

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CHARLES RIVER LABORATORIES, INC.,
Petitioner,

v.

SEIKAGAKU CORPORATION,
Patent Owner.

IPR2025-00440
Patent No. 11,236,318

DECLARATION OF SIDDHARTHA JAIN, PH.D.

CRL Exhibit 1002 CRL v. Seikagaku

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I, Dr. Siddhartha Jain, declare:

1. I have been retained by Wolf, Greenfield & Sacks, P.C., counsel for Petitioner Charles River Laboratories, Inc., (“Petitioner”) to assess claim 21 (the challenged claim) of U.S. Patent No. 11,236,318 (“the ’318 patent”). I am being compensated for my time at my standard rate, plus actual incurred expenses. My compensation is not dependent in any way upon the outcome of this *inter partes* review of the ’318 patent.

I. PERSONAL AND PROFESSIONAL BACKGROUND

2. I have over 18 years of experience in the field of CMC (“Chemistry, Manufacturing, and Controls”) development and manufacturing for recombinant protein biologics drugs including significant experience with different formats of endotoxin assays such as the LAL (limulus amoebocyte lysate) assay and assays involving recombinant Factor C.

3. I graduated from Massachusetts Institute of Technology in 2006 with a Ph.D. in Bioengineering, Biological Engineering Department where my thesis focused on the interface of chemical engineering / material science (polymeric microparticle and nanoparticle-based delivery systems) and life sciences (immunology) to develop a vaccine technology using pathogen-mimetic systems. Prior to that, I graduated from the Indian Institute of Technology in 2001 with a 5-Year Integrated Master of Technology degree in Biochemical Engineering and

Biotechnology. My first exposure to the endotoxin assays was during my Ph.D. when I tested the polymeric microspheres for endotoxin using a chromogenic LAL assay to ensure that the immune cells are not activated nonspecifically.

4. Since completing my education in Bioengineering, I have worked in a number of industry roles for leading pharmaceutical and biotechnology companies. Through my work in these roles I have engaged firsthand with analytical assays and protocols including endotoxin testing as well as development and manufacturing of recombinant proteins that I understand to be the subject matter of the present dispute (i.e., use of a recombinant protein for endotoxin assay) for which I submit this declaration.

5. From July 2006 to July 2008, I worked for Bristol-Myers Squibb Company as a Bioprocess Engineer in the Manufacturing Sciences department. Then from July 2008 to January of 2012, I worked at Novartis Vaccines and Diagnostics as a Research Investigator for Novartis' Viral Vaccine Formulation Development group. In this role, I was responsible for the development and testing of various vaccine formulations and adjuvants that were evaluated in animal studies. Vaccine formulations were tested for endotoxin content by me and my team to ensure low pyrogenicity of vaccine formulations. I primarily utilized the gel clot and chromogenic formats of the LAL assay for testing the vaccine formulations. For a novel vaccine adjuvant, I invented a formulation

(US20130101629A1) that utilized a viscosity enhancing suspension agent that resulted in background interference (enhancement) in the LAL assay. To resolve this interference as well as to potentially incorporate a high throughput endotoxin assay for routine testing of vaccines and adjuvants, I evaluated the recombinant Factor C endotoxin kit for its comparability with the gel clot endotoxin method.

6. Between January and June of 2012, I then worked as an independent consultant developing a strategic plan for a large pharmaceutical company.

Starting in June of 2012, I then spent over five years until September of 2017 leading CMC operations management team at Regeneron Pharmaceuticals by working at the interface of and with R&D, Manufacturing, Quality Assurance, Quality Control, Regulatory, Procurement, Process Sciences and alliance partners for biologics clinical and commercial manufacturing operations.

7. In October of 2017, I moved into the role of Director for Strategy Planning, Operations Management and Innovation, Quality at Novartis Institutes for BioMedical Research (“NIBR”), a position I held until April of 2019.

8. From April of 2019 until December of 2021, I was the Director of Manufacturing Science and Technology (“MSAT”) at Sanofi where I led digital transformation and automation of the global MSAT laboratories, and built new capabilities such as risk-based cleaning validation and material science. Finally, from December 2021 until May of 2023, I held the role of Head (Senior Director)

of Process Development & Manufacturing at Generate Biomedicines where I led the development and manufacturing of recombinant protein drug candidates that were computationally designed using Artificial Intelligence/Machine Learning.

9. Since May 2023 I have been leading Shoolin Management Company. Through the consulting arm of Shoolin Management Company, I provide consulting service to various biopharmaceutical companies, consulting firms and health authorities about biopharmaceutical CMC and digital technologies.

10. By way of professional affiliations, I held the position of Chair of the American Chemical Society for the Eastern New York Section from 2016 to 2017 and I have co-founded companies across technical disciplines, including the company Sequesco, a clean energy company that converts greenhouse gases into biofuels. Since 2023, I have mentored multiple US-based early-stage companies through MassChallenge.

11. An up-to-date version of my curriculum vitae is provided as Exhibit 1003.

II. MATERIALS REVIEWED AND CONSIDERED

12. My findings, as explained below, are based on my years of education, research, experience, and background in the field of biological and biochemical engineering and as well as my independent investigation and study of relevant materials for this declaration. When developing the opinions set forth in this

declaration, I assumed the perspective of a person having ordinary skill in the art (“POSA”), as set forth in V.C. below. In forming my opinions, I have studied and considered the materials identified in the list below.

Exhibit	Description
1001	U.S. Patent No. 11,236,318 (“the ’318 patent”)
1002	Declaration of Siddhartha Jain, Ph.D., PMP (“Jain”)
1003	Curriculum Vitae of Dr. Siddhartha Jain, Ph.D., PMP
1004	Prosecution History for U.S. Application No. 16/399,386
1005	International Patent Publication No. WO 2018/074498 A1
1006	U.S. Patent Application No. 14/650,767, as filed June 9, 2015
1007	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.
1008	Gan et al., “Analysis of Protein Sequence/Structure Similarity Relationships,” <i>Biophys J.</i> 2002 Nov; 83(5):2781-91.
1009	U.S. Patent Application Publication No. 2015/0307864
1010	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.
1011	Transition Certification for U.S. Patent Application No. 15/983,725, filed May 31, 2019.
1012	U.S. Patent No. 5,716,834
1014	U.S. Patent No. 5,858,706
1015	International Patent Publication No. WO 2000/026366 A1
1016	Iwanaga, Sadaaki, “Biochemical principle of <i>Limulus</i> test for detecting bacterial endotoxins,” <i>Proc. Jpn. Acad., Ser. B. Phys Biol Sci.</i> 2007 May; 83(4):110-9.
1019	Japanese Priority Document JP2012-269840 (certified translation, plus original)
1020	U.S. Application No. 14/650,767, addition to PCT specification
1021	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.

Exhibit	Description
1022	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.
1023	Levin, J., Bang, F., “The role of endotoxin in the extracellular coagulation of <i>Limulus</i> blood,” <i>Bulletin of John’s Hopkins Hospital</i> , 1964 Sep: 115:265-74.
1024	Walls, E. et al, The Horseshoe Crab, <i>Limulus polyphemus</i> : 200 Million Years of Existence, 100 Years of Study, <i>Reviews in Fisheries Science</i> , 2002; 10(1):39-73.
1025	Carmichael, R., et al., “Changing Global Perspectives on Horseshoe Crab Biology, Conservation, and Management,” Springer, Ch. 29: “Biotechnology Efforts to Conserve Horseshoe Crabs Through the Development of Recombinant Factor C-Based Endotoxin Test” pp. 501-512 (2015).
1028	Ding et al, “A new era in pyrogen testing,” <i>Trends in Biotechnology</i> , 2001 Aug; 19(8):277-81.
1029	Declaration Under 37 C.F.R. § 1.132, U.S. Patent Application. No.: 14/650,767, filed Feb. 22, 2018.
1030	Barrabes 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 et al., “Production of Recombinant Proteins: Challenges and Solutions,” <i>Methods Mol Biol</i> . 2004: 267:15-51.
1032	U.S. Patent No. 6,645,724
1033	Mizumura, H., et al., “Genetic engineering approach to develop next-generation reagents for endotoxin quantification,” <i>Innate Immunity</i> . 2017 Feb; 23(2):136-146.
1034	Ding et al., “Expression of full length and deletion homologues of <i>Carcinoscorpius rotundicauda</i> Factor C in <i>Saccharomyces cerevisiae</i> : immunoreactivity and endotoxin binding,” <i>Journal of Endotoxin Research</i> . 1997; 4(1): 33-43.
1035	International Patent Publication No. WO 2014/092079 A1 (“the ‘082 PCT”)
1036	Wang et al., “Modular Arrangement and Secretion of a Multidomain Serine Protease,” <i>J Biol Chem</i> . 2002 Sep 27;277(39):36363-72.

Exhibit	Description
1037	Marengo-Rowe, Alain, J., “Structure-function relations of human hemoglobins,” <i>Proc (Bayl Univ Med Cent)</i> 2006 Jul; 19(3):239–245.
1038	Sekiguchi, K. and Shuster, C.N., “Limits on the Global Distribution of Horseshoe Crabs (Limulacea): 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.

III. MY UNDERSTANDING OF LEGAL PRINCIPLES

13. In developing my opinions, I discussed various relevant legal principles with Petitioner’s attorneys. Though I am not an attorney and I do not purport to have prior knowledge of such legal principles, I understood them when they were explained to me and have relied upon such legal principles, as explained to me, in the course of forming the opinions set forth in this declaration. My understanding in this respect is as follows:

14. I understand that an “*inter partes* review” (“IPR”) is a proceeding before the United States Patent & Trademark Office for evaluating the patentability of an issued patent’s claims based on prior art patents and printed publications.

15. I understand that, in this proceeding, Petitioner has the burden of proving that the challenged claim (claim 21) of the ’318 patent is unpatentable by a preponderance of the evidence. I understand that “preponderance of the evidence” means that a fact or conclusion is more likely true than not true.

16. I understand that, in IPR proceedings, claim terms in a patent are typically given their ordinary and customary meaning as understood by a POSA in the context of the entire patent and the prosecution history pertaining to the patent. I have interpreted claim terms in accordance with their ordinary and customary meaning as understood by a POSA in light of the specification and prosecution history of the '318 patent.

A. Priority (Effective Filing) Date

17. I understand that determining whether a particular patent or printed publication constitutes prior art to a challenged patent claim can require determining the effective filing date (sometimes referred to as the “priority date”) to which the challenged claim is entitled.

18. I understand that for a patent claim to be entitled to the benefit of the filing date of an earlier application to which the patent claims priority, the earlier application must have *described* the claimed invention in sufficient detail to convey with reasonable clarity to the POSA that the inventor had possession of the full scope of the claimed invention as of the earlier application’s filing date and that the inventor, through his description of the invention, has *enabled* POSAs to make and use that invention without undue experimentation. These “written description” and “enablement” requirements for determining effective filing date are the same as those set forth in Sections III.B. and III.C. below.

19. I also understand that for an invention claimed in a patent to be patentable, it must be, among other things, not obvious in view of the prior art. My understanding of this legal standard is set forth below.

B. Written Description

20. I have been informed that the written description requirement under 35 U.S.C. § 112(a) is based on the text of the statute requiring that:

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same...

21. I understand that a patent application, as of its filing date (and if entitlement to an earlier priority date is asserted, as of the filing date of that earlier application) must contain a disclosure that describes the claimed invention. I understand this is known as the “written description” requirement. I understand that the written description requirement is satisfied if the disclosure of the patent application leads a person of ordinary skill in the art to recognize that the inventor(s) possessed the full scope of what is claimed.

22. It is also my understanding that the written description requirement may be satisfied by such descriptive means as text, structure, figures, diagrams,

formulas or other disclosures that set forth the claimed invention. I further understand that a description that merely renders the invention obvious does not satisfy the written description requirement. What is required is a description that leads a person of ordinary skill in the art can clearly conclude that the inventor(s) had conceptual possession of what is claimed.

23. Conversely, I understand that an application's disclosure does not satisfy the written description requirement if it omits even one element of the claimed invention, if it does not describe the claimed subject matter as an integrated whole rather than as a collection of independent limitations, or if the claimed limitations must be plucked selectively from the application with the benefit of hindsight of knowing the claimed invention.

C. Enablement

24. As noted above, I also understand that whether a patent claim is entitled to the benefit of an earlier filed application date, the earlier application must also satisfy the enablement requirement—that is, it must enable a person of ordinary skill in the art to make and use the invention of the claim without undue experimentation.

25. I have been informed that the enablement requirement under 35 U.S.C. § 112(a) is based on the text of the statute requiring that:

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same...

26. I have been informed that a specification does not comply with the enablement requirement under 35 U.S.C. § 112(a) with respect to a particular claimed invention if the specification does not teach a POSA how to make and use the invention as broadly as it is claimed, without undue experimentation, as of the relevant date. In the case of assessing whether a patent claim is entitled to the benefit of an earlier-filed application, I have been informed that the relevant timeframe to consider is the date of the earlier application (as discussed above in section III.A.).

27. I have been informed that a finding of undue experimentation must consider multiple factors to determine if there is sufficient evidence to support such a determination. I have been informed that these factors, which are referred to as *Wands* factors, include (1) the nature of the invention, (2) the relative skill of those in the art, (3) the breadth of the claims, (4) the presence or absence of working samples, (5) the amount of direction or guidance presented, (6), the state

of the prior art, (7) the predictability or unpredictability of the art, and (8) the quantity of experimentation necessary.

28. I have been informed that the specification need not teach what is well known in the art.

29. Finally, I have been informed that the determination of non-enablement must be based on the evidence as a whole.

D. Obviousness

30. I understand that a patent claim may be unpatentable if it would have been obvious in view of a single prior-art reference or a combination of prior-art references as of the effective filing date of that claim.

31. I understand that a patent claim is obvious if the differences between the subject matter of the claim and the prior art are such that the subject matter as a whole would have been obvious to a person of ordinary skill in the relevant field at the time the invention was made. Specifically, I understand that the obviousness question involves a consideration of:

- the scope and content of the prior art;
 - the differences between the prior art and the claims at issue;
 - the knowledge of a person of ordinary skill in the pertinent art; and
 - if present, objective factors indicative of non-obviousness,
- sometimes referred to as “secondary considerations.” I understand

that the Patent Owner to the '318 patent has argued multiple times and in multiple different cases to the patent examiner that certain “secondary considerations” counsel against a finding that the invention of the '318 patent or related patents is obvious. I address these arguments below as relevant to the '318 patent.

32. I understand that in order for a claimed invention to be considered obvious, a POSA must have had a reason for combining teachings from multiple prior-art references (or for altering a single prior-art reference, in the case of obviousness in view of a single reference) in the fashion proposed.

33. I further understand that in determining whether a prior-art reference would have been combined with other prior art or with other information within the knowledge of a POSA, the following are examples of approaches and rationales that may be considered:

- combining prior-art elements according to known methods to yield predictable results;
- simple substitution of one known element for another to obtain predictable results;
- use of a known technique to improve similar devices in the same way;
- applying a known technique to a known device ready for improvement to yield predictable results;

- applying a technique or approach that would have been “obvious to try,” i.e., choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success.
- known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art;
- some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior-art reference or to combine prior-art reference teachings to arrive at the claimed invention. I understand that this teaching, suggestion or motivation may come from a prior-art reference or from the knowledge or common sense of one of ordinary skill in the art.

34. I understand that for a single reference or a combination of references to render the claimed invention obvious, a POSA must have had, (1) a reason to combine the prior art teachings, and (2) a reasonable expectation of success in arriving at the claimed invention by altering or combining the applied references. Certainty and absolute predictability are not required to establish a reasonable expectation of success.

IV. TECHNOLOGY BACKGROUND

35. The '109 patent relates to endotoxin testing for the quality assurance of medical products, product testing, and environmental safety. It is directed to recombinantly-produced Factor C proteins—enzymes useful in detecting the presence of biologically harmful substances (endotoxins) that can contaminate medical products (such as medical devices or pharmaceuticals). It claims recombinant Factor C proteins—enzymes sourced from the hemolymph (invertebrate equivalent of blood) of horseshoe crabs which had been discovered to undergo a detectable clotting reaction in the presence of a harmful bacterial substance known as endotoxin. Ex. 1001, 1:27-54.

36. This detectable clotting phenomenon was discovered in the 1960's and has since been relied upon by the biomedical industry to generate tests for the detection and quantitation of endotoxin that may have contaminated medical products. Ex. 1001:41-54; Ex. 1023, 265.

37. The challenged claim in this Petition (claim 21) recites Factor C proteins from the *Limulus polyphemus* species of horseshoe crab that are recombinantly produced in HEK293 cells and exhibit a particular “residual activity” function relating to the protein's ability to detect endotoxin in the presence of otherwise inhibitory ions. Ex. 1001, cl. 1.

38. “Recombinant” production involves using the genetic information for a particular protein (either the gene coding sequence or the amino acid sequence of that protein), and “programming” a host cell (like HEK293) with this information such that the host cell produces (or expresses) the protein in vitro (in a lab).

39. In the decade leading up to the earliest effective filing date of the ’318 patent, and in view of the labor-intensive methods required to obtain Factor C protein from horseshoe crabs directly, many scientists turned to the recombinant expression of Factor C as an alternative source of this protein. Singaporean researchers Ding et al. disclosed using mammalian cells to recombinantly express Factor C from *Carcinoscorpius rotundicauda* crabs more than a decade prior to the ’318 patent. Ex. 1014 (U.S. Patent No. 5,858,706), Abstract; Ex. 1015 (WO 2000/026366), 25-27, 29-30; Ex. 1034, Abstract.

40. The gene and amino acid sequences for Factor C and related proteins for *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* species of horseshoe crab were known prior to the earliest claimed priority date of the ’318 patent. Ex. 1012 (U.S. Patent No. 5,716,834), 2:64-3:10; Ex. 1007, 6556.

41. The gene and amino acid sequences encoding full-length Factor C for the horseshoe crab species *Limulus polyphemus* (native to North America), however, had **not** been described or fully characterized until much more recently. Ex. 1010 (McClymont Thesis, 2017) (disclosing full protein sequences for Factor

C in *L. polyphemus* horseshoe crabs), 36 (“The sequence of *Limulus polyphemus* Factor C is not publicly available. For this reason, it was necessary to assemble the lpFC gene from the raw data kindly provided from Washington University, St Louis, in order to synthesize full-length lpFC.”), 159-60; Ex. 1005 (’498 PCT), ¶¶17, 194-195, pp. 49-75 (SEQ ID NOS. 1-4).

V. THE ’318 PATENT

42. The ’318 patent discusses the production of recombinant horseshoe crab Factor C protein using commonly used mammalian cell lines. Ex. 1001, 18:25-54. According to the specification, expression of Factor C from known mammalian industrial production strains, including Chinese Hamster Ovary (“CHO”), purportedly solves the problem of ion interference with the Factor C signaling cascade that limits endotoxin recovery (thus underestimating endotoxin levels). The inventors state that they “have found that a recombinant horseshoe crab Factor C can be produced by use of human cells or Chinese hamster cells as host cells, and that endotoxin can be measured by use of the thus-produced recombinant Factor C while reaction inhibition in the presence of a salt (ion) is mitigated.” Ex. 1001, 3:12-18; Ex. 1001, 35:26-43.

43. This concept of “reaction inhibition in the presence of a salt (ion) is mitigated” is captured in the ’318 patent claims by the term “residual activity” which the ’318 patent says is a measurement of the activity of a reconstituted

Factor C cascade in detecting a given amount of endotoxin in an ion solution relative to the activity of the reconstituted cascade in detecting the same amount of endotoxin in water. Ex. 1001, 18:8-15.

44. The '318 patent inventors tested the residual activity of *Tachypleus tridentatus* Factor C protein recombinantly produced via various expression systems and exposed to particular concentrations of various ionic solutions. They reported the results of the residual activity measurements of the recombinant Factor C protein (from *Tachypleus tridentatus*) from three expression systems and the horseshoe crab amebocyte lysate (aka native horseshoe crab Factor C protein) in a single figure (FIG. 7). Ex. 1001, 34:39-35:43, FIG. 7.

45. Claim 21 recites that the claimed Factor C protein “exhibits a residual activity of at least one selected from the group consisting of:

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

Ex. 1001, cl. 21.

This claim encompasses recombinantly produced Factor C protein of the *Limulus polyphemus* species satisfying either one of these wide and open-ended ranges for the functional characteristic of “residual activity.”

46. I understand that the '318 patent was filed as U.S. Patent Application No. 16/399,386 (“the '386 Application”) on April 30, 2019. The '386 Application

is a continuation application of U.S. Patent Application No. 15/983,725 (“the ’725 Application”) which was filed on May 18, 2018, and is now U.S. Patent No. 10,982,202. The ’725 Application is a continuation application of U.S. Patent Application No. 14/650,767 (“the ’767 Application”) which was filed as International Patent Application No. PCT/JP2013/083082 (“the ’082 PCT”) (Ex. 1035) on December 10, 2013, and is now U.S. Patent No. 10,144,923. The December 10, 2013 filing of the ’082 PCT was within one year of the claimed priority document for the ’318 patent which is the ’840 JP Priority Application No. 1012-269840, filed on December 10, 2012.

47. In view of the above chain of applications, I have been informed that the ’318 patent claims, on its face, a priority date of December 10, 2012. As I discuss in detail in Section VI., below, claim 21 is not entitled to this earliest effective filing date of December 10, 2012, but rather is entitled to an effective filing date of no earlier than the actual filing date of the ’318 patent—April 30, 2019.

A. Challenged Claim

48. I have been asked to provide my opinion regarding challenged claim 21 of the ’318 patent. Claim 21 is reproduced below:

49. A horseshoe crab Factor C protein having activity of Factor C, wherein the horseshoe crab is *Limulus polyphemus*, and wherein the horseshoe crab Factor C protein is produced

through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, and wherein the Factor C protein exhibits a residual activity of at least one selected from the group consisting of:

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

B. Prosecution History

50. Though I am not an attorney, I was provided with the back-and-forth correspondence between Patent Owner and the USPTO when Patent Owner was working to secure the '318 patent and I understand the following aspects of that correspondence.

51. Patent Owner filed the '386 Application with 18 claims. As-filed claim 1 recited:

1. A horseshoe crab Factor C protein having activity of Factor C, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*, and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary (CHO) DG44 cell or HEK cell.

Ex. 1004, 515.

52. Original claim 1 said nothing about the “residual activity” of the horseshoe crab Factor C. Claim 2 recited several amino acid sequences as set forth

in the specification and included a limitation reciting “wherein the Factor C protein exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.” Ex. 1004, 515-16. Claim 11 recited, “The Factor C protein according to claim 2, which contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding native horseshoe crab Factor C protein and/or a corresponding horseshoe crab Factor C protein recombinantly expressed using Sf9 as a host cell” (hereafter, the “Greater Sialic Acid Limitation”). Ex. 1004, 517.

53. In the first Office Action on the merits (June 30, 2020), the Examiner rejected claims 2-9, 12, and 13 for failing to comply with the written description requirement. Ex. 1004, 371-76. The Examiner noted that the rejected claims recited a broad genus with insufficient disclosure of representative species and a failure to link the “residual activity” function claimed with some structural feature, namely the Greater Sialic Acid Limitation found in other pending claims. Ex. 1004, 375-76. The Examiner also rejected claims 1-13 as obvious over the prior art. Ex. 1004, 376-84.

54. In response, Patent Owner amended claim 2 to incorporate the Greater Sialic Acid Limitation of claim 11. Ex. 1004, 227-28. Patent Owner also submitted a declaration from first-named inventor Dr. Hikaru Mizumura who testified that the inventors had “succeeded in cloning cDNA Factor C from *Limulus polyphemus* (=LpFC) hemocyte” and cited U.S. Pub. No. 2019/0241629, the 2019 U.S.

Publication of the '498 PCT in which Patent Owner claimed separately to have finally invented recombinant *Limulus polyphemus* Factor C protein. Ex. 1004, 245-46. Dr. Mizumura also submitted newly-acquired experimental data of *Limulus polyphemus* Factor C recombinantly produced in the CHO cell mammalian expression system. Ex. 1004, 245-48.

55. The Examiner subsequently withdrew the written description rejection but maintained the obviousness rejection as to pending claims 1-10, 12, 13, and 19. Ex. 1004, 185-202.

56. Patent Owner then amended *claim 1* to now *also* recite the Greater Sialic Acid Limitation. Ex. 1004, 168. Patent Owner submitted yet another declaration by Dr. Mizumura alleging that the present invention provides “unexpected superior results over” the cited prior art. Ex. 1004, 149-53.

57. The Examiner then rejected claims 1-9, 12, 13, and 19-23 as obvious over the cited prior art again. Ex. 1004, 101-15.

58. In response, on August 24, 2021, Patent Owner added new claim 24 (issued claim 21) which now did not require the presence of (α -2,3)-linked terminal sialic acid despite the multitude of earlier amendments adding this limitation to resolve the Examiner’s written description rejection. Ex. 1004, 73. Patent Owner argued yet again that the purported invention demonstrated unexpectedly superior results achieved with Factor C. Ex. 1004, 75-78. Patent

Owner also filed a third Inventor Declaration by Dr. Mizumura, again alleging unexpectedly superior results achieved with the claimed Factor C. Ex. 1004, 82-87.

59. In a striking about-face, both Patent Owner *and* Dr. Mizumura conceded that “superior salt tolerance can be reproduced *irrespective of* presence of (α -2,3)-linked terminal sialic acid, at least for *Limulus [polyphemus]* Factor C recombinantly produced by using HEK 293 cell as a host.” Ex. 1004, 77, 86.

60. Thereafter, an Examiner-Initiated Interview took place in which the Examiner proposed amendments to claims 14 and 18, which were authorized by Patent Owner. Ex. 1004, 25. The Examiner thereafter allowed the claims. Ex. 1004, 14.

C. Person of Ordinary Skill in the Art (“POSA”)

61. I have been informed and understand that for purposes of assessing whether prior-art references would have rendered the claim obvious, and/or describe and enable the full scope of the challenged claims, the patent and the prior-art references must be assessed from the perspective of a person having ordinary skill in the art (“POSA”) to which the patent is related, based on the understanding of that person at the time of the patent claim’s priority date.

62. I have been informed and understand that a POSA is presumed to be aware of all pertinent prior art and the conventional wisdom in the art, and is a

person of ordinary creativity. I have applied this standard throughout my declaration.

63. The '318 patent involves technology in the field of protein biology, biochemical engineering and biotechnology. I have been asked to provide my opinions as to the state of the art in this field for purposes of determining what the inventors disclosed as their invention as of December 10, 2013, which is the date that the international patent application giving rise to the '318 patent was filed. I use this timeframe because while the face of the '318 patent indicates an earliest claimed priority date of December 10, 2012 (JP2012-269840, the "'840 JP Priority Application"), as I discuss below, the disclosure appearing in that Application is encompassed by the material found in the PCT application filed about one year later, on December 10, 2013 (PCT/JP2013/083082, "the '082 PCT"). Thus, if the disclosure in the December 10, 2013 filing of the '082 PCT fails to demonstrate that the inventors possessed and enabled the invention of claim 21, the disclosure in the '840 JP Priority Application is equally inadequate.

64. For purposes of assessing whether claim 21 of the '318 patent is entitled to claim priority to either of these dates (its '840 JP Priority Application or PCT filing date), I assess the sufficiency of the disclosure made in the '767 Application (a disclosure that includes the material of the '840 JP Priority Application and is identical to that found in the '082 PCT¹). Whenever I offer an

¹ I note that when the Patent Owner filed the English version of the disclosure found in PCT/JP2013/083082 as the '767 Application, it added the following paragraph just before Paragraph 19 of the '767 Application (Ex. 1020, 2-3):

In the present invention, a series of reactions wherein endotoxin activates Factor C to generate active-type Factor C; the active-type Factor C activates Factor B to generate active-type Factor B; and the active-type Factor B activates Pro-clotting enzyme to generate a corresponding Clotting enzyme; may be referred to as “cascade reaction”. In the present invention, Factor C, Factor B, and Pro-clotting enzyme may be collectively referred to as “factor”. In the present invention, a cascade reaction system constituted by combining the factors may be referred to as “reconstituted system”. In such a reconstituted system, an effect of a contaminant contained in an amebocyte lysate of a horseshoe crab, e.g. Factor G, on the cascade reaction can be eliminated.

This paragraph does not appear in the publication of the '767 Application (US2015/0307864), however I have considered this additional paragraph in my analysis herein regarding the ability of Patent Owner to claim a December 10, 2013 priority date for claim 1 of the '318 patent.

opinion in this declaration about the knowledge of a POSA, the manner in which a POSA would have understood the claims of the '318 patent or its description, the manner in which a POSA would have understood the prior art, or what a POSA would have been led to do based on the prior art, I am referencing this December 10, 2013 timeframe, even if I do not say so specifically in each case.

65. In my opinion, a person of ordinary skill in the art in the December 10, 2013 timeframe ("POSA") would have had a Ph.D. degree in biological, chemical or biochemical engineering, molecular biology, or biochemistry and at least two years of experience in the field of utilizing or assessing horseshoe crab Factor C (lysate, purified native and/or recombinantly produced) for endotoxin detection and quantitation. More education could substitute for experience to a certain extent, and vice versa. This person would have been capable of understanding and applying the teachings of the '318 patent and the prior-art references discussed in this declaration.

66. By December 10, 2013, I held a Ph.D. in Bioengineering with a focus at the interface of chemical engineering / material science and life sciences, and I had over seven years of work experience in industry focusing on recombinant protein drugs and vaccines, including two years of experience with manufacturing of sialylated recombinant drugs using CHO cells (Orencia® and Nulojix®), over three years of experience working with lysate-based endotoxin assays

incorporating Factor C, and one year of experience evaluating Recombinant Factor C assay for endotoxin measurements. Therefore, I was a person of more than ordinary skill in the art during the relevant timeframe. I have also regularly interacted and worked with many people who fit the characteristics of the POSA, and I am familiar with their level of skill. When developing the opinions set forth in this declaration, I assumed the perspective of a POSA, as set forth above.

D. Described Embodiments

67. I have reviewed the specification of the '318 patent (identical to the disclosure found in the '767 Application) and the examples and experiments described therein.

68. The '318 patent specification describes a single "Example" via 10 sub-parts in which the inventors purport to describe the results of experiments expressing and testing recombinant Factor C of the "present invention." Ex. 1001, 29:30-39:17. In all cases, these experiments were conducted with Factor C from the horseshoe crab species, *Tachypleus tridentatus*. Comparative data on, for example, molecular weights, was provided from prior art publications about Factor C from the horseshoe crab species, *Carcinoscorpius rotundicauda*. Ex. 1001, 37:1-24 (Table 1). The '318 patent provides gene and amino acid sequence information of Factor C for these two species of horseshoe crab (*Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*). Ex. 1001, 40:4-25 (SEQ ID NOS.

1-4). However, other than a passing and general mention of the species *Limulus polyphemus* (Ex. 1001, 1:41-45; 13:30-39), the '318 patent does not include any discussion, examples, or data describing the expression of, use of, or experimentation with this species of horseshoe crab Factor C, nor does it include any gene or amino acid sequence information about Factor C from this species of horseshoe crab.

69. As I discuss further below, the gene and amino acid sequences of *Tachypleus tridentatus* Factor C and *Carcinoscorpius rotundicauda* Factor C were known by December 10, 2013. Ex. 1007 (Muta et al., J. Biol. Chem., 266 (10), 6554-6561 (1991)), 6556; Ex. 1012 (US5716834, issued Feb. 10, 1998), 5:50-57, cols. 27-54.

70. The full length gene and amino acid sequence of Factor C for the horseshoe crab species *Limulus polyphemus*, however, had not been fully elucidated until much more recently. Ex. 1010, 159-60 (disclosing full gene and protein sequences for Factor C in *L. polyphemus* horseshoe crabs); Ex. 1005 ('498 PCT), ¶¶17, 194-195, pp. 49-75 (SEQ ID NOS. 1-4).

E. Claim Interpretation

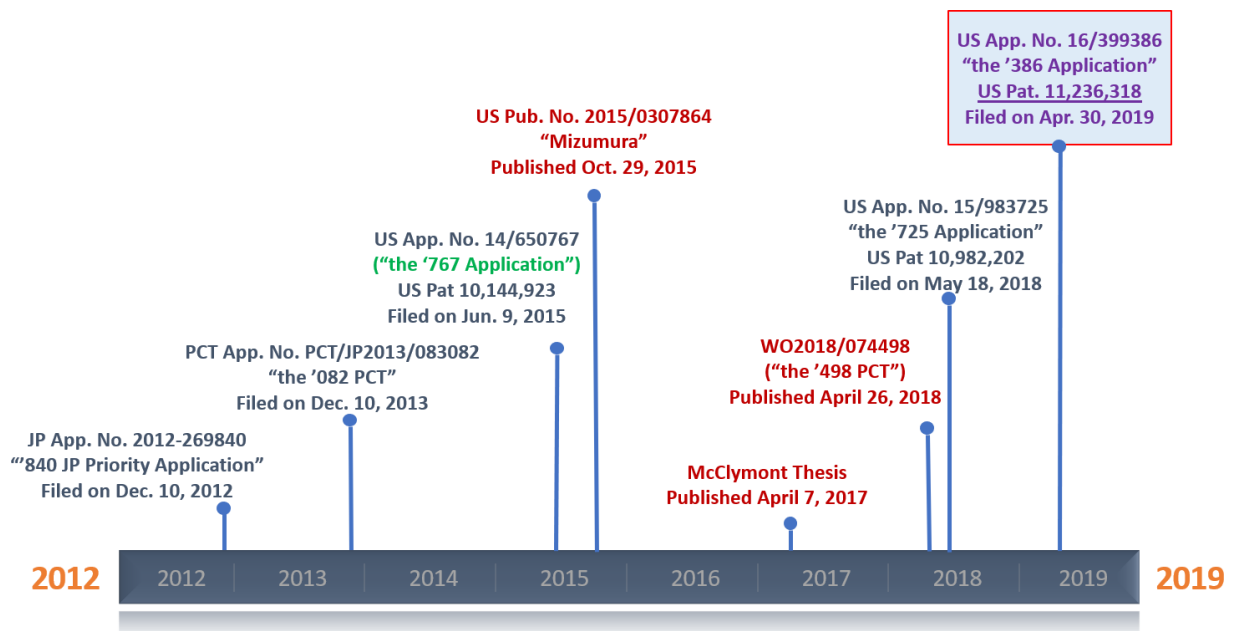
71. I understand that, in IPR proceedings, claim terms in a patent are typically given their ordinary and customary meaning as understood by a POSA in the context of the entire patent and the prosecution history pertaining to the patent.

I have interpreted claim terms in accordance with their ordinary and customary meaning as understood by a POSA in light of the specification and prosecution history of the '318 patent.

VI. CLAIM 21 IS ENTITLED TO AN EFFECTIVE FILING DATE NO EARLIER THAN THE '318 PATENT'S ACTUAL FILING DATE.

A. The '318 Patent's Priority Claim

72. As shown in the timeline below, the '318 patent claims priority through a series of near-identical continuation applications to a Japanese Priority Application (2012-269840) ("the '840 JP Priority Application") (Ex. 1019) filed December 10, 2012.



73. The '840 JP Priority Application is incorporated by reference into each application in the '318 patent priority chain. It discloses less than the later-filed utility applications (for example, it includes the experiments and results numbered (1)-(5), including Figure 7, but not experiments (6)-(10)). Compare Ex. 1019, ¶¶0096-134 with Ex. 1035. While the '840 JP Priority Application discloses residual activity in the presence of sodium citrate using language of “greater than 10%” (as opposed to “10% or greater” as later claimed), it provides no mention of a residual activity range or percent limitation for magnesium sulfate at all. Ex. 1019, ¶¶0017-18.

74. Upon U.S. entry of the '082 PCT on July 9, 2015, Patent Owner amended the specification to add a paragraph relating to the Factor C, Factor B, Pro-clotting enzyme cascade reaction. Ex. 1020, 2-3. This paragraph appears in the subsequent '725 and '386 Applications, and in the '318 patent specification. Ex. 1001, 13:8-21.

75. For purposes of analyzing entitlement to the claimed priority date of December 10, 2013, I analyze and cite to the '767 Application (including the added paragraph, Ex. 1020) as representative of each disclosure (including the '840 JP Priority Application) in the priority chain extending back to December 10, 2012. If the '767 Application fails to describe and enable claim 21, the '840 JP Priority

Application and all other applications in the priority chain fail for the same reasons (including the June 9, 2015 filing date of the '767 Application itself).

B. The '767 Application Lacks Written Description Support for the Recombinant *Limulus Polyphemus* Factor C Proteins Having Residual Activity Across the Full Scope of the Claimed Range.

76. In my opinion, the representative '767 Application lacks written description support for issued claim 21 as it does not demonstrate to a POSA that the inventors possessed the alleged invention of that claim as of December 10, 2013. April 30, 2019 (the actual filing date of the '318 patent) is therefore the effective filing date of that patent. 35 U.S.C. § 100(i)(1).

1. The Inventors Did Not Possess *Limulus Polyphemus* Factor C Proteins With Claim 21's Functional Requirements as of 2013.

77. Claim 21 requires that the *Limulus polyphemus* Factor C protein “exhibits a residual activity of at least one selected from the group consisting of:

- (1) 10% or higher in the presence of 21 mM sodium citrate; and
- (2) 15% or higher in the presence of 16 mM magnesium sulfate.” Ex. 1001, cl. 21.

78. This claim encompasses a genus of recombinantly produced *Limulus polyphemus* Factor C proteins satisfying either one of these exceedingly wide ranges for the functional characteristic of “residual activity.”

79. The '767 Application purports to disclose experimental protocols for making—and data demonstrating that recombinant Factor C of the *Tachypleus tridentatus* species, when produced in HEK293 cells, yields—Factor C proteins with a “residual activity” falling within the claimed range. Ex. 1006, 83-99.

80. For sodium citrate, the '767 Application describes only an HEK-produced *Tachypleus tridentatus* recombinant Factor C protein with a residual activity of approximately 25%. Ex. 1006, 96-99, 122 (FIG. 7). It describes no other example of such a protein (HEK-expressed recombinant Factor C from a different horseshoe crab species) with a residual activity of any other value within the wide range of “10% or higher.”

81. For magnesium sulfate, the '767 Application describes only an HEK-produced *Tachypleus tridentatus* recombinant Factor C protein with a residual activity of approximately 21%. Ex. 1006, 96-99, 122 (FIG. 7). It describes no other example of such a protein (HEK-expressed recombinant Factor C from a different horseshoe crab species) with a residual activity of any other value within the wide range of “15% or higher.”

82. A POSA would not expect as default that such protein expression protocols would yield recombinant proteins in soluble and functional form from a *different* species of crab (whose full sequence information was not even known as of 2013), let alone expect as default that such unknown and uncharacterized

proteins would exhibit a function—residual activity—falling in and across the full scope of the claimed range without performing an experimental verification or elucidating a verified structural explanation for such function across species.

83. Similarly, a POSA would not expect the '318 patent inventors to have been in possession of such proteins with such residual activities above the noted thresholds and across the entirety of the ranges recited in claim 21 when the protein or gene sequence of *Limulus polyphemus* Factor C was not even available until much later.

84. The species delineation of these horseshoe crabs is based on geography (Japanese, Singaporean, North American species). Given this geographical separation, a POSA would have expected each species to have evolved distinct characteristics, including differences in certain protein functions. Ex. 1038, 6-7, 10-16. While a POSA in 2013 may have expected the native members of these varied horseshoe crab species to behave similarly in terms of their hemolymph ability to detect endotoxin, recombinant generation of the proteins responsible for such endotoxin detection function and the specifics of how such recombinant proteins would function on more specific features such as “residual activity” would have been highly speculative and not amenable to extrapolation between species.

85. And while the *Limulus polyphemus* Factor C sequence information was elucidated and disclosed in 2017, years after the claimed priority date of the '318 patent, my understanding is that such “later-acquired knowledge” about a gene is inadequate to demonstrate possession of an invention flowing from that gene.

a. The '767 Application Does Not Mention or Describe a Single Example of the Claimed Recombinant *Limulus polyphemus* Factor C Proteins, Let Alone One Satisfying the Claimed Function.

86. The '767 Application does not disclose *any* example (hypothetical or experimental) of *any* recombinant protein from *Limulus polyphemus* (*i.e.*, the American horseshoe crab). Instead, it focuses entirely on a different and geographically segregated species of crabs—*Tachypleus tridentatus* (*i.e.*, the Japanese horseshoe crab). Even *Carcinoscorpius rotundicauda* (*i.e.*, the Singaporean horseshoe crab) is mentioned only for molecular weight comparison. Ex. 1006, 103. Every experimental example, table entry, and gene and amino acid sequence listing in the '767 Application concerns the Japanese and Singaporean species.

87. Without the ability to make or describe the function of any recombinant Factor C protein from the claimed *Limulus polyphemus* species of horseshoe crab—whose gene and amino acid sequence was not yet known or disclosed by 2013—the inventors did not and could not describe *a single example*

of a *Limulus polyphemus* Factor C protein recombinantly produced via HEK293 cells (or by any other mammalian cell) that falls within the claimed range of residual activity in the presence of the recited ions.

88. Where geography separated these species of crabs (belonging to distinct taxonomic families Limulidae and Tachypleinae), giving rise to genetic and morphological distinctness, and where existing and limited sequence data for the *Limulus polyphemus* crab showed **differences** between its Factor C amino acid sequence and those of other species (discussed below), the failure to provide any experimental example or verified structural link between species giving rise to such similar function only confirms a lack of possession Ex. 1038, 6-7, 10-16 (discussing the geographic and genetic discreteness of Family Limulidae and Family Tachypleinae); Ex. 1022, 157.

b. Differences In Species and Known Differences in Sequence Would Be Expected To Yield Differences in Protein Function.

89. As of 2013, a POSA would have expected that differences in gene or amino acid sequence (as would be expected across different horseshoe crab species) lead to differences in protein structure, and, in turn, differences in protein function. As a result, using sequence homology (or, presumed similarity where a sequence is not yet characterized) to assume the existence of functional identity is an over-simplified understanding of protein function and subject to error. Thus,

due to the criticality of the sequence information to produce the recombinant Factor C protein from *Limulus polyphemus* with the claimed Factor C residual activity, in the absence of such information, a POSA would not have expected that the inventors possessed recombinant Factor C with the residual activity function as recited in claim 21 (from *Limulus polyphemus* crabs) simply because they possessed recombinant Factor C protein for other species.

90. Protein molecules interact with other molecules in 3-dimensional state such as an enzyme interacting with its substrate, antibody binding to its antigen and receptor binding to a ligand. There are two commonly referenced models to describe enzyme-substrate interaction—lock & key model and induced fit model. In both models, the substrate fits into the enzyme's binding site and has precise interactions with the residues that form the catalytic site. Therefore, what the substrate sees is the enzyme's 3-dimensional structure, not a 1-dimensional sequence, since the enzyme's active site is defined by the catalytic residues as well as by multiple other amino acid residues that define its 3-dimensional structure. Thus, even small changes in protein sequence can result in unexpected changes to protein structure which can have unexpected impacts on protein function (abrogation or enhancement).

91. Indeed, POSAs at the time would have been familiar with the cautionary concept that “function similarity generally increases with sequence

similarity but with a high degree of variability.” Ex. 1021, 1; Ex. 1008, 2781.

Without the full gene and amino acid sequence information for *Limulus polyphemus* Factor C in 2013 from which **any comparison** could even be made to any other species, any view about the resulting functional characteristics (specifically, residual activity) of a recombinantly produced protein from *Limulus polyphemus* would have been mere speculation.

92. A POSA would not expect that such protocols would yield recombinant proteins in soluble and functional form from a **different** species of crab (whose full sequence information was not even known as of 2013) and that too with a similar residual activity falling in that range without performing an experimental verification. For example, Wang et. al. (2002) demonstrated that the mutation of only two amino acids in the *Carcinoscorpius rotundicauda* Factor C impaired their expression in the insect cells. Ex. 1036, 36365-36366.

93. Without the full gene and/or amino acid sequence information for *Limulus polyphemus* Factor C in 2013 from which any comparison could be made to any other species, any view about the functional characteristics of a recombinantly produced protein from *Limulus polyphemus* would have been mere speculation. Put differently, a POSA in 2013 would have understood that relying on sequence homology (or, similarity) to assume the existence of functional identity would have been an over-simplified understanding of protein function and

subject to error. Even a single amino acid substitution can be critical enough to change the function and biophysical-biochemical properties of a protein such as in the case of hemoglobin that results in sickle cell disease with a single amino acid substitution. Ex. 1037, 239-241. Hence, a POSA could not have been assured of functional similarity between Factor C from different species of horseshoe crab without experimental verification, especially in the absence of complete *Limulus polyphemus* sequence information or any experimental data in the '767 Application to be able to apply the residual activity data across species.

94. The '767 Application itself references “conservative variation” as differences in amino acid sequences between species “where the function of the protein is appropriately maintained.” Ex. 1006, 47. However, as of 2013, a POSA would have known from limited sequencing information publicly available about *Limulus polyphemus* Factor C proteins, that certain sequence differences between that species and *Tachypleus tridentatus* were **non-conservative** (i.e., potentially giving rise to changes in function in terms of enzymatic activity of Factor C). Ex. 1022, 157.

95. Thus, by December 2013, and based on partial and limited amino acid sequence information for *Limulus polyphemus* Factor C compared with the corresponding sequence for *Tachypleus tridentatus* Factor C by Tokunaga 1991, it would have been apparent to POSAs that non-conservative mutations (including,

as reported in Tokunaga 1991, a difference in a carbohydrate attachment site) between species exist. A POSA would have understood as well that such non-conservative mutations between species could potentially cause structure and function differences between the recombinantly produced Factor C proteins of those species as well. Ex. 1022, 157 (Supp. FIG. 7).

96. For example, Supplementary Figure 7S from Tokunaga 1991 provides a side-by-side comparison of a partial amino acid sequence of Factor C for *Limulus polyphemus* (labeled as L.p.) and corresponding regions of *Tachypleus tridentatus* (labeled as T.t.).

7S

H chain

	1		10		20																
<i>T. t.</i>	R	G	V	D	L	G	L	C	D	E	T	R	F	E	C	K	C	G	D	P	-
<i>L. p.</i>	X	G	V	D	L	G	L	C	D	D	T	R	F	E	X	K	C	G	D	P	-

L and A chains

	1		10		20																
<i>T. t.</i>	S	S	Q	P	S	T	V	D	L	A	S	K	V	K	L	P	E	G	H	Y	-
<i>L. p.</i>	S	A	P	P	S	T	V	D	L	S	S	K	V	K	L	-	-	-	-	-	

B chain

	1		10		20																
<i>T. t.</i>	I	W	N	G	N	S	T	E	I	G	Q	W	P	W	Q	A	G	I	S	R	-
<i>L. p.</i>	I	V	N	G	L	G	L	-	-	-	-	-	-	-	-	-	-	-	-	-	

97. As was known by December 2013, the middle panel (L and A chains) depicts the region immediately after the Proline-rich region (PRR) and the last panel (B chain) depicts the serine protease region immediately after the autocatalytic cleavage site. Referencing the list of conservative substitutions in the '318 patent, a POSA would see that the partial amino acid sequence contains non-conservative mutations—the middle panel has a Q → P (Gln → Pro) mutation and the last panel has W → V (Trp → Val) mutation.

98. These two non-conservative mutations would have been reasonably expected by a POSA to cause differences in protein structure. Since prolines are known to disrupt the secondary structures, Q → P may be expected to contribute to a structural change. Similarly, since tryptophan is a bulky and aromatic amino acid residue compared to valine, W → V is also expected to have contributed to a structural difference. Notably, W → V mutation is present adjacent to the Factor C autocatalytic cleavage site, which is a critical region for the activation Factor C and a structural difference at the cleavage site could translate to a functional difference. The last panel also shows a change from NST → LGL (Asn-Ser-Thr → Leu-Gly-Leu) which are not only non-conservative mutations but also removes an N-glycosylation site (NST).

99. Thus, by December 2013, and based on limited amino acid sequence for *Limulus polyphemus* Factor C (compared with the corresponding regions for *Tachypleus tridentatus* Factor C), it would have been apparent to POSAs that non-conservative mutations (including, for example, difference in a carbohydrate attachment site) between these species of horseshoe crabs exist, and they had a potential to cause structure and function differences between the recombinantly produced Factor C proteins. Ex. 1022, 157 (Supp. FIG. 7); Ex. 1010, 31-35.

100. Moreover, while the Tokunaga paper suggests that the “immunological and biochemical properties of the purified factor C [from *Limulus*

polyphemus] are indistinguishable from those of factor C isolated from *Tachypleus* hemocytes” (Ex. 1022, 151), a POSA in 2013 would recognize this to be a conclusion based on only the gross biophysical-biochemical properties of similar molecular weight, amino acid composition (with large differences of > 10% in the composition of at least one chain, H chain or L chain, for Ser, Pro and Arg), presence of two-chain structure and ability to bind rabbit Factor C antiserum. These characteristics would not have been granular enough to elucidate the protein structure and enzymatic activity differences that may be caused by the non-conservative mutations as per the partial sequencing, and so from this information, a POSA would not have been able to predict the functional properties (particularly the specific property of residual activity in presence of specific ions) of a recombinant Factor C whose full amino acid sequence was not yet known.

101. The fundamental question raised by the knowledge available as early as 1991 was not whether *Limulus polyphemus* Factor C has a similar biological function as *Tachypleus tridentatus* Factor C, but instead the question is whether, by 2013, a POSA would have understood the inventors to be in ***possession*** of HEK293-produced ***recombinant*** *Limulus polyphemus* Factor C with the claimed residual activity despite an admitted lack of possession of the underlying gene and amino acid sequences for that protein and despite the known unpredictability of sequence differences that would have counseled against extrapolating from

recombinant *Tachypleus tridentatus* Factor C data. As a POSA in 2013, that answer would be a resounding no – the inventors of claim 21 did not possess HEK293-expressed recombinant *Limulus polyphemus* Factor C proteins at all, let alone across the full scope of specified residual activity based on the disclosure they included in the '767 Application. And there is certainly no disclosure or experimental evidence demonstrating that enzymatic activity (including residual activity) of recombinant Factor C proteins between the two species would be predictable so as to assure a POSA that a hypothetical recombinant *Limulus polyphemus* Factor C protein would fall within the claimed ranges.

102. Without the full gene and/or amino acid sequence information for *Limulus polyphemus* Factor C in 2013 from which any comparison could even be made to any other species, any view about the functional characteristics of a recombinantly produced protein from *Limulus polyphemus* would have been no more than speculation. Accordingly, a POSA in 2013 would have understood that knowledge of a protein sequence from one species (i.e., *Tachypleus tridentatus*) did not equate to possession of a protein sequence from a different, uncharacterized, species (i.e., *Limulus polyphemus*) across a wide range of a specific functional property (residual activity).

103. A POSA reading the '767 Application disclosure as of December 10, 2013—years before sequence information was known or published for *Limulus*

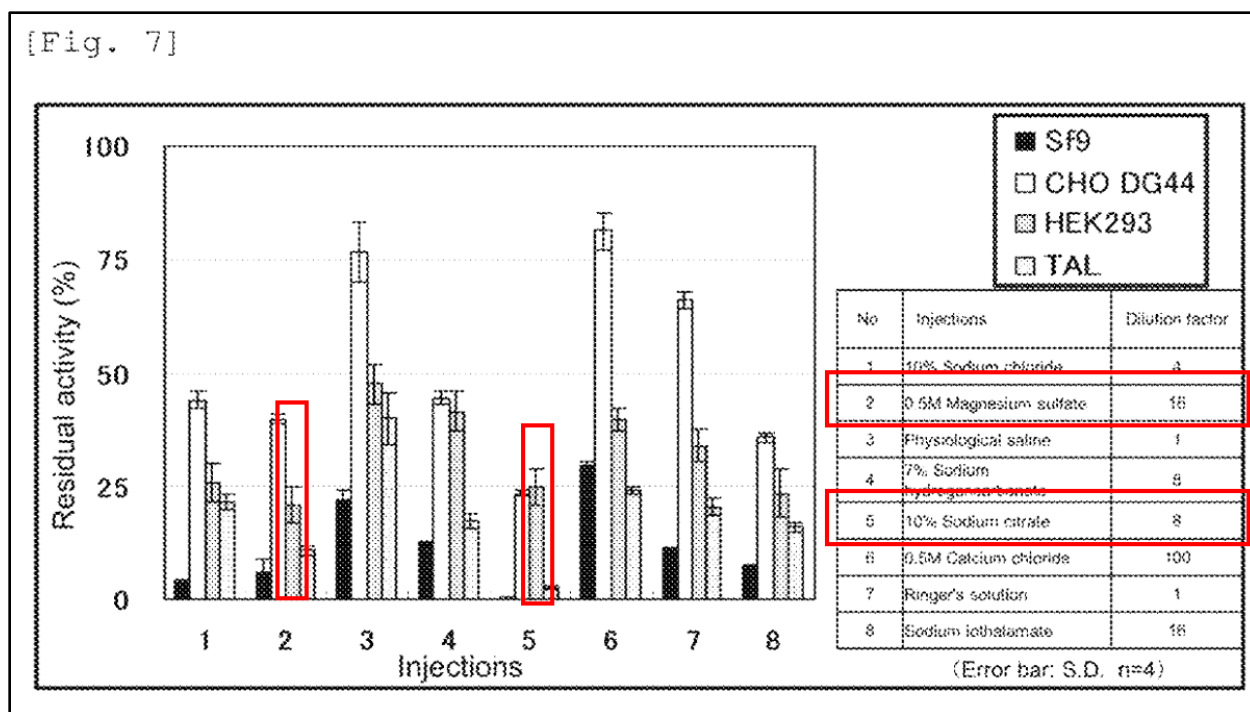
polyphemus—would not have understood Patent Owner to have been in possession of *Limulus polyphemus* recombinant Factor C proteins with the residual activity function recited in challenged claim 21. Moreover, a POSA would not have simply relied upon the *Tachypleus tridentatus* data of the '767 Application to conclude comparable functional activity for recombinant *Limulus polyphemus* Factor C without experimental verification because a POSA in 2013 would have understood that knowledge of a protein sequence from one species (i.e., *Tachypleus tridentatus*) did not equate to possession of a protein sequence with similar function from a different, uncharacterized, species (i.e., *Limulus polyphemus*).

104. Accordingly, a POSA in 2013 would have understood that knowledge of a protein sequence giving rise to a particular function for one species (i.e., *Tachypleus tridentatus*) did not equate to possession of a protein sequence with a comparable function from a different, unknown, uncharacterized, species (i.e., *Limulus polyphemus*), let alone possession of such protein across an exceedingly wide range of residual activity function.

c. The Inventors Failed To Provide A Structural Feature Common to the Claimed Genus That Correlates with Function From Which A Reasonable Expectation of Functional Comparability Could be Made.

105. Claim 21 encompasses a genus of HEK-expressed recombinant *Limulus polyphemus* Factor C protein whose residual activity is *either* 10% or

higher in the presence of 21 mM sodium citrate *or* 15% or higher in the presence of 16 mM magnesium sulfate. Ex. 1001, cl. 1. The '840 JP Priority Application lacks any mention of any residual activity range or percent limitation in the presence of magnesium sulfate whatsoever and so fails to support claim 21. Ex. 1019. In addition, the '767 Application provides only a single example of HEK-expressed recombinant Factor C proteins (all derived from *Tachypleus tridentatus*) falling within each of these optional ranges.



Ex. 1006, 122 (FIG. 7) (annotated).

106. Even these two examples, however, were obtained via production of recombinant Factor C protein from *Tachypleus tridentatus* horseshoe crabs, and *not* *Limulus polyphemus* horseshoe crabs. The '767 Application provides no other

explanation or structural feature common to such proteins that would indicate to a POSA that the inventors were in possession of the full scope of proteins with residual activities falling above the claimed thresholds (from 10% and 15% all the way up to 85% and 99%, for example) for a different species of horseshoe crab.

107. Though the '767 Application speculates that the addition of post-translation (α -2,3)-linked terminal sialic acid via expression in mammalian systems (such as HEK293 cells) could contribute to such increase in residual activity, Patent Owner and the inventors explicitly abandoned this theory for *Limulus polyphemus* proteins expressed in HEK293 cells in securing issuance of claim 21. Ex. 1004 (FH 8.24.2021 Reply to Office Action), 77 (“[S]uperior salt tolerance can be reproduced irrespective of presence of (α -2,3)-linked terminal sialic acid, at least for *Limulus* Factor C recombinantly produced using HEK293 cell as a host.”), 86 (same).

108. Indeed, with the benefit of after arising data, the inventors and Patent Owner revert to claiming that “salt tolerance of *Limulus* Factor C produced by HEK 293 cells is increased not dependent on presence or absence of (α -2,3)-linked terminal sialic acid.” Ex. 1004, 86. They claim instead that “increase of salt tolerance depends on factors other than (α -2,3)-linked terminal sialic acid.” Ex. 1004, 86. Such speculation about “other” factors only underscores the unpredictability that a POSA would have understood about these functionally-

claimed proteins as of 2013, when the *Limulus polyphemus* sequence was still unknown and undisclosed.

109. Ultimately, a POSA in 2013 would have recognized that there were known differences between Factor C from *Tachypleus tridentatus* and Factor C from *Limulus polyphemus* and would have further understood that the full gene and amino acid sequences for the latter remained unknown and undisclosed. As such, a POSA would not have understood the '318 patent inventors to be in possession of the full scope of functionally-defined recombinant *Limulus polyphemus* proteins of claim 21

110. Because the '767 Application discloses no examples of Factor C proteins satisfying either of the broad “residual activity” thresholds of claim 21, and because it also fails to disclose structural features common to this claimed set of proteins that would lead a POSA to understand the members of this genus in the absence of any actual examples, the '767 Application fails to satisfy the written description requirement for patentability.

2. The '318 Patent Inventors Admitted that the Invention of Recombinant *Limulus polyphemus* Factor C Was Not Completed Until Years After the Claimed Priority Date.

111. That the inventors did not possess recombinant *Limulus polyphemus* Factor C proteins having the claimed functional property is confirmed by their own admissions to the patent office.

112. On October 18, 2017, two of the three inventors named on the '318 patent (Mizumura and Oda) filed International Application No. WO 2018/074498 entitled, "Recombinant Proteins Derived From Genus *Limulus*, and DNA Molecules Encoding the Same" ("the '498 PCT") (Ex. 1005). In this PCT, the inventors detailed the struggle the field encountered in accomplishing the recombinant production of *Limulus polyphemus* proteins—" [t]here have been no reports of a reagent using recombinant proteins derived from *Limulus polyphemus*, which is widely used as a lysate reagent." Ex. 1005, ¶¶0007-09. The '498 PCT explains that "[t]he reason is that in *Limulus polyphemus*, the proteins and their genes involved in the mechanism of coagulation caused by endotoxin have not been completely identified **structurally or functionally**." Ex. 1005, ¶0010.

113. Instead the '498 PCT inventors explained that a predictable correlation between structure and function of *Limulus polyphemus* Factor C proteins was not well known by the priority date of the '318 patent and explicitly attribute this unpredictability to biodiversity. Ex. 1005, ¶¶0012-13 (" [D]ue to the various differences and diversity of biomolecules depending on the genus and species of horseshoe crab, it has not been possible to accurately determine the full-length sequence structure, expression, and function of these proteins and genes using conventional methods.").

114. In the '498 PCT, however, inventors Mizumura and Oda tout their own more **recent** success: “in obtaining full-length cDNAs encoding all of the protein factors involved in the coagulation mechanism of *Limulus polyphemus*, something that no one had succeeded in doing for many years...thereby completing the present invention.” Ex. 1005, ¶0017. By their own admission, these inventors did not possess (by 2013) the invention of claim 21—a recombinant protein from *Limulus polyphemus* defining boundaries **excluding** every example (related to the entirely separate *Tachypleus* species) that the '767 Application discloses.

115. As discussed in Section VI.B.1. above, there were **known** amino acid sequence differences between *Limulus polyphemus* crabs and other species such that predictions about functional activity would have been speculative. Ex. 1022, 157. In my opinion, such variation between species demonstrates a lack of possession of functional proteins in the absence of complete sequence information.

116. In my opinion, the '318 patent inventors did not possess the claimed recombinant *Limulus polyphemus* Factor C protein in 2013—a fact they freely admitted when they later—having acquired the gene and amino acid sequence information for this species of crab—attempted to patent this very invention. At best, the '767 Application invites a POSA to try to: (1) sequence the *Limulus polyphemus* Factor C gene despite the prior difficulty of doing so, (2)

recombinantly produce this Factor C protein of claim 21 using the new information they discovered, (3) measure the functional “residual activity” of the resulting proteins to determine whether such falls within the range claimed, (4) unearth a structural feature driving protein’s function as claimed. In my opinion, this invitation does not amount to the inventors’ possession of the full scope of recombinant Factor C protein encompassed by claim 21.

C. The ’767 Application Fails to Enable the Full Scope of Factor C Proteins Across the Claimed Range of Residual Activity Values.

117. As noted above, I understand that the enablement requirement instructs that the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without “undue experimentation.”

118. Undue experimentation factors include “(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.”

119. Challenged claim 21 sweeps in recombinant Factor C proteins with residual activity values falling anywhere above very low thresholds (10% and 15%) when in the presence of defined concentrations of two ionic solutions—

sodium citrate and magnesium sulfate, respectively. Ex. 1001, cl. 1. The '767 Application describes only a ***single*** residual activity measurement of Factor C in each of the recited ionic solutions (sodium citrate and magnesium sulfate), but for an ***unclaimed*** species of crab. The '767 Application describes ***zero*** examples or residual activity measurements of any Factor C in any ionic solution for the claimed *Limulus polyphemus* horseshoe crab. And the '767 Application merely reports on such results when the proteins are expressed via insect or mammalian expression systems, rather than explain ***why*** such values were obtained in each case.

120. In my opinion, to do so, a POSA would have needed to engage in significant experimentation to make proteins exhibiting a residual activity across such a wide claimed range. Such would have amounted to a trial and error research project.

121. Indeed, while the inventors had surmised that such residual activity values were due to the post-translational addition of (α -2,3) sialic acid when such proteins were expressed in mammalian cells, they abandoned this theory to secure claim 21, telling the Patent Office that such theory ***did not apply to Limulus polyphemus recombinant proteins***.

122. In addition, as discussed in Section VI.B., above, the field of recombinant protein technology involved significant experimentation by 2013 due

to the complex and unpredictable relationship between protein sequence, three-dimensional protein structure, post-translational modifications, and resulting protein function. As such, there was still a significant amount of research and experimentation required to generate a particular protein, with a particular functional capability, across a number of different expression systems.

123. In particular, production of recombinant proteins with specific functional properties is achieved in the biopharmaceutical and biomedical industry through the control of input parameters - cell line, production scale, cell culture media, feed and supplements and process parameters (including but not limited to pH, temperature, dissolved oxygen, mixing / agitation speed, initial cell density, culture duration etc.). Control of input parameters is manifested as control of biophysical-biochemical properties of the recombinant protein including expression level (protein concentration), aggregation, impurities, any fragments and isoforms, and post-translational modifications such as glycosylation which are verified to demonstrate consistent protein characteristics. Challenged claim 21 claims a set of proteins falling within a broad range of residual activity function but fails to specify what parameters will achieve proteins of this set. In essence, claim 21 requires only that a POSA express *Limulus polyphemus* Factor C in HEK293 cells. But such POSA could otherwise produce the protein using any condition and at any scale. The '767 Application, however, provides no guidance

on how a POSA would set or manipulate such input parameters to achieve any structural attribute or to achieve the specific residual activity function claimed. Instead, to reach the full range of residual activity values claimed, a POSA would have to engage in an enormous amount of trial-and-error—adjustments to various input parameters, testing of outcomes, and further iteration. In view of this, the production of recombinant proteins from an animal such as a horseshoe crab in 2013 required significant trial and error, experimentation, modification, and optimization in order to produce proteins across such a broad range of a specific functional feature like residual activity.

124. It must be noted here that residual activity is not a biophysical-biochemical property of Factor C that can be directly predicted from the protein sequence. Rather, as noted above, it is an outcome of one or more structural properties / attributes of Factor C expressed from *Tachypleus tridentatus* using HEK293 and CHO cells. While the inventors theorized that the (α -2,3) sialic acid was the structural property / attribute giving rise to functional characteristics (even if setting aside the fact that this structural element was not included in claim 21 and the theory was abandoned by the inventors), there is no explanation of *why* the (α -2,3) sialic acids lead to such residual activity, what is the minimum number of sialic acids required to achieve the desired residual activity, and whether there are specific N-glycosylation sites that are preferred over others. *Tachypleus tridentatus*

Factor C contains six potential N-glycosylation sites of which one was confirmed to not be glycosylated (Ex. 1007, 6555-6556). Each of the remaining five N-glycosylation sites can theoretically contain up to four sialic acids (Ex. 1030, 2904) which gives the range of potential sialic acids from zero to twenty in a range of zero to five sites. This is a wide range for a POSA to navigate to generate Factor C from a different species that would behave in a manner similar to the one from *Tachypleus tridentatus* relative to the full scope of claimed residual activity.

125. In my opinion, therefore, the '767 Application does not teach a POSA how to make or use *Limulus polyphemus* Factor C proteins with any particular residual activity value (whether that be inside or outside the claimed range, or at a value slightly higher than the threshold, or a value approaching 90% or 100%, both of which are claimed).

126. To do so, a POSA would have needed to engage in significant experimentation to make proteins exhibiting a residual activity across such a wide and open-ended claimed range. Such would have amounted to a trial and error research assignment requiring an unreasonable amount of experimentation.

127. As noted above, I have been informed that a certain set of factors are examined in order to determine whether the amount of experimentation required to complete an invention based on the disclosure provided in the specification would

have been “unreasonable” or “undue”. These undue experimentation factors include:

- (1) the quantity of experimentation necessary,
- (2) the amount of direction or guidance presented,
- (3) the presence or absence of working examples,
- (4) the nature of the invention,
- (5) the state of the prior art,
- (6) the relative skill of those in the art,
- (7) the predictability or unpredictability of the art, and
- (8) the breadth of the claims.

128. In my opinion, each of these factors supports the conclusion that the amount of experimentation required of a POSA to make and use the claimed Factor C proteins with the claimed residual activity properties for *Limulus polyphemus* as of December 10, 2013 would have been undue.

129.

1. Wands Factor Analysis

a. Claim 21 is Broad (Factor 8).

130. Claim 21 broadly encompasses Factor C proteins satisfying a wide range of values of residual activity in the presence of certain ions. While the inventors abandoned the one structural characteristic offered in the '767

Application to potentially explain the purported functional advantage of the claimed invention—and eliminated this characteristic from claim 21—they provided no additional disclosure to enable a POSA to make and use the full scope of resulting proteins in a manner amounting to anything more than a trial-and-error research assignment to “see what works.”

131. Instead, the '767 Application provides inconsistent statements about the role of post-translational modifications such as the addition of sialic acid relative to the functional properties of recombinantly produced proteins. The '767 Application states, “The test results indicate that Factor C expressed in mammalian cells can have low susceptibility to activity inhibition by ions. Such a property is particularly advantageous for detection of endotoxin contamination in production of injections.” Ex. 1006, 99. The '767 Application links the type of host cell employed to differences in post-translational modifications (additions of sugar chains on the proteins) as evidenced by the differing weights of the resulting protein. Ex. 1006, 93 (“The difference in molecular weight among the above prepared four types of Factor C is conceived to be attributed to the difference in post-translation modification (sugar chain modification) depending on the type of host cells employed. For example, it has been known that sialic acid is linked to the N-type sugar chain after translation in mammalian cells, whereby a

recombinant protein produced in mammalian cells has dimensions larger than those of a recombinant protein produced in insect cells.”).

132. However, the ’767 Application also indicates that *Tachypleus tridentatus* Factor C proteins produced via HEK293 cells **have the same weight** as native (horseshoe crab-produced) Factor C proteins. Ex. 1006, 101. Given these identical weight profiles, the ’767 Application leaves the POSA to wonder the extent of sialylation on the HEK293-expressed *Tachypleus tridentatus* Factor C protein vis-à-vis native Factor C, and whether and how such post-translational structural modification impacts the function of HEK293-recombinantly produced proteins of claim 21.

133. Moreover, the Patent Owner simply walked away from this sialic acid modification theory during prosecution of the ’386 Application and claimed recombinant Factor C proteins broadly (both with and without post-translational sialic acid). Throughout prosecution, the Applicant underscored the importance of having sialic acid as a distinguishing feature of the claimed Factor C during prosecution of the ’318 patent in an attempt to overcome the cited prior art. For example, the Applicant stated in an Amendment filed on September 17, 2020 that “the claimed invention using the recombinant Factor C having (α -2,3)-linked terminal sialic acid in a greater amount for the endotoxin assay reagent exhibits effects of providing improved sensitivity in the presence of the salt compared with

the endotoxin assay using the native Factor C (*see* Fig. 7 of the present specification), which effects are unexpected from Ding and Current Protocols.” Ex. 1004 (Page 12 of Amendment filed on September 17, 2020), 236. The Applicant then reiterated on February 3, 2021 that “the claimed Factor C protein provides an unexpected result in terms of salt tolerance compared to the native Factor C, that is, neither Ding nor Current Protocols discloses or suggests the effects of the claimed invention of providing improved sensitivity of endotoxin assay in the presence of a salt, by increasing terminal sialic acid of Factor C contained in its endotoxin assay reagents.” Ex. 1004 (Page 9 of Amendment filed on February 3, 2021), 175.

134. Notably, however, claim 21 is the only independent claim in the ’318 patent that is agnostic to the sialic acid status of the claimed Factor C. Ex. 1004, 77, 86 (conceding that “superior salt tolerance can be reproduced *irrespective of* presence of (α -2,3)-linked terminal sialic acid, at least for *Limulus [polyphemus]* Factor C recombinantly produced by using HEK 293 cell as a host.”).

135. Reaching the full scope of claim 21 therefore requires an extensive amount of experimentation across multiple fronts: (1) to discover and characterize the gene and amino acid sequence for *Limulus polyphemus* Factor C itself, which was not known as of 2013 (Section VI.B.2., above), (2) to use this as-yet undiscovered genetic information to recombinantly generate Factor C protein

across the full scope of residual activity function characteristic as defined by claim 21.

b. The Nature of the Invention, Lack of Information in the Prior Art, and Unpredictability of the Field Left Uncertainty About The Residual Activity Across the Wide Range Claimed (Factors 4, 5, 7).

136. Per the '318 patent inventors' own admission years after the '767 Application was filed, the field had struggled to sequence *Limulus polyphemus* Factor C protein and could not make accurate predictions as to its structural and functional properties in view of the biodiversity between horseshoe crab species. Ex. 1005 ('498 PCT), ¶0012-17 ("in *Limulus polyphemus*, even though more than 20 years have passed since the report in the genus *Tachypleus*, complete identification of these proteins and genes in terms of both structure and function has not been achieved. This is because due to the various differences and diversity of biomolecules depending on the genus and species of horseshoe crab, it has not been possible to accurately determine the full-length sequence structure, expression, and function of these proteins and genes using conventional methods.").

137. In addition, while recombinant technology was not brand new as of 2013, a significant amount of research and experimentation was still required to generate a particular protein, with a particular functional capability, across a number of different expression systems, through screening of expression systems

and optimization of cell culture conditions (including cell culture media composition). Ex. 1031, 16 (“Even when hundreds of proteins are produced at commercial scale, the production of recombinant proteins still constitutes a challenge in many cases... A necessary condition for adequate recombinant protein production is the efficient expression of the gene of interest. However, expression can be lost due to structural changes in the recombinant gene or disappearance of the gene from host cells.”); Ex. 1030, Abstract (“PTMs directly influence the 2-DE pattern, and knowledge of the rules of protein separation is required in order to understand the protein distribution in a 2-DE gel. Glycosylation is the most common PTM and can modify both the molecular weight and the pI of a protein. In particular, the effect of charged monosaccharides (mainly sialic acids, SAs) on the 2-DE pattern of a protein is of major interest since changes in sialylation are regularly observed in comparative studies. Little is known about the pI shift of a glycoprotein induced by the presence of SAs, or whether this shift is the same for all glycoproteins.”). Ex. 1039, 936 (Noting that “[t]here have been numerous reports on the effects of cell type, culture process, and culture media toward the resulting glycoform profile” and summarizing same).

138. The '767 Application provides no guidance, whatsoever, on how to control for this functional characteristic—how to drive it higher or lower in HEK293 recombinant systems—and certainly provides no guidance on this in the

Limulus polyphemus species of crab, specifically. In my opinion, a POSA would not have been able to dictate, predict, or expect a particular level of “residual activity” from a *Limulus polyphemus* Factor C protein produced as of 2013.

c. The Level of Ordinary Skill in the Art Is High (Factor 6).

139. As I discuss above, the level of ordinary skill in the art in the field of this purported invention is high: an ordinary skilled artisan would have had a Ph.D. degree in biological, chemical or biochemical engineering, molecular biology, or biochemistry and at least two years of experience in the field of utilizing or assessing horseshoe crab Factor C (lysate, purified native and/or recombinantly produced) for endotoxin detection.

140. However, designing, expressing, and testing recombinant proteins is laborious, time-consuming, complicated, and highly unpredictable due to the many effects that variations in amino acid sequence (and subsequent post-translational modifications) can have on protein structure and function. Moreover, raw material variability impacting protein glycosylation has been well-known and documented such that even replicating an expression process for the same recombinant protein and same cell line but different batch of cell culture media (or serum, if applicable) may lead to differing structure and functional activity. In the event of such raw material variability between two expression processes, a POSA would not expect the same residual activity for both proteins. Additionally, making a recombinant

Factor C protein that actually has **any** desired activity (i.e., ability to detect endotoxin) was known to be difficult in view of challenges, for example, with getting such proteins to be secreted by the host cells themselves. Ex. 1026, 360; Ex. 1027, 71. In view of this, making a recombinant Factor C protein with endotoxin detection function ***at or above a particular level*** was highly speculative.

141. Thus, in my opinion, reaching the full scope of claim 21 would require a high level of skill. While the level of skill typically required to practice in this field is already high, because of the lack of guidance in the '767 Application itself and elsewhere in the prior art, as discussed in at least Section VI.B above, such highly skilled individuals may ***attempt*** to practice the claimed invention but ***still*** be left with nothing but a “research assignment[]” to generate *Limulus polyphemus* Factor C proteins with varying levels of sialic acid and to identify which version achieves a residual activity falling in the claimed range.

d. The Absence of Guidance or Examples in the '767 Application Necessitates Extensive and Undue Experimentation to Practice the Full Scope of Claim 21 (Factors 1-3).

142. While claim 21 includes *Limulus polyphemus* recombinant proteins across a wide range of “residual activity” levels, the '767 Application never describes or instructs a POSA how to make or use **any** recombinant proteins from this species of horseshoe crab at all. The '767 Application provides no working examples of such proteins. And nothing in the application suggests any steps the

inventors envisioned for ensuring that such *Limulus polyphemus* Factor C proteins (when they were eventually made) would satisfy the claimed residual activity requirement.

143. Even assuming the '318 patent inventors had a plan for generating these *Limulus polyphemus* proteins with the claimed functional properties, they provided no guidance whatsoever on how to execute that plan.

144. As of 2013, the generation of recombinant Factor C proteins from a different species of crab and across a wide range of functional capabilities would have required literature review, the obtaining of sequences, the preparation of precursors, optimization of the experimental conditions, the obtaining and purifying of Factor C with various levels of sialic acid, and the verification of residual activity at lower, moderate, and higher values with then claimed “10% or higher” and “15% or higher” thresholds. However, the '767 Application makes no mention of what logic or protocol a POSA would use to generate recombinant Factor C proteins with the functional characteristics across the full scope of the alleged claimed invention (e.g., 90% or 95%).

145. In addition, the function of residual activity in claim 21, as explained through the '767 Application, is calculated based on measurement of the product of the full cascade reaction shown in FIG. 4. Ex. 1006, 52. Thus, in addition to providing no explanation of how to express recombinant *Limulus polyphemus*

Factor C in HEK293 cells to achieve proteins with residual activity function across the full scope of the claimed range, the '767 Application provides no guidance whatsoever on how to express *Limulus polyphemus* Factor B and Pro-clotting enzyme to generate the cascade from which residual activity can be measured. Moreover, not only does the '767 Application not provide gene or amino acid sequence information for Factor B and Pro-clotting enzyme for *Limulus polyphemus* horseshoe crabs, it also provides no evidence or discussion about whether and how the claimed *Limulus polyphemus* Factor C would react with Factor B and Pro-clotting enzyme from *Tachypleus tridentatus* horseshoe crabs (produced in insect cells, as the '767 Application describes) to trigger the cascade reaction required to measure the claimed "residual activity." The inventors undoubtedly left it to the POSA to complete the invention defined by claim 21.

146. In the absence of any disclosure from which a POSA could make or use the full scope of recombinant *Limulus polyphemus* Factor C proteins defined by the range of function set forth in claim 21, it is my opinion that the '767 Application fails to enable the full scope of claim 21.

VII. PRIOR-ART TO THE '318 PATENT

147. I briefly introduce in the Section below the prior-art references discussed in this declaration.

A. United States Publication US 2015/0307864: “Novel Recombinant Factor C and Method for Producing the Same, and Method for Measuring Endotoxin,” Published October 29, 2015 (“Mizumura”) (Ex. 1009)

148. The reference I refer to as “Mizumura” is the United States publication of the '767 Application. Excepting the paragraph added with the filing of the '767 Application (Ex. 1020, 2-3), Mizumura has an identical disclosure to the '318 patent (*compare* Ex. 1009, *with* Ex. 1001).

149. Mizumura would have itself been filed confidentially on June 9, 2015, thus being inaccessible to the public until Mizumura was published on October 29, 2015. The disclosure and content of Mizumura, however, is identical in all relevant respects to the content of the '767 Application and the '082 PCT. As noted in Footnotes 1 and 2, I have considered the paragraph added when filing the '767 Application in the United States in my analysis of Mizumura, even though it does not appear in the publication itself.

150. Mizumura published on October 29, 2015. I have been informed, therefore, that if challenged claim 21 is not entitled to an effective filing date of earlier than April 30, 2019, then Mizumura is indisputable prior art to claim 21 because it was published more than one year prior to April 30, 2019.

B. PCT Publication WO2018/074498: Recombinant Proteins Derived From Genus *Limulus*, and DNA Molecules Encoding Same, Published April 26, 2018 (“The ’498 PCT”) (Ex. 1005)

151. The reference I refer to as “the ’498 PCT” is the publication of International Application PCT/JP2017/037629, which was filed on October 18, 2017, and published on April 26, 2018. I understand that if claim 21 is not entitled to an effective filing date of earlier than April 30, 2019, then the ’498 PCT is indisputable prior art to claim 21 because it was published more than one year prior to April 30, 2019.

152. The “present invention” of the ’498 PCT “relates to recombinant proteins derived from the genus *Limulus*, DNA encoding the same, and methods of use thereof.” Ex. 1005 (’498 PCT), ¶ 0001. The ’498 PCT specifies that *Limulus polyphemus* is the only member of the genus *Limulus* and discusses using mammalian expression systems (CHO cells experimentally, and HEK293 cells generally) for the recombinant production of *Limulus polyphemus* Factor C. Ex. 1005 (’498 PCT), ¶¶ 0004 (“The organisms belonging to the genus *Limulus* are assigned to only one species: *Limulus polyphemus*.”), 0039 (“The host cell other than a horseshoe crab is not particularly limited as long as it can functionally express the protein from the first cDNA. Examples of the host cell other than a horseshoe crab include mammalian cells and insect cell. Examples of mammals include rodents and primates. . . . Examples of the human cells include a human

embryonic kidney cell-derived cell line (HEK). Examples of the HEK include HEK293.”).

C. McClymont 2017 (Ex. 1010)

153. The reference I refer to as “McClymont” is a University of Glasgow Ph.D. Thesis by Karen McClymont, dated March, 2017 (deposited as of April 7, 2017). I understand that if claim 21 of the ’318 patent is not entitled to an effective filing date of earlier than April 30, 2019, then McClymont is prior art to claim 21 because it was published and publicly accessible more than one year prior to April 30, 2019.

154. McClymont discusses the need for complete sequence information for Factor C from *Limulus polyphemus* horseshoe crabs given intra-species variability in binding site structure. Ex. 1010 (McClymont), 36 (“Despite the fact that other Factor C based tests use protein from the *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* species, the commercial LAL test is made from *Limulus* Factor C. As there are known variances between different LPS binding sites, *it is beneficial to study lpFC to identify any differences that may arise between the species in terms of sensitivity or specificity of binding.*”).

McClymont reported that “[n]either the amino acid, nor the genomic DNA or cDNA sequences for Factor C from *Limulus polyphemus* were available in any public databases” and described the research and results of her endeavor “to

assemble the *Limulus polyphemus* Factor C coding sequence and gene” in 2017. Ex. 1010, 38-39, 159-60 (Appx. B).

155. I understand that McClymont was publicly accessible to POSAs at least as of April, 2017. Ex. 1010, 1.

VIII. GROUND 1: CLAIM 21 IS OBVIOUS OVER MIZUMURA IN VIEW OF THE ’498 PCT

156. Mizumura’s disclosure is identical to both the ’767 Application and the ’318 patent specification² and it discloses recombinantly produced Factor C having the residual activity properties of challenged claim 21. Ex. 1009, ¶¶0035, 0099, 0179, 0203, 0311, FIG. 7.

157. The ’498 PCT is an international publication disclosing the production of *Limulus polyphemus* recombinant Factor C via mammalian recombinant expression systems, including examples in CHO cells and reference to HEK293 cells as an additional option. Ex. 1005, ¶¶0037-39, 0155-185.

158. By April 30, 2019, it would have been obvious for a POSA to have replaced the *Tachypleus tridentatus* Factor C proteins of Mizumura using the by-then-known *Limulus polyphemus* genetic and/or amino acid sequences for

² Excepting the paragraph that was added with the U.S. conversion of the ’082 PCT to the filing of the ’767 Application. Section VI.A., above., This paragraph does not impact the determination of obviousness.

recombinant protein production. This is similar to the fact that the inventors of '318 patent produced *Tachyplesus tridentatus* Factor C in Sf9 insect cells based on a previous disclosure of producing a soluble and functional *Carcinoscorpius rotundicauda* Factor C in Sf9 cells because the amino acid sequence for *T. Tridentatus* was known by that time. Ex. 1032, Abstract; Ex. 1007, 6556.

159. The LAL lysate test (incorporating native *Limulus polyphemus* Factor C sourced directly from crabs) had been one of the premier assays for endotoxin detection since its discovery in the 1960. Ex. 1023, 265, 272-73; Ex. 1005, ¶¶0003-4. By 2018 the field had accomplished recombinant production of Factor C from both *Tachyplesus tridentatus* and *Carcinoscorpius rotundicauda*. Ex. 1009, ¶¶0288-290; Ex. 1032, Abstract.

160. In view of the movement in the field of Factor C protein use for endotoxin testing toward the generation of recombinant Factor C proteins as a substitute for harvesting blood from native crabs (Ex. 1005, ¶¶0008-09), by April of 2019, POSAs would have had reason to combine Mizumura and the '498 PCT to produce recombinant *Limulus polyphemus* Factor C proteins and would have had a reasonable expectation of success that such proteins would exhibit the residual activity of claim 21.

1. Mizumura and the '498 PCT Collectively Disclose all of the Limitation of Claim 21.

161. As shown below, Mizumura and the '498 PCT together disclose the various characteristics of recombinantly produced Factor C proteins recited in claim 21. As discussed below, a POSA would have a reason to combine these two disclosures by April 30, 2019, in order to express *Limulus polyphemus* Factor C in HEK293 cells and would have reasonably expected such resulting protein to have the residual activity characteristics claimed.

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
[21PRE] A horseshoe crab Factor C protein having activity of Factor C,	<i>“The Factor C of the present invention is a Factor C derived from a horseshoe crab.</i> The Factor C of the present invention is a recombinant protein. The term ‘recombinant protein’ refers to a protein obtained through introduction of a gene encoding the protein into a host cell and heterologous expression of the gene. <i>The Factor C of the present invention has activity of Factor C.</i>” EX1009, ¶0187.	“Recombinant proteins derived from genus <i>Limulus</i>, and DNA molecules encoding same.” EX1005, Title. “The present invention relates to recombinant proteins derived from the genus <i>Limulus</i>, DNA encoding the same, and a methods of use thereof.” EX1005, ¶0001. “An example of a protein having factor C activity is protein 1. <i>The first protein is specifically any of the following proteins:</i> <i>(I) A protein comprising the amino acid sequence of the protein having factor C activity of Limulus</i>

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
		<i>polyphemus</i>.” EX1005, ¶¶0082-83. “As a result, there was a success for the first time in expressing and obtaining a protein from <i>Limulus polyphemus</i> that may have factor C activity (sequence number 2), a protein that may have factor B activity (sequence number 6), and a protein that may have pro-clotting enzyme activity (sequence number 14).” EX1005, ¶¶0179-80.
[21A] wherein the horseshoe crab is <i>Limulus polyphemus</i>,	“Meanwhile, it has been known that the American horseshoe crab (<i>Limulus polyphemus</i>) undergoes blood clotting when it is infected with a Gram-negative bacterium. This phenomenon has been conventionally employed for the detection of endotoxin.” EX1009, ¶¶0003, 0188.	“The present invention relates to recombinant proteins derived from the genus <i>Limulus</i>, DNA encoding the same, and methods of use thereof.” EX1005, ¶0001. “As a result, there was a success for the first time in expressing and obtaining a protein from <i>Limulus polyphemus</i> that may have factor C activity (sequence number 2).” EX1005, ¶¶0179-80. “The genus <i>Limulus</i> contains only one species, <i>Limulus polyphemus</i>. The genus <i>Tachypleus</i> contains only three species: <i>Tachypleus tridentatus</i>, <i>Tachypleus</i>

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
		<i>gigas</i>, and <i>Tachypleus rotundicauda</i> (also called <i>Carcinoscorpius rotundicauda</i>).” EX1005, ¶¶0004, 0017, 0155-0185 pp. 49-75. <i>See also</i>, EX1010, 46-48, 159-160.
[21B] and wherein the horseshoe crab Factor C protein is produced through being recombinantly expressed from a Human Embryonic Kidney (HEK) 293 cell, and	“The Factor C of the present invention may be produced through, for example, expression in a mammalian cell as a host....The mammalian cell is not particularly limited, so long as the cell can functionally express a horseshoe crab Factor C....The mammalian cell is preferably a cell of a mammal selected from the group consisting of a rodent and a primate.... Of these, human is preferred. Examples of the human cell include human embryo kidney (HEK) cell line. Examples of HEK include HEK293.” EX1009, ¶¶0205-06. “FIG. 6 A table and a graph showing the activity of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>).” EX1009, ¶0178. “FIG. 7 A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and	“Host cells other than horseshoe crab are not particularly limited, so long as they are capable of functionally expressing a protein from the first cDNA. Host cells other than those of horseshoe crab include, for example, mammalian cells and insect cells....Human cells include human embryonic kidney (HEK) derived cell lines. An example of HEK is HEK293.” EX1005, ¶¶0039, 155-185.

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
	TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>).” EX1009, ¶0179. <i>See also</i> EX1009, ¶¶0288-90.	
[21C] wherein the Factor C protein exhibits a residual activity of at least one selected from the group consisting of:	“The Factor C of the present invention may have low susceptibility to inhibition of activity by an ion....The ‘residual activity’ is defined as a relative reactivity (%) observed for a measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to an ion-containing test specimen, with respect to the reactivity observed for another measurement specimen (endotoxin concentration: 0.05 EU/mL) prepared by adding endotoxin to injection water as 100%, in the assay system shown in FIG. 4.” EX1009, ¶0203. “Activity of Factor C was measured through the procedure shown in FIG. 4. A residual activity was defined as a relative reactivity (%) for endotoxin in an injection specimen, with respect to the reactivity for endotoxin in the corresponding control specimen as 100%.” EX1009, ¶0311.	
[21C1] (1) 10% or higher in the presence of 21 mM	“The above-described Factor C, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.” EX1009, ¶0099.	

Claim Term	Mizumura (EX1009)	'498 PCT (EX1005)
sodium citrate; and	“FIG. 7 A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>).” EX1009, ¶0179, FIG. 7 (showing bar graphs for No. 5 “Sodium citrate” as around 25% (for HEK293 cells)).	
[21C2] (2) 15% or higher in the presence of 16 mM magnesium sulfate.	“The above-described Factor C, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.” EX1009, ¶0035. “FIG. 7 A table and a graph showing the activity inhibition by injections of the four types of Factor C (Sf9, CHO, HEK, and TAL) derived from Japanese horseshoe crab (<i>Tachypleus tridentatus</i>).” EX1009, ¶0179, FIG. 7 (showing bar graphs for No. 2 “Magnesium sulfate” as around 20% (for HEK293 cells)).	

162. I note that the material disclosed in Mizumura was also published as a peer-reviewed journal publication in 2017. Ex. 1033, 140-145.

1. By April 30, 2019, A POSA Would Have Had Reason to Combine Mizumura and the '498 PCT.

163. By 2019, POSAs not only had the full Factor C gene and amino acid sequence for *Limulus polyphemus* available to them, but they had reason to use such information to develop recombinant *Limulus polyphemus* Factor C via HEK293 cells with the “residual activity” described in Mizumura. Such reasons include (1) the widely regarded success of the *Limulus polyphemus* Factor C as a component in the then-popular and trusted LAL endotoxin detection assay, (2) a general movement in the field of recombinant protein science to generate naturally sourced proteins in a much more controlled, characterized, and predictable way through known recombinant expression systems, and (3) to mitigate the enhancement effect caused by the presence of Factor G in LAL endotoxin detection assay. These reasons were apparent across the prior art as of 2019, including within Mizumura, the '498 PCT, and throughout literature in the field.

a. *Limulus polyphemus* Factor C Was Known as the Critical Component of Existing and Widely-Used Endotoxin Assays.

164. As noted above, use of Factor C sourced from *Limulus polyphemus* crabs in the LAL (Limulus amoebocyte lysate) test dated back to the early 1960's. Ex. 1023, 265. This advancement—based on a natural characteristic of *Limulus polyphemus* crabs—is widely regarded as being of critical importance to the biomedical industry. Ex. 1024, 54-58 (“The discovery, commercialization, and use

of LAL has been an important improvement to the pharmaceutical industry....[It] provides a means to detect endotoxins without having to kill or disable animals.”).

165. By the first decades of the 21st century, the value of hemolymph from *Limulus polyphemus* horseshoe crabs, specifically, was clear. Ex. 1016, 110, 116-117 (“*Limulus* test...has been widely employed as a simple and very sensitive assay method for bacterial endotoxins.”).

166. The medical device and pharmaceutical industries relied on the public trust and confidence in their products that was made possible by the LAL endotoxin assay. Ex. 1028, Abstract. Indeed, the inventors of Mizumura and the ’498 PCT touted the value that native *Limulus polyphemus* Factor C had provided as a critical component of endotoxin detection since the early 1960’s. Ex. 1023; Ex. 1009, ¶0003 (“[I]t has been known that the American horseshoe crab (*Limulus polyphemus*) undergoes blood clotting when it is infected with a Gram-negative bacterium. This phenomenon has been conventionally employed for the detection of endotoxin.”).

167. The ’498 PCT similarly states, “[A]moeocyte lysates are widely used as a highly sensitive reagent for detecting endotoxin in the quality control of pharmaceuticals and the like.” Ex. 1005, ¶0005.

b. Limitations of the LAL Assay Prompted Research for Alternative Endotoxin Detection Methods.

168. As important as the LAL assay has been to the biomedical industry, it is not without flaws. As noted above, “lack of specificity” of the test to particular endotoxins was identified as a limitation prompting research into more specific alternatives. Ex. 1025, 501 (“Problems with the specificity of LAL to endotoxin and the potential for negative effects on horseshoe crab populations have called for an alternative reliable endotoxin test.”), 507-8; Ex. 1028, Abstract (“[V]ariations in sensitivity and specificity of LAL to endotoxin, and the limited supply of *limulus* (horseshoe crabs) has called for an alternative pyrogen test.”); Ex. 1010, 26.

169. Thus arose efforts to generate synthetic versions of the zymogen proteins (Factor B, Factor C, and Pro-clotting enzyme) involved in the clotting cascade of horseshoe crabs. This included utilizing increasingly popular recombinant technology to generate recombinantly-produced versions of Factor C (“rFC”) from particular species of crabs. Ex. 1028, Abstract; Ex. 1025, 507 (“The rapid and specific detection of endotoxin is urgently needed for the development and production of clinical drugs and medical devices where state-of-the-art pyrogen-free facilities are required.”).

170. But, of course, in order to match or outperform the detection ability of the existing LAL test, such recombinantly produced *Limulus polyphemus* proteins had to demonstrate the functional ability to detect endotoxin. Ex. 1025, 507.

171. Given the biomedical research industry’s (and society’s) reliance on the ability to detect endotoxin in medical products so as to avoid associated adverse events, the desire to maximize endotoxin detection activity is and has been a core motivation in this field. Ex. 1025, 507-8.

172. As such, in my opinion, POSAs focusing on producing recombinant Factor C would have had reason to maximize the endotoxin detection capabilities of such protein—including by maximizing the claimed “residual activity” in the presence of inhibitory ions—in an effort to produce recombinant horseshoe crab proteins to replace those natively-sourced.

173. Of course, as discussed above, knowledge about how to accomplish the recombinant production of Factor C from different species of horseshoe crab was acquired at different times. While prior to 2017, scientists had the “idea to artificially produce proteins in amebocyte lysate using recombinant technology” and while certain companies had already commercialized products involving the recombinant proteins from the *Tachypleus* and *Carcinoscorpius* species of crabs, (Ex. 1005, ¶¶0008), as the ’498 PCT explains, the structural and functional characterization of the proteins involved in clotting from *Limulus polyphemus* horseshoe crabs remained elusive. Ex. 1005, ¶¶0008-13.

174. As the '498 PCT elaborates, while the gene and amino acid sequences of various *Tachypleus* species of crabs had been reported and widely available to the field by 1991:

in *Limulus polyphemus*, even though more than 20 years have passed since the report in the genus *Tachypleus*, complete identification of these proteins and genes in terms of both structure and function has not been achieved. This is because due to the various differences and diversity of biomolecules depending on the genus and species of horseshoe crab, it has not been possible to accurately determine the full-length sequence structure, expression, and function of these proteins and genes using conventional methods.

In fact, a comprehensive analysis of the genes of *Limulus polyphemus* is being conducted as part of the invertebrate genome project using next-generation sequencers

(<http://genome.wustl.edu/genomes/detail/limulus-polyphemus/>). The full-length sequences of all proteins and genes involved in the coagulation mechanism of *Limulus polyphemus* have not yet been elucidated. This fact also demonstrates the difficulty in accurately determining the full-length sequence structures of these proteins and genes in *Limulus polyphemus*. Ex. 1005, ¶¶ 0012-13.

175. But by 2017-2018 this identification was accomplished. By the April 26, 2018 publication of the '498 PCT, those inventors described that “The present inventors have succeeded in obtaining full-length cDNAs encoding all of the protein factors involved in the coagulation mechanism of *Limulus polyphemus*,

something that no one had succeeded in doing for many years, and in expressing all of the recombinant proteins, and have further discovered that these can be used for a variety of purposes, thereby completing the present invention.” Ex 1005, ¶10017.

176. Moreover, as the inventors also noted in adding a paragraph to the ’082 PCT with the U.S. national phase entry of the ’767 Application on June 9, 2015, recombinant production of Factor C proteins and use in a reconstituted system avoided contaminants associated with the natural lysate of a horseshoe crab, e.g. Factor G, on the cascade. Ex. 1020, 2-3.

177. Thus, by April 30, 2019, a POSA considering Mizumura’s data on HEK293 produced recombinant Factor C from *Tachypleus tridentatus*, as well as the ’498 PCT’s explicit disclosure of production of functional *Limulus polyphemus* recombinant Factor C in HEK293 cells would have ample reason—including the wide adoption of and reliance upon the LAL test but also including the specificity challenges with that test and the desire to avoid contaminants from native lysate such as Factor G—to develop a recombinant Factor C protein with a residual activity value similar to that shown in Mizumura’s FIG. 7 *for Limulus polyphemus* species of horseshoe crab.

2. A POSA Would Have Had a Reasonable Expectation of Success in Using *Limulus* Constructs to Generate the HEK293 Produced Recombinant Factor C of Claim 21.

178. By April 2019, POSAs would have reasonably expected to successfully generate recombinant Factor C of the *Limulus polyphemus* species via HEK293 cells where that recombinant Factor C has a residual activity falling within the range defined in claim 21. Such expectation stems at least from:

- Knowledge of the full-length amino acid sequence for *Limulus polyphemus* and the ability to actually compare such sequence to others;
- The inventors' own statements about the feasibility of using recombinant techniques for generating *Limulus polyphemus* proteins by 2017; and
- Dr. Mizumura's testimony indicating that, as of the actual filing date of the '318 patent, a POSA would reasonably expect to successfully generate recombinant *Limulus polyphemus* Factor C and would have expected it to have the same "residual activity" properties as demonstrated with *Tachypleus tridentatus*.

a. Knowledge of the Full Length Amino Acid Sequence Would Have Allowed a POSA to Do a Full Comparison to A Different Species and Form a Reasonable Expectation of Similarity in Function.

179. By the 2017 filing date of the '498 PCT, the inventors describe the successful “obtaining [of] full-length cDNAs encoding all protein factors involved in the coagulation mechanism of *Limulus polyphemus*.” EX1005, ¶¶0017, 0173 (“[T]he first successful elucidation of the full-length cDNA sequence (sequence number 1, sequence number 3) encoding a protein that may have *Limulus polyphemus* C factor activity, and the full-length amino acid sequence of that protein (sequence number 2, sequence number 4).”).

180. Having the complete sequences in hand by April 30, 2019 would have allowed a POSA to affirmatively compare them with those of other, well-studied, species such as *Tachypleus tridentatus* to make reasonable predictions about whether recombinantly-produced proteins would exhibit similar functional characteristics such as “residual activity.” This would have enabled a POSA to reasonably expect that recombinantly-produced *Limulus* Factor C protein would exhibit similar functional characteristics such as endotoxin detection or “residual activity.” Ex. 1010, 38, 48 (“Determining the sequence for lpFC also allowed comparisons to be made between the different species to identify any differences that may exist between the LPS binding sites. The high-level of similarity between

the three N-terminal regions supports the assumption that all three proteins are likely to bind lipid A in the same way.”).

181. While no such comparison could be fully predictive regarding the differences in conservative versus non-conservative mutations between sequences and their potential impacts on structure and function (as explained above, Section VII.B.3, above), having both sequences to compare would have provided a POSA with starting information leading to a reasonable expectation of achieving a protein with the residual activity function of *Limulus polyphemus* recombinant Factor C. Ex. 1010 (McClymont), 48.

182. In my opinion, it would have been well within the skill and experience of a POSA as of 2019 to compare amino acid sequences for the then-known *Limulus polyphemus* horseshoe crab and the already-studied and experimented *Tachypleus tridentatus* horseshoe crab and, given similarities overall, expect the two to be comparable in terms of residual activity.

b. The '498 PCT Describes How to Recombinantly Produce *Limulus Polyphemus* Factor C in a Mammalian Expression System Using the Known Amino Acid Sequence.

183. The '498 PCT describes the various gene and protein sequences, including “cDNA for a protein having *Limulus polyphemus* Factor C activity” (Ex. 1005, ¶¶0019) from which recombinant protein is generated. Ex. 1005, ¶¶0020-21 (“A recombinant first protein is a recombinant protein having factor C activity. The

recombinant first protein is specifically a recombinant protein which is...(I) a recombinant protein that contains an amino acid sequence of the protein of *Limulus polyphemus* having factor C activity.”); Ex. 1005, pp. 49-75.

184. The ’498 PCT then provides a detailed description of how such cDNA sequences were then expressed recombinantly. Ex. 1005, ¶¶0155-185; 0178 (“The cDNA (SEQ ID NO: 13) obtained in the above (5) was ligated to a vector pCA7 for protein expression in mammalian cells, whereby an expression plasmid was obtained. The 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 cDNA of SEQ ID NO: 1 was integrated) obtained in the above (3-7), and Expi CHO™ Expression System (Thermo Fisher Scientific), recombinant proteins thereof were obtained as culture supernatant fractions.”).

185. And the ’498 PCT inventors confirmed the endotoxin detecting function of such recombinantly-expressed *Limulus polyphemus* proteins, describing that it was “demonstrated ***for the first time*** that the use of each of the three recombinant proteins [produced via cDNA from *Limulus polyphemus*] enables quantitative detection of endotoxin with high sensitivity and high accuracy.” *Id.*, Examples 6-7, ¶¶0178-85.

186. While the '498 PCT experimental disclosure relates to the CHO mammalian expression system, it would have been well within the skill of a POSA at the time to carry out expression with the also-listed HEK293 expression system. Mizumura describes expression of Factor C of the *Tachypleus tridentatus* species of horseshoe crab using **both** the CHO and HEK293 cells. Ex. 1009, ¶¶0282-90 (“(2) Expression of Factor C in Mammalian Cells (CHO DG44)...(3) Expression of Factor C in Mammalian Cells (HEK293)”); Ex. 1005, ¶¶ 0205-0206 (“The Factor C of the present invention may be produced through, for example, expression in a mammalian cell as a host... The mammalian cell is not particularly limited...[examples] include human embryo kidney (HEK) cell line. Examples of HEK include HEK293.”).

187. In my opinion, by 2019, a POSA would have had a reason to recombinantly express the then-elucidated full-length amino acid sequence of *Limulus polyphemus* horseshoe crabs via the HEK293 system described in Mizumura in order to generate proteins and would have reasonably expected such proteins to exhibit similar values of residual activity as those reported in Mizumura for *Tachypleus tridentatus*.

c. The '318 Patent's Lead Inventor Testified To a Reasonable Expectation of Success.

188. Once the sequence information for *Limulus polyphemus* Factor C protein was known, a POSA could have compared such information to that of other

known horseshoe crab species and formed a reasonable expectation that the recombinantly-produced proteins would exhibit similar residual activity.

189. Such expectation would have been consistent with Dr. Mizumura's *own statements* during prosecution of applications in the '318 patent family, testifying that POSAs would reasonably have expected such success. In a February 22, 2018 Declaration submitted in support of prosecution *of the '767 Application* (grand-parent application to the '318 patent), Dr. Mizumura provided an amino acid alignment for Factor C as between *Limulus polyphemus* and *Tachypleus tridentatus*. Ex. 1029, 2-4.

190. Dr. Mizumura testified that “a [POSA] would readily recognize that the presently claimed invention could also work when Factor C is the Factor C of *Limulus polyphemus*.” Ex. 1029, 4. He further indicated that this expectation of success would extend to the “residual activity” property of such proteins. Ex. 1029, 4-5 (“[T]he above-described evidence supports that the Factor C of *Limulus polyphemus* would also have unexpectedly superior property of being less susceptible to inhibition by ions, as exhibited by Factor C of *Tachypleus tridentatus*.”).

191. In view of Dr. Mizumura's own testimony over a year before Patent Owner filed the application giving rise to the '318 patent (the '386 Application), a POSA would have had a reasonable expectation of success in combining

Mizumura and the '498 PCT to generate a recombinant Factor C protein with the residual activity characteristic recited in challenged claim 21.

IX. GROUND 2: CLAIM 21 IS OBVIOUS OVER MIZUMURA IN VIEW OF MCCLYMONT.

192. In my opinion, claim 21 is also obvious over Mizumura in view of the 2017 disclosure by McClymont of the full gene coding and amino acid sequences of the *Limulus polyphemus* Factor C protein. As with the '498 PCT, McClymont discloses the full cDNA and amino acid sequence for *Limulus polyphemus* Factor C protein and discloses expressing such protein using several common expression systems (including yeast and insect cells). Ex. 1010, 84-96, 159-60 (Appx. B). To my knowledge, McClymont is not associated with Patent Owner or any inventors. In my opinion, and for the same reasons discussed above in Ground 1, POSAs would have had reason to utilize the sequence information from McClymont to generate a recombinant Factor C protein of the *Limulus polyphemus* species in HEK293 cells as disclosed for *Tachypleus tridentatus* in Mizumura, and would have reasonably expected the resulting proteins to have residual activity described in Mizumura and falling within the claimed range. For this additional reason, challenged claim 21 is obvious.

X. SECONDARY CONSIDERATIONS DO NOT OVERCOME THE FOREGOING DEMONSTRATION OF OBVIOUSNESS.

193. Patent Owner offered two declarations from Dr. Mizumura in support of its argument during prosecution of the '318 patent that unexpected results associated with the invention rebutted the Examiner's *prima facie* case of obviousness and rendered the claims of the '318 patent (including challenged claim 21) patentable.

194. I disagree that this evidence demonstrates that the claims are not obvious. I understand that for so-called evidence of secondary considerations (or indicia) of non-obviousness to rebut a finding of obviousness, such evidence must be sufficiently linked to, and commensurate in scope with, the purported invention of *that particular claim*. Such evidence—for example, purportedly unexpected results of the invention—must also be relative to, as compared with, the results of the closest prior art. I disagree that any of Patent Owner's evidence of secondary considerations of non-obviousness meet these requirements.

195. For at least these reasons, the evidence of unexpected results offered by Patent Owner during prosecution does not save claim 21 from obviousness. I am unaware of any other secondary considerations evidence.

A. The January 8, 2021 Mizumura Declaration Does Not Establish Unexpected Results of the Subject Matter of Claim 21.

196. Dr. Mizumura's January 8, 2021 declaration was submitted "to explain and demonstrate that [their] invention provides unexpected superior results over" the Examiner's then-pending obviousness rejection. Ex. 1004, 150. Claim 21 had not yet been introduced in prosecution when the January 8, 2021 Mizumura declaration was submitted.

197. This January 8 declaration introduced new data for recombinantly produced *Tachypleus tridentatus* Factor C (from CHO-S) cells to demonstrate that the CHO DG44 recombinantly produced Factor C performed *better* than the data offered already in Figure 7 of the application. Ex. 1004, 150-52. It concludes by reiterating the hypothesis offered in the specification that "differences in molecular weight of Factor C expressed in different host cells such as Sf9, CHO-DG44 or HEK cells is attributable to the differences in post-translation modification (for instance, sugar chain modification) employed in each of the cells. *See* paragraph [0127] and Figure 2 of the specification." Ex. 1004, 153. "Therefore, a Factor C produced in CHO DG44 cells is *structurally different* from a Factor C produced in CHO-S, HEK and Sf9 cells." Ex. 1004, 153. (emphasis original).

198. The January 8, 2021 Declaration provides no evidence whatsoever of unexpected results with respect to the genus of Factor C proteins encompassed by

claim 21, both because the data offered was from recombinant Factor C of the *Tachypleus tridentatus* species (and not *Limulus polyphemus*) **and** because the data offered was from Factor C recombinantly produced in CHO cells (and not HEK293) cells.

199. Thus, in my opinion, the January 8, 2021 Declaration does not demonstrate the invention of claim 21 to have provided unexpectedly superior results over the prior art.

B. The August 11, 2021 Mizumura Declaration Does Not Establish Unexpected Results of Claim 21 Relative to the Closest Prior Art.

200. Dr. Mizumura's August 11, 2021 declaration was again submitted "to further explain and demonstrate that [their] invention provides unexpected and superior results over" the Examiner's then-pending obviousness rejection. Ex. 1004, 82. In the August 11 Declaration, Dr. Mizumura noted that "[a]s shown in Fig. 7 of the specification, *T. tridentatus* Factor C recombinantly produced by using CHO cell or HEK cell as a host has superior salt tolerance compared to native *T. tridentatus* Factor C (TAL) or *T. tridentatus* Factor C recombinantly produced by using Sf9 [insect] cell as a host." Ex. 1004, 83. Dr. Mizumura then offered "additional experimental data show[ing] that the same is true in the case of *L. polyphemus* Factor C recombinantly produced by using CHO cell." Ex. 1004, 83.

201. Dr. Mizumura concluded: “As shown above, *L. polyphemus* Factor C recombinantly produced by using CHO cells as a host have superior salt tolerance compared to native *L. polyphemus* Factor C (LAL) and *L. polyphemus* Factor C recombinantly produced by using Sf9 as a host. Especially, *L. polyphemus* Factor C recombinantly produced by using CHO DG44 cell has superior salt tolerance even compared to *L. polyphemus* Factor C recombinantly produced by using CHO-S cell as a host.” Ex. 1004, 85.

202. Claim 21 requires *Limulus polyphemus* Factor C recombinantly produced *via HEK293* (not CHO) cells, so the new data offered in Figure 1 of the August 11, 2021 Mizumura Declaration are not relevant to any unexpected properties of the claimed proteins, specifically.

203. Dr. Mizumura also pointed to a set of “Inhibition data” which was cited in a February 3, 2021 IDS. Ex. 1004, 85-86.

204. He argued, based on this data, that “salt tolerance of *Limulus* Factor C produced by HEK293 cell [mammalian] is superior to LAL and *L. polyphemus* Factor C recombinantly produced by using Sf9 cell [insect] as a host.” Ex. 1004, 85-86. However, such statement fails to explain how the comparison between the separate “Inhibition data” and the data in Figure 1 (from expression in CHO and Sf9) was made. There is no explanation as to whether the protocols, materials, or methods were similar so as to allow for any valid comparison. Moreover, even if

such comparison could be made, it fails to demonstrate any difference *in kind* in “residual activity” as between *Limulus polyphemus* recombinant Factor C produced in HEK293 cells versus insect cells (as opposed to a difference of degree).

205. Finally, in my opinion, this comparison is immaterial to how such results compare to the closest prior art as of 2019, which would be recombinant Factor C protein produced via other mammalian expression systems. The ’767 Application already disparages native Factor C (LAL) and recombinant Factor C expressed using insect host cells as susceptible to ion inhibition and thus having a low residual activity under such conditions, and so a POSA would look to closer prior art. Dr. Mizumura’s data fails to show unexpectedly superior results of recombinant *Limulus polyphemus* Factor C expressed in HEK293 cells relative to recombinant Factor C expressed in, for example, CHO cells.

206. Because Patent Owner provided no evidence of any unexpected results of the claimed Factor C proteins of claim 21 relative to the closest prior art, and because all other purported evidence of “unexpected results” stems from data on Factor C proteins that are not within the scope of claim 21, it is my opinion that claim 21 is unpatentable as obvious over the prior art.

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

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

Dated: 15 January 2025

/Siddhartha Jain/
Siddhartha Jain, Ph.D.