articulate a separate reason why a POSA would not be able to "separately express" the three zymogen proteins "in individually established host cells" as recited in claim 9. *Id.* Indeed, this would have been routine experimentation for a POSA. Therefore, in my opinion, claim 9 is enabled for the same reasons that claim 1 is enabled.

I declare under penalty of perjury of the laws of the United States that the foregoing is true and correct.


Brian C. Schaefer, Ph.D.

12/01/2025
Date