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Please find below and/or attached an Office communication concerning this application or proceeding.

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DETAILED CORRESPONDENCE

Status of the Application

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1-20 are pending in the application.

Election/Restrictions

Applicant's election without traverse of Group I, claims 1-17, in the reply filed on September 10, 2019 is acknowledged.

Claims 18-20 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on September 10, 2019.

Applicant's election with traverse of the species A), SEQ ID NO: 2, in the reply filed on September 10, 2019 is acknowledged. The traversal is on the ground(s) that the invention works irrespective of the amino acid sequences of A) SEQ ID NO: 2 and B) SEQ ID NO: 4, and species A) SEQ ID NO: 2 and B) SEQ ID NO: 4 are related. This is not found persuasive because, as noted in the previous Office action and undisputed by the applicant, the species of A) and B) are independent or distinct because each of these species is a different and distinct amino acid sequence; these species are not obvious variants of each other based on the current record; and there is a search and/or examination burden for the patentably distinct species at least because a separate

keyword and sequence search is required for each of the species of A) and B). The requirement is still deemed proper and is therefore made FINAL.

Claims 1-17 are being examined on the merits with claims 7 and 16 being examined only to the extent the claims read on the elected subject matter.

Priority

This application is a continuation application of U.S. non-provisional application no. 14/650,767, filed on June 9, 2015, now U.S. Patent 10,144,923, which is 35 U.S.C. 371 national stage filing of PCT/JP2013/083082, filed on December 10, 2013, which claims foreign priority under 35 USC § 119(a)-(d) to Japanese application 2012-269840, filed on December 10, 2012. A certified copy of the foreign priority document has been filed in the prior-filed application no. 14/650,767 on June 9, 2015.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on May 18, 2018, September 6, 2018, September 7, 2018, and January 24, 2019 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Citations 11-15, 32, and 33 of the IDS filed on May 18, 2018 have been lined through because, according to the transmittal letter filed on September 7, 2018, the IDS filed on September 7, 2018 has been filed to correct citations 11-15, 32, and 33 of the IDS filed on May 18, 2018.

Citation 37 of the IDS filed on May 18, 2018 has been lined through because there is no copy of the cited reference in this application or the prior-filed application no. 14/650,767.

Specification/Informalities

The applicant is requested to update the status of prior-filed application no. 14/650,767 in the disclosure of the specification at p. 1. According to USPTO records, the application no. 14/650,767 is now issued as U.S. Patent 10,144,923.

Claim Objections

Claims 1, 3-7, 9, 10, and 12-16 are objected to because of the following informalities:

Claims 1 and 9 are objected to in the recitation of “(α-2,3)-liked”, which appears to be a misspelling of the term “(α-2,3)-linked”.

Claims 3-7 and 12-16 are objected to in the inconsistent recitation of “wherein the Factor C” in claims 3-6 and 12-15 and “wherein the horseshoe crab Factor C” in claims 7 and 16 and in the interest of improving claim form and consistency, it is suggested that the phrase “wherein the Factor C” in claims 3-6 and 12-15 be amended to recite “wherein the horseshoe crab Factor C”.

Claims 7 and 16 are objected to in the recitation of “wherein the horseshoe Factor C is a protein” and in the interest of improving claim form, it is suggested that the noted phrase be amended to recite, *e.g.*, “wherein the horseshoe Factor C expressed in a mammalian cell”.

Claim 10 is objected to in the recitation of “recovering the expressed horseshoe Factor C as a solution” and in the interest of improving claim form, it is suggested that the noted phrase be amended to recite, *e.g.*, “recovering the horseshoe Factor C expressed in a mammalian cell as a solution”.

Claim Rejections - 35 USC § 112(b)

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

Claims 1-17 are rejected under 35 U.S.C. 112(b) as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor regards as the invention.

Claims 1 (claims 2-8 dependent therefrom) and 9 (claims 10-17 dependent therefrom) are indefinite in the recitation of “a horseshoe crab Factor C expressed using Sf9 as a host cell”. It is unclear as to whether the applicant intends for “a horseshoe crab Factor C expressed using Sf9 as a host cell” is the same horseshoe crab Factor C that is expressed in the mammalian cell, or encompasses horseshoe crab Factor C proteins that are different from the horseshoe crab Factor C that is expressed in the mammalian cell. In the interest of compact prosecution, if the applicant intends for the phrase “a horseshoe crab Factor C expressed using Sf9 as a host cell” to be interpreted as meaning the same horseshoe crab Factor C that is expressed in the mammalian cell, the applicant may consider amending the phrase “a horseshoe crab Factor C expressed using Sf9 as a host cell” in claims 1 and 9 to recite “a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell”.

Claim Rejections - 35 USC § 112(a)

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(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, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

Claims 1-17 are rejected under 35 U.S.C. 112(a) as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor at the time the application was filed, had possession of the claimed invention.

MPEP 2163.II.A.2.(a).i) states, “Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention”.

For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical

and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

MPEP § 2163 further states that “[s]atisfactory disclosure of a ‘representative number’ depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus... Instead, the disclosure must adequately reflect the structural diversity of the claimed genus, either through the disclosure of sufficient species that are ‘representative of the full variety or scope of the genus,’ or by the establishment of ‘a reasonable structure-function correlation.’ Such correlations may be established ‘by the inventor as described in the specification,’ or they may be ‘known in the art at the time of the filing date.’”

The factors considered in the Written Description requirement are (1) level of skill and knowledge in the art, (2) partial structure, (3) physical and/or chemical properties, (4) functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the (5) method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the

conclusion that the applicant was in possession of the claimed species is sufficient."

MPEP § 2163.

Claims 1-8 are drawn to a method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Claims 9-17 are drawn to a method for producing a water soluble horseshoe crab Factor C for use in endotoxin assay in the presence of a salt ion, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Regarding claims 1-17, the specification discloses an actual reduction to practice of the following representative species of the claimed methods to achieve a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility – recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a

human embryonic kidney (HEK) cell as an expression host. Other than these representative species, the specification and the prior art fail to disclose the reduction to practice of any other species of the claimed methods to achieve a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility. The specification supports a high level of unpredictability regarding the recombinant expression of a horseshoe crab Factor C in a mammalian cell to achieve a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility. For example, the specification discloses that a *Carcinoscorpius rotundicauda* horseshoe crab Factor C that is recombinantly expressed in a COS-1 cell (a monkey kidney cell) is reported to be recovered in an insoluble fraction (p. 86, paragraph [0132]).

Further regarding claims 1-6 and 9-15, the amino acid sequences of the genus of recited horseshoe crab Factor C polypeptides are unlimited and considered to be widely variant, encompassing any horseshoe crab Factor C polypeptide expressed from any mammalian host cell. In view of the relative few disclosed representative species as noted above, and given that the effects of amino acid substitution are highly unpredictable (applicant's attention is directed to MPEP 2144.08.11.A.4.(c) for a description of the unpredictable effects of amino acid substitution on the function of a polypeptide), one of skill in the art would not accept the disclosure of recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell as an

expression host as being representative of other methods to produce a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility as recited in the claims.

Regarding claims 7 and 16, with the aid of a computer, one of skill in the art could conceivably identify all of the amino acid sequences that have at least 90% identity with the amino acid sequence of SEQ ID NO: 2. However, there is no prior-art or disclosed teaching regarding which 10% (or 102) of the amino acids can vary from SEQ ID NO: 2 and still result in a protein that has (α -2,3)-linked terminal sialic acid in a greater amount, has salt ion resistance or water solubility, and retains activity of Factor C as recited in the claims. Given what is known in the art about the likely outcome of substitutions on structure, conservation of structure is not necessarily a surrogate for conservation of function (applicant's attention is directed to MPEP 2144.08.11.A.4.(c) for a description of the unpredictable effects of amino acid substitution on the function of a polypeptide). In this case, there is no disclosed correlation between amino acid sequence and the features of having (α -2,3)-linked terminal sialic acid in a greater amount, having salt ion resistance or water solubility, and retaining activity of Factor C as recited in the claims. While general knowledge in the art may have allowed one of skill in the art to identify other proteins expected to have the same or similar tertiary structure of a polypeptide that has Factor C activity, there is no general knowledge in the art about having (α -2,3)-linked terminal sialic acid in a greater amount, having salt ion resistance or water solubility, and retaining activity of Factor C to suggest that general similarity of structure confers the activity. Accordingly, one of skill in the art

would not accept the disclosure of SEQ ID NO: 2 as being representative of other polypeptides having (α -2,3)-linked terminal sialic acid in a greater amount, having salt ion resistance or water solubility, and retaining activity of Factor C Factor C activity as encompassed by the claims.

As such, the specification, taken with the pre-existing knowledge in the art of amino acid substitution, fails to satisfy the written description requirement of 35 U.S.C. 112(a).

Claims 1-17 are rejected under 35 U.S.C. 112(a) because the specification, while being enabling for a method for increasing resistance to a sodium or magnesium salt ion or a method for producing a water soluble horseshoe crab Factor C by recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell as an expression host, does not reasonably provide enablement for all methods as broadly encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

“The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue.” *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of

one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors considered to be most relevant to the instant rejection are addressed in detail below.

The nature of the invention: According to the specification, “there has never been known a case where functional Factor C is produced using mammalian cells as host cells” (paragraph [0011]) and “[t]he present inventors 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” (paragraph [0016]).

The breadth of the claims: Claims 1-8 are drawn to a method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Claims 9-17 are drawn to a method for producing a water soluble horseshoe crab Factor C for use in endotoxin assay in the presence of a salt ion, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: The specification supports a high level of unpredictability regarding the salt ion resistance (*i.e.*, maintaining Factor C activity in the presence of a salt ion) and solubility of a horseshoe crab Factor C that is recombinantly expressed in a mammalian cell. For example, the specification discloses that a horseshoe crab Factor C that is recombinantly expressed in COS cells (a monkey kidney cell) is insoluble (p. 86, paragraph [0132]).

Further, the Factor C amino acid sequences of claims 1-6 and 9-15 are unlimited and it is well-known in the prior art that the amino acid sequence of a polypeptide determines the polypeptide's structural and functional properties. Predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity/utility requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (*i.e.*, expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The positions within a protein's sequence where modifications can be made with a reasonable expectation of success in obtaining a polypeptide having the desired activity/utility are limited in any protein and the result of such modifications is highly unpredictable. In addition, one skilled in the art would

expect any tolerance to modification for a given protein to diminish with each further and additional modification, *e.g.*, multiple substitutions.

It is well-known in the art that even a single amino acid alteration can alter the folding of a polypeptide. See, *e.g.*, MPEP 2144.08.II.A.4.(c), which states, “[i]n the area of biotechnology, an exemplified species may differ from a claimed species by a conservative substitution (“the replacement in a protein of one amino acid by another, chemically similar, amino acid... [which] is generally expected to lead to either no change or only a small change in the properties of the protein.” Dictionary of Biochemistry and Molecular Biology 97 (John Wiley & Sons, 2d ed. 1989)). The effect of a conservative substitution on protein function depends on the nature of the substitution and its location in the chain. Although at some locations a conservative substitution may be benign, in some proteins only one amino acid is allowed at a given position. For example, the gain or loss of even one methyl group can destabilize the structure if close packing is required in the interior of domains. James Darnell et al., *Molecular Cell Biology* 51 (2d ed. 1990).”

The amount of direction provided by the inventor and The existence of working examples: The specification discloses the following working examples of a method as encompassed by the claims – a method for increasing resistance to a sodium or magnesium salt ion or a method for producing a water soluble horseshoe crab Factor C by recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell as an expression host. Other than these working examples, the specification fails to disclose any other working examples of a horseshoe crab Factor C as

encompassed by the claims and other than *Tachypleus tridentatus* Factor C expressed in CHO cells or HEK cells, the specification fails to provide guidance regarding the combination of horseshoe crab Factor C and expression hosts, particularly mammalian expression hosts, that are useful in accordance with the invention. Regarding amino acid sequence, the specification fails to identify any alterations to the amino acid sequence of SEQ ID NO: 2 that can be made and still have the identity of a horseshoe crab Factor C polypeptide and as described above, the same horseshoe crab Factor C expressed in different host cells can have very different characteristics and even be non-functional.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, and the state of the prior art, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102/103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale or otherwise available to the public before the effective filing date of the claimed invention.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-17 are rejected under 35 U.S.C. 102(a)(1) as anticipated by or, in the alternative, under 35 U.S.C. 103 as obvious over Ding et al. (US 5,858,706; cited on the IDS filed on May 18, 2018; hereafter “Ding”) as evidenced by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on May 18, 2018; hereafter “Mizumura”). See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103 when the prior art product seems to be identical except that the prior art is silent as to an inherent characteristic. See also MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 1-8 are drawn to a method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Regarding claims 1-8, the reference of Ding discloses a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding discloses mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has a salt ion resistance as recited in claims 1 and 2 and exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claims 3-6, respectively. However, Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application show that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10% sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10%

sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab

Factor C expressed using Sf9 as a host cell, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10% sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab

Factor C expressed using Sf9 as a host cell.

Ding also does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claim 1. However, the specification discloses that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 7, Ding does not disclose the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2 of this application. However, evidentiary reference of Mizumura is cited to show that the amino acid

sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Claims 9-17 are drawn to a method for producing a water soluble horseshoe crab Factor C for use in endotoxin assay in the presence of a salt ion, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a human cell or a Chinese hamster cell as a host cell,

wherein the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Regarding claims 9-17, the reference of Ding discloses a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1). Ding discloses mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells is water soluble as recited in claim 9. However, according to the disclosure of the specification of this application, the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells is recovered in a soluble fraction (paragraph [0112]), *i.e.*, is water-soluble as defined in the specification at paragraph [0036]). As such, since *T. tridentatus* factor C recombinantly expressed in

CHO cells is water-soluble, it follows that Ding's Factor C polypeptide recombinantly expressed in CHO cells is also water-soluble.

Ding also does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells is for use in endotoxin assay in the presence of a salt ion as recited in claims 9 and 11 and exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claims 12-15, respectively. However, Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application show that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10% sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10% sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also exhibits a higher residual activity in the presence of 10% sodium citrate, 7% sodium hydrogencarbonate, 10% sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Ding also does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater

amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claim 9. However, the specification discloses that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 10, Ding does not expressly disclose "recovering the expressed horseshoe crab Factor C as a solution". However, Ding discloses using solutions and purification media for purifying the Factor C polypeptide (column 7, lines 46-67) and one of ordinary skill in the art would recognize that Ding is disclosing recovering the expressed horseshoe crab Factor C as a solution.

Regarding claim 16, Ding does not disclose the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2 of this application. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

This anticipates claims 1-17 as written.

Claim Rejections - Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(I)(1) - 706.02(I)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-17 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 2, 5-7, and 14-17 of U.S. Patent No. 10,144,923 B2 (cited on Form PTO-892). Although the claims at issue are not identical, they are not patentably distinct from each other for the reasons that follow.

Regarding claims 1, 2, and 8 of this application, the combined limitations of claims 1, 2, and 6 of the patent anticipate claims 1, 2, and 8.

Regarding claim 3 of this application, the combined limitations of claims 1, 2, and 6 of the patent anticipate claim 3 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 4 of this application, the combined limitations of claims 1, 6, and 14 of the patent anticipate claim 4 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 5 of this application, the combined limitations of claims 1, 6, and 15 of the patent anticipate claim 5 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 6 of this application, the combined limitations of claims 1, 6, and 16 of the patent anticipate claim 6 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a horseshoe

crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 7 of this application, the combined limitations of claims 1, 2, and 7 of the patent anticipate claim 7.

Regarding claims 9, 11, and 17 of this application, the combined limitations of claims 1, 2, 5, and 6 of the patent anticipate claims 9, 11, and 17.

Regarding claim 10 of this application, although the claims of the patent do not recite “recovering the expressed horseshoe crab Factor C as a solution”, since claim 5 of the patent recites the Factor C protein is water soluble, it would have been obvious to one of ordinary skill in the art to recover the horseshoe crab Factor C produced by the method of claim 6 of the patent as a solution. One would have been motivated to and would have had a reasonable expectation of success to recover the horseshoe crab Factor C produced by the method of claim 6 of the patent as a solution because claim 5 of the patent recites the Factor C protein is water soluble.

Regarding claim 12 of this application, the combined limitations of claims 1, 2, 5, and 6 of the patent anticipate claim 12 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 13 of this application, the combined limitations of claims 1, 5, 6, and 14 of the patent anticipate claim 13 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual

activity in the presence of 52 mM sodium hydrogencarbonate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 14 of this application, the combined limitations of claims 1, 5, 6, and 15 of the patent anticipate claim 14 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 15 of this application, the combined limitations of claims 1, 5, 6, and 16 of the patent anticipate claim 15 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 16 of this application, the combined limitations of claims 1, 2, 5, and 7 of the patent anticipate claim 16.

Claims 1-17 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 6-8, and 12 of copending Application No. 16/399,386 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because for the reasons that follow.

Regarding claims 1, 2, 7, and 8 of this application, the combined limitations of claims 1, 2, and 12 of the reference application anticipate claims 1, 2, 7, and 8.

Regarding claim 3 of this application, the combined limitations of claims 1, 2, and 12 of the reference application anticipate claim 3 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 4 of this application, the combined limitations of claims 1, 2, 6, and 12 of the reference application anticipate claim 4 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 5 of this application, the combined limitations of claims 1, 2, 7, and 12 of the reference application anticipate claim 5 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 6 of this application, the combined limitations of claims 1, 2, 8, and 12 of the reference application anticipate claim 6 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher

residual activity in the presence of 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claims 9, 11, 16, and 17 of this application, the combined limitations of claims 1-3 and 12 of the reference application anticipate claims 9, 11, 16, and 17.

Regarding claim 10 of this application, although the claims of the reference application do not recite “recovering the expressed horseshoe crab Factor C as a solution”, since claim 3 of the patent recites the Factor C protein is water soluble, it would have been obvious to one of ordinary skill in the art to recover the horseshoe crab Factor C produced by the method of claim 12 of the reference application as a solution. One would have been motivated to and would have had a reasonable expectation of success to recover the horseshoe crab Factor C produced by the method of claim 12 of the reference application as a solution because claim 3 of the reference application recites the Factor C protein is water soluble.

Regarding claim 12 of this application, the combined limitations of claims 1-3 and 12 of the reference application anticipate claim 12 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 13 of this application, the combined limitations of claims 1-3, 6, and 12 of the reference application anticipate claim 13 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher

residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 14 of this application, the combined limitations of claims 1-3, 7, and 12 of the reference application anticipate claim 14 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

Regarding claim 15 of this application, the combined limitations of claims 1-3, 8, and 12 of the reference application anticipate claim 15 given that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell (see Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application).

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Citation of Relevant Art

The art made of record and not relied upon is considered pertinent to applicant's disclosure. Mizumura et al. ("Genetic engineering approach to develop next-generation reagents for endotoxin quantification", *Innate Immunity* 23:136-146, 2017; cited on Form PTO-892) discloses recombinant expression of Factor C using CHO DG44 and HEK293

cells.

Conclusion

Status of the claims:

Claims 1-20 are pending.

Claims 18-20 are withdrawn from consideration.

Claims 1-17 are rejected.

No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656

AZZ33066

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Query Match      100.0%;  Score 5619;  DB 19;  Length 1019;
Best Local Similarity 100.0%;
Matches 1019;  Conservative 0;  Mismatches 0;  Indels 0;  Gaps 0;
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[illegible]

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PGR2025-00023



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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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DETAILED CORRESPONDENCE

Status of the Application

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claims 1-6, 8, 9, 11-15, and 17-22 are pending in the application.

Applicant's amendment to the claims, filed on December 12, 2019, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's remarks filed on December 12, 2019 in response to the non-final rejection mailed on October 8, 2019 have been fully considered.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Election/Restrictions

Claims 18-20 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on September 10, 2019.

Claims 1-6, 8, 9, 11-15, 17, 21 and 22 are being examined on the merits with claims 1 and 9 being examined only to the extent the claims read on the elected subject matter.

Claim Objections

Claims 1, 9, 21, and 22 are objected to because of the following informalities:

Claims 1 and 9 are objected as ending with two periods.

Claims 21 and 22 are objected to in the recitation of "the mammalian cell is CHO or HEK" and in the interest of improving claim form, it is suggested that the noted phrase be amended to recite, *e.g.*, "the mammalian cell is a Chinese Hamster ovary (CHO) cell or a human embryonic kidney (HEK) cell."

Claim Rejections - 35 USC § 112(b)

Claim 11 is rejected under 35 U.S.C. 112(b) as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor regards as the invention.

Claim 11 recites the limitation "the salt ion". There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 112(a)

Claims 1-6, 8, 9, 11-15, and 17 are rejected under 35 U.S.C. 112(a) as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor at the time the application was filed, had possession of the claimed invention.

MPEP 2163.II.A.2.(a).i) states, "Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to

be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention”.

For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

MPEP § 2163 further states that “[s]atisfactory disclosure of a ‘representative number’ depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus... Instead, the disclosure must adequately reflect the structural diversity of the claimed genus, either through the disclosure of sufficient species that are ‘representative of the full variety or scope of the genus,’ or by the establishment of ‘a reasonable structure-function correlation.’ Such correlations may be established ‘by the

inventor as described in the specification,' or they may be 'known in the art at the time of the filing date.'"

The factors considered in the Written Description requirement are (1) level of skill and knowledge in the art, (2) partial structure, (3) physical and/or chemical properties, (4) functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the (5) method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient." MPEP § 2163.

As amended, claims 1-6 and 8 are drawn to (in relevant part) a method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

- recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell, and
- recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C,
- wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,
- wherein the mammalian cell is a Chinese hamster cell or a human cell, and
- wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C.

As amended, claims 9, 11-15, and 17 are drawn to (in relevant part) a method for producing an endotoxin assay agent comprising a water soluble horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell, and

recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,

wherein the mammalian cell is a Chinese hamster cell or a human cell, and
wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A) or (B):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; or

(B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C.

The specification discloses an actual reduction to practice of the following representative species of the claimed methods to achieve a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab

Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility – recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell as an expression host. Other than these representative species, the specification and the prior art fail to disclose the reduction to practice of any other species of the claimed methods to achieve a Factor C that contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell and that has salt ion resistance or water solubility.

The claims have been amended to recite the limitation “wherein the mammalian cell is a Chinese hamster cell or a human cell”, which is interpreted as meaning a Chinese hamster cell or human cell from any part (*e.g.*, any organ or tissue) of a Chinese hamster or human, respectively. The reference of Mizumura et al. (*Innate Immunity* 23:136-146, 2017; cited on Form PTO-892 mailed on October 8, 2019) discloses that recombinant proteins, even with the same amino-acid sequences, are well known to exhibit different activity and stability depending on their different post-translational modifications (p. 143, column 1). According to the applicant’s instant remarks, it has been commonly known that N-glycosylation such as sialic acid modification highly affects properties of proteins (remarks at p. 14, middle). The reference of Blomme et al. (*J. Hepat.* 50:592-603, 2009; cited on Form PTO-892) discloses that the functional role(s) of N-linked carbohydrate moieties of glycoproteins is/are often not well understood, but N-glycosylation is a necessity in the correct folding of certain proteins and that aberrant protein folding affects various physicochemical and functional properties of proteins including protein stability and protein solubility (p. 593,

column 2). The art acknowledges a high level of unpredictability in the N-glycosylation profile among different human cells. For example, the reference of Holst et al. (*Sci. Rep.* 7, 16623 (2017), 15 pages; cited on Form PTO-892) discloses that an analysis of the N-glycan profiles among different pancreatic ductal adenocarcinoma (PDAC) cell lines revealed *major* differences in the N-glycosylation of the different PDAC cells as well as compared to the control cell line (p. 1, abstract). The reference of Blomme (*supra*) discusses the alteration of protein glycosylation in diseased hepatic cells (see whole document). The applicant's evidence in the prior-filed application 14/650,767 even shows a substantial difference in the ion resistance of a horseshoe crab Factor C expressed in differing CHO cell lines (see p. 4 of the Declaration under 37 CFR 1.132, filed on October 12, 2017 in the '767 application) and the applicant's specification discloses that a *Carcinoscorpius rotundicauda* horseshoe crab Factor C that is recombinantly expressed in a COS-1 cell (a monkey kidney cell) is reported to be recovered in an insoluble fraction (p. 86, paragraph [0132]), which is presumably due to differences in glycosylation of the recombinantly expressed horseshoe crab Factor C polypeptide. As such, one of skill in the art would recognize a high level of unpredictability that the recited horseshoe crab Factor C polypeptide produced by *any* Chinese hamster or human cell would have (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell.

In this case, other than recombinant expression of the recited horseshoe crab Factor C in a CHO cell line or a HEK cell line, there is no other disclosed Chinese hamster or human cell that produces the recited horseshoe crab Factor C having (α -

2,3)-linked terminal sialic acid in a greater amount, having salt ion resistance or water solubility, and retaining activity of Factor C as recited in the claims. Given the wide variation in glycosylation among Chinese hamster or human cells, the respective genus of Chinese hamster or human cells is considered to be widely variant and given the high level of unpredictability regarding N-glycosylation using any Chinese hamster cell or any human cell, one of skill in the art would not accept the specification's disclosure of recombinantly expressing *Carcinoscorpius rotundicauda* or *Tachypleus tridentatus* Factor C using a Chinese Hamster Ovary (CHO) cell or a human embryonic kidney (HEK) cell as an expression host as being representative of methods of recombinantly expressing the recited horseshoe crab Factor C using any Chinese hamster or human cell to achieve a greater (α -2,3)-linked terminal sialic acid, salt ion resistance or water solubility, and retaining activity of Factor C as encompassed by the claims.

As such, the specification, taken with the pre-existing knowledge in the art of amino acid substitution, fails to satisfy the written description requirement of 35 U.S.C. 112(a).

RESPONSE TO REMARKS: The applicant argues that given that sialic acid modification sites were known before the effective filing date and sialyltransferase activity to add (α -2,3)-linked terminal sialic acid was not confirmed in Sf9 cell, one skilled in the art would recognize that the recited horseshoe crab Factor C expressed in CHO cell or HEK cell meets the "Requirement 1". The applicant argues that the claims have been amended to limit the mammalian cell to a Chinese hamster cell or a human cell as a host cell, which excludes a COS-1 cell. The applicant argues independent

claims 1 and 9 have been amended to incorporate the structural limitations of now-canceled claims 7 and 16. The applicant argues that in view of the Declaration under 37 CFR 1.132 by joint inventor Mizumura, one of skill in the art would recognize that a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 or 4 maintains the Factor C activity and high reactivity with endotoxin under a high salt concentration. According to the applicant, given that sialic acid modification sites were known, one skilled in the art could understand that the recited horseshoe crab Factor C produced by “a Chinese hamster cell or a human cell” as a host cell receives same kinds of glycosylation (sialic acid modification) and would exhibit the same properties of salt ion resistance and water solubility.

Applicant’s argument is not found persuasive. To the extent the rejection was based on the structure (*i.e.*, amino acid sequence) of the recited horseshoe crab Factor C, the applicant’s argument is found persuasive. However, as noted above, claims 1 and 9 have been amended to recite the limitation “wherein the mammalian cell is a Chinese hamster cell or a human cell”, which is interpreted as meaning any type of Chinese hamster cell or human cell (*e.g.*, normal cells or cancer cells) from any part (*e.g.*, any organ or tissue) of a Chinese hamster or human, respectively, and there is a high level of unpredictability that the recited horseshoe crab Factor C polypeptide produced by *any* Chinese hamster or human cell would have (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell. While COS-1 cells are excluded by the recitation of “wherein the mammalian cell is a Chinese hamster cell or a human cell”,

the specification's disclosure that a *Carcinoscorpius rotundicauda* horseshoe crab Factor C recombinantly expressed in a COS-1 cell is present in an insoluble fraction still supports unpredictability in the art. Even if a Chinese hamster or human cell comprises a sialyltransferase that adds (α -2,3)-linked terminal sialic acid, given that N-glycosylation is required for correct folding and the properties of protein stability and protein solubility, yet N-glycosylation differs among different Chinese hamster or human cells, there is still a high level of unpredictability that the recited horseshoe crab Factor C polypeptide produced by *any* Chinese hamster or human cell would have the recited salt ion resistance and/or water solubility. The applicant's Declaration under 37 CFR 1.132 by joint inventor Mizumura has been fully considered, however, the results do not provide any evidence beyond CHO cell as a host cell for recombinant production of a horseshoe crab Factor C having a greater (α -2,3)-linked terminal sialic acid, salt ion resistance or water solubility, and retaining activity of Factor C.

For these reasons, it is the examiner's position that the specification fails to adequately describe the claimed invention.

Claim Rejections - 35 USC § 102/103

Claims 1-6, 8, 9, 11-15, 17, 21 and 22 are rejected under 35 U.S.C. 102(a)(1) as anticipated by or, in the alternative, under 35 U.S.C. 103 as obvious over Ding et al. (US 5,858,706; cited on the IDS filed on May 18, 2018; hereafter "Ding") as evidenced by Mizumura et al. (WO 2012/118226 A1; cited on the IDS filed on May 18, 2018; hereafter "Mizumura").

See MPEP 2112.III regarding a rejection under 35 U.S.C. 102/103 when the prior art product seems to be identical except that the prior art is silent as to an inherent characteristic. See also MPEP 2131.01 regarding a multiple reference 35 U.S.C. 102 rejection.

Claims 1-6, 8, and 21 are drawn to (in relevant part) a method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell, and

recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,

wherein the mammalian cell is a Chinese hamster cell or a human cell, and
wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A) or (B):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; or
(B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C.

Regarding the claim 1 limitation "recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell... wherein the mammalian cell is a Chinese hamster cell or a human cell", the reference of Ding discloses a mammalian host cell

transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 9, Example 1). Ding discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1).

Regarding the claim 1 limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C”, the examiner has reviewed the specification to determine the applicant’s intended meaning of “recovering a culture supernatant” in the context of the claim. There are several instances in the specification of centrifugation of a cell culture for recovering a culture supernatant (*e.g.*, p. 78, paragraph [0112]). In accordance with the specification, the limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” is interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C (*i.e.*, the limitation “containing the horseshoe crab Factor C” refers to the mammalian cell, not the culture supernatant).

Ding discloses that recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, except that the physical disruption steps can be omitted, as mammalian cells lack a cell wall (column 9, Example 1). Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifuging to obtain a cell pellet and resuspending the cell pellet in a buffer (column 10, lines 39 and 40; paragraph bridging columns 10 and 11). Given that Ding discloses recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian

cells can be performed substantially as described for purification from yeast cells and that Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifugation, one would have recognized that Ding is disclosing a method for recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells, which includes a step of centrifugation.

Regarding the claim 1 limitation “wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A) or (B): (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; (B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C”, Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20). Ding does not disclose the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2 of this application. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding the claim 1 limitation “imparting a salt ion resistance for a horseshoe crab Factor C” and the limitations of claims 2-6, Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has a salt ion resistance as recited in claims 1 and 2 and exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claims 3-6, respectively. However, Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this

application show that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claims 12-15, respectively. As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. Since the Office does not have the facilities for examining and comparing applicants' protein with the protein of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed method and the method of the prior art. See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

Regarding the claim 1 limitation "wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell", Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with

a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding claim 8, as noted above, Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Regarding claim 21, as noted above, Ding discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 9, Example 1).

Claims 9, 11-15, 17, and 22 are drawn to (in relevant part) a method for producing an endotoxin assay agent comprising a water soluble horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell, and

recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,

wherein the mammalian cell is a Chinese hamster cell or a human cell, and
wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A) or (B):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2; or
- (B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C.

Regarding the claim 9 limitation “recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell... wherein the mammalian cell is a Chinese hamster cell or a human cell”, the reference of Ding discloses a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 9, Example 1). Ding discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 9, Example 1).

Regarding the claim 9 limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent”, the examiner has reviewed the specification to determine the applicant’s intended meaning of “recovering a culture supernatant” in the context of the claim. There are several instances in the specification of centrifugation of a cell culture

for recovering a culture supernatant (*e.g.*, p. 78, paragraph [0112]). In accordance with the specification, the limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” is interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C, and wherein the horseshoe crab Factor C is a component of the endotoxin assay agent (*i.e.*, the limitation “containing the horseshoe crab Factor C as a component of the endotoxin assay agent” refers to the mammalian cell, not the culture supernatant).

Ding discloses that recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, except that the physical disruption steps can be omitted, as mammalian cells lack a cell wall (column 9, Example 1). Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifuging to obtain a cell pellet and resuspending the cell pellet in a buffer (column 10, lines 39 and 40; paragraph bridging columns 10 and 11). Given that Ding discloses recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells and that Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifugation, one would have recognized that Ding is disclosing a method for recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells, which includes a step of centrifugation.

Regarding the claim 9 limitation “wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A) or (B): (A) a protein

comprising the amino acid sequence shown in SEQ ID NO: 2; (B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C”, Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20). Ding does not disclose the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2 of this application. However, evidentiary reference of Mizumura is cited to show that the amino acid sequence of *T. tridentatus* factor C is identical to SEQ ID NO: 2 herein (see Appendix A sequence alignment).

Regarding the claim 9 limitation “wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell”, Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. However, the specification discloses that factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3) linked terminal sialic acid (paragraph [0154]), while *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid (paragraph [0154]). As such, because *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid and since *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell does not have any (α -2,3) linked terminal sialic acid, it follows that Ding’s Factor C polypeptide recombinantly expressed and recovered from CHO cells

also has a greater amount of (α -2,3)-linked terminal sialic acid than *T. tridentatus* factor C polypeptide expressed in Sf9 as a host cell.

Regarding the claim 9 limitation “comprising a water soluble horseshoe crab Factor C”, Ding does not disclose the Factor C polypeptide recombinantly expressed and recovered from CHO cells is water soluble as recited in claim 9. However, according to the disclosure of the specification of this application, the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells is recovered in a soluble fraction (paragraph [0112]), *i.e.*, is water-soluble as defined in the specification at paragraph [0036]). As such, since *T. tridentatus* factor C recombinantly expressed in CHO cells is water-soluble, it follows that Ding’s Factor C polypeptide recombinantly expressed in CHO cells is also water-soluble.

Regarding claim 11, claim 9 does not provide antecedent basis for “the salt ion” and in view of the indefiniteness of this phrase and in the interest of compact prosecution, claim 11 is included in this rejection.

Regarding claims 12-15, Ding does not disclose the Factor C polypeptide exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell as recited in claims 12-15, respectively. However, Figure 7 and the supporting disclosure at specification paragraphs [0136]–[0138] of this application show that *T. tridentatus* factor C polypeptide recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as

compared with a horseshoe crab Factor C expressed using Sf9 as a host cell. As such, since *T. tridentatus* factor C recombinantly expressed and recovered from CHO cells exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell, it follows that Ding's Factor C polypeptide recombinantly expressed and recovered from CHO cells also exhibits a higher residual activity in the presence of 21 mM sodium citrate, 52 mM sodium hydrogencarbonate, 214 mM sodium chloride, or 16 mM magnesium sulfate as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

Regarding claim 17, as noted above, Ding discloses the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Regarding claim 22, as noted above, Ding discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 9, Example 1).

This anticipates claims 1-6, 8, 9, 11-15, 17, 21 and 22 as written.

RESPONSE TO REMARKS: The applicant argues that while the examiner has relied on an inherency rationale to support the rejection, the Examiner has not provided any objective evidence to show that Ding inherently teaches the claimed method and properties. The applicant argues that rather than objective evidence, the Examiner used improper hindsight by relying upon the disclosure of the present application. According to the applicant, since Ding does not teach or suggest the claimed properties, the properties would have been unknown to one of ordinary skill in the art based on the teaching of Ding.

The applicant further argues the amended claims 1 and 9 recite the limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C”. According to the applicant, Ding does not teach or suggest this limitation because Ding disclose the recombinantly produced Factor C is recovered from within the host cell as an insoluble protein, not from “a culture supernatant” as a secreted protein as recited in claims 1 and 9.

The applicant further argues that since Ding’s horseshoe crab Factor C is produced within the cell as an insoluble protein and is not secreted into a culture medium and because Ding’s soluble fraction is a fraction obtained by solubilizing an insoluble horseshoe crab Factor C by detergents, Ding does not disclose a soluble fraction as “a culture supernatant”. According to the applicant, the claimed invention provides unexpected effects such that the endotoxin assay with mitigating reaction inhibition by ions in a sample can be accomplished by using the Factor C produced by the mammalian cells as a host rather than the Factor C produced by insect cells.

Applicant’s argument is not found persuasive. The examiner has compared Ding’s method with the disclosed method and since Ding’s method is encompassed by the claims, it is the examiner’s position that the horseshoe crab Factor C polypeptide produced by the method of Ding would inherently have the same properties and functions of the horseshoe crab Factor C polypeptide recited in the claims. MPEP 2112.II makes clear that “[t]here is no requirement that a person of ordinary skill in the art would have recognized the inherent disclosure *at the time of invention*, but only that the subject matter is in fact inherent in the prior art reference”. Thus, contrary to the examiner’s position, the examiner *has* provided a basis in fact and/or technical

reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. The applicant has presented no evidence or line of reasoning to the contrary.

Although the applicant argues the examiner has relied on an improper hindsight analysis and the applicant asserts an unexpected result, it is noted that the instant rejection is *not* an obvious rejection. As such, the applicant's allegation of improper hindsight analysis and unexpected results cannot rebut the instant rejection. In this case, the specification of this application is not relied upon to support an obviousness rationale. Rather, the specification of this application is relied upon in accordance with MPEP 2112.V and MPEP 2112.02.I to compare the disclosed and claimed method with the method of the prior art in order to support inherent characteristics of Ding's recombinantly produced horseshoe crab Factor C polypeptide.

It appears the applicant intends for the claim limitation "recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C" to be interpreted as meaning that the horseshoe crab Factor C is secreted by the mammalian cell into and is soluble in a culture medium and the culture supernatant comprising the secreted, soluble horseshoe crab Factor C is isolated from the mammalian cell. However, the claims do not recite a limitation that corresponds to the applicant's intended meaning. As written and given a broadest reasonable interpretation in light of the specification (as described in detail above), the claim 1 limitation "recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C" has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C. Similarly, the claim 9 limitation

of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C, and wherein the horseshoe crab Factor C is a component of the endotoxin assay agent. For the reasons set forth above, the method of Ding is encompassed by the claims in view of this broad yet reasonable interpretation.

Claim Rejections - Double Patenting

Claims 1-6, 8, 9, 11-15, 17, 21 and 22 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 2, 5-7, and 14-17 of U.S. Patent No. 10,144,923 B2 (cited on Form PTO-892 mailed on October 8, 2019) in view of Ding (*supra*). Although the claims at issue are not identical, they are not patentably distinct from each other for the reasons that follow.

The difference between the claims of the patent and the claims of this application is that the claims of the patent do not recite the step of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” in claim 1 or the step of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” in claim 9. As written and given a broadest reasonable interpretation in light of the specification (as described in detail above), the claim 1 limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise

the horseshoe crab Factor C and the claim 9 limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C, and wherein the horseshoe crab Factor C is a component of the endotoxin assay agent.

Ding discloses that recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, except that the physical disruption steps can be omitted, as mammalian cells lack a cell wall (column 9, Example 1). Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifuging to obtain a cell pellet and resuspending the cell pellet in a buffer (column 10, lines 39 and 40; paragraph bridging columns 10 and 11). Given that Ding discloses recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells and that Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifugation, one would have recognized that Ding is disclosing a method for recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells, which includes a step of centrifugation.

In view of the teachings of Ding, it would have been obvious to one of ordinary skill in the art before the effective filing date to modify the method of producing a horseshoe crab Factor C of the patent to centrifuge the cell culture to obtain a cell pellet. One would have been motivated to do this as a step in the recovery of the

recombinant horseshoe crab Factor C. One would have had a reasonable expectation to modify the method of producing a horseshoe crab Factor C of the patent to centrifuge the cell culture to obtain a cell pellet because of the teachings of Ding. Therefore, the methods of claims 1-6, 8, 9, 11-15, 17, 21 and 22 of this application are unpatentable over the claims of the patent.

Claims 1-6, 8, 9, 11-15, 17, 21 and 22 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 6-8, 12, and 13 of copending Application No. 16/399,386 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because for the reasons that follow.

The difference between the claims of the reference application and the claims of this application is that the claims of the reference application do not recite the step of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” in claim 1 or the step of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” in claim 9. As written and given a broadest reasonable interpretation in light of the specification (as described in detail above), the claim 1 limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C and the claim 9 limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a component of the endotoxin assay agent” has been interpreted as

meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C, and wherein the horseshoe crab Factor C is a component of the endotoxin assay agent.

Ding discloses that recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells, except that the physical disruption steps can be omitted, as mammalian cells lack a cell wall (column 9, Example 1). Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifuging to obtain a cell pellet and resuspending the cell pellet in a buffer (column 10, lines 39 and 40; paragraph bridging columns 10 and 11). Given that Ding discloses recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells and that Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifugation, one would have recognized that Ding is disclosing a method for recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells, which includes a step of centrifugation.

In view of the teachings of Ding, it would have been obvious to one of ordinary skill in the art before the effective filing date to modify the method of producing a horseshoe crab Factor C of the reference application to centrifuge the cell culture to obtain a cell pellet. One would have been motivated to do this as a step in the recovery of the recombinant horseshoe crab Factor C. One would have had a reasonable expectation to modify the method of producing a horseshoe crab Factor C of the reference application to centrifuge the cell culture to obtain a cell pellet because of the

teachings of Ding. Therefore, the methods of claims 1-6, 8, 9, 11-15, 17, 21 and 22 of this application are unpatentable over the claims of the reference application.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

RESPONSE TO REMARKS: The applicant argues that the amended claims are not obvious and are patentable over the claims of the patent and reference application. According to the applicant, the amended claims 1 and 9 recite the limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C”, which limitation is not recited in the claims of the patent or reference application.

Applicant’s argument is not found persuasive. As noted above, it appears the applicant intends for the claim limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” to be interpreted as meaning that the horseshoe crab Factor C is secreted by the mammalian cell into and is soluble in a culture medium and the culture supernatant comprising the secreted, soluble horseshoe crab Factor C is isolated from the mammalian cell. However, the claims do not recite a limitation that corresponds to the applicant’s intended meaning. As written and given a broadest reasonable interpretation in light of the specification (as described in detail above), the claim 1 limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C. Similarly, the claim 9 limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C as a

component of the endotoxin assay agent” has been interpreted as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C, and wherein the horseshoe crab Factor C is a component of the endotoxin assay agent. For the reasons set forth above, the methods of claims 1-6, 8, 9, 11-15, 17, 21 and 22 of this application are unpatentable over the claims of the patent and reference application.

Conclusion

Status of the claims:

Claims 1-6, 8, 9, 11-15, and 17-22 are pending.

Claims 18-20 are withdrawn from consideration.

Claims 1-6, 8, 9, 11-15, 17, 21, and 22 are rejected.

No claim is in condition for allowance.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **DAVID J STEADMAN** whose telephone number is (571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an

interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656

AZZ33066

AZZ33066 standard; protein; 1019 AA.
 XX
 AC AZZ33066;
 XX
 DT 25-OCT-2012 (first entry)
 XX
 DE Tachypleus tridentatus factor C, SEQ ID 2.
 XX
 KW factor C; protein production; protein quantitation; BOND_PC;
 KW limulus factor C precursor; limulus factor C; GO4252; GO5215; GO5515;
 KW GO5529; GO6508; GO6810; GO7155; GO8233; GO16020; GO16021; GO16787;
 KW GO42381.
 XX
 OS Tachypleus tridentatus.
 XX
 CC PN WO2012118226-A1.
 XX
 CC PD 07-SEP-2012.
 XX
 CC PF 28-FEB-2012; 2012WO-JP055728.
 XX
 PR 28-FEB-2011; 2011US-0447556P.
 XX
 CC PA (SEKG) SEIKAGAKU CORP.
 XX
 CC PI Mizumura H, Aizawa M, Oda T;
 XX
 DR WPI; 2012-L75079/61.
 DR N-PSDB; AZZ33065, AZZ33071.
 DR PC:NCBI; gi585398.
 DR PC:SWISSPROT; P28175.
 XX
 CC PT Agent useful for measuring endotoxin, comprises factor C derived from
 CC PT Tachypleus tridentatus, factor B of horseshoe crab and proclotting enzyme
 CC PT of horseshoe crab.
 XX
 CC PS Claim 3; SEQ ID NO 2; 72pp; English.
 XX
 CC The present invention relates to a novel agent, useful for measuring
 CC endotoxin. The agent comprises: (a) a factor C derived from Tachypleus
 CC tridentatus, wherein factor C does not have his-tag sequence at the C-
 CC terminal region; (b) a factor B derived from horseshoe crab; and (c) a
 CC proclotting enzyme derived from horseshoe crab. The invention also
 CC provides: a method for producing the endotoxin measuring agent by (a)
 CC incorporating a DNA sequence encoding the factor C, factor B and
 CC proclotting enzyme, into a viral DNA, (b) infecting insect cells with the
 CC virus incorporated with the DNA, (c) allowing the infected insect cells
 CC to express the protein; and a method for measuring endotoxin in a test
 CC sample. The present sequence represents a specifically claimed Tachypleus
 CC tridentatus factor C, which can be produced by the above mentioned
 CC endotoxin measuring agent production method, for measuring endotoxin.
 CC
 CC Revised record issued on 18-OCT-2012 : Enhanced with precomputed
 CC information from BOND.
 XX
 SQ Sequence 1019 AA;

Query Match 100.0%; Score 5619; DB 19; Length 1019;
Best Local Similarity 100.0%;
Matches 1019; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

[illegible]

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Charles River Laboratories, Inc. v. Seikagaku Corporation
PGR2025-00023

**AMENDMENT UNDER 37 C.F.R. § 1.116
EXPEDITED PROCEDURE
GROUP 1656
PATENT APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q233613

Hikaru MIZUMURA, et al.

Appln. No.: 15/983,725

Group Art Unit: 1656

Confirmation No.: 5544

Examiner: STEADMAN, DAVID J

Filed: May 18, 2018

For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE
SAME, AND METHOD FOR MEASURING ENDOTOXIN

**AMENDMENT UNDER 37 C.F.R. § 1.116
FILED UNDER THE AFCP 2.0 PROGRAM**

MAIL STOP AF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated February 12, 2020, please amend the above-identified application as follows on the accompanying pages. A Request for Consideration under the Office's After Final Consideration Pilot Program 2.0 is being filed concurrently.

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CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0			
Practitioner Docket No.:	Q233613	Application No.:	15/983,725
First Named Inventor:	Hikaru MIZUMURA	Filing Date:	May 18, 2018
Title:	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN		
APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.			
1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (e.g., a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c). 2. The above-identified application contains an outstanding final rejection. 3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect. 4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection. 5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response. 6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web). 7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, e.g., extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.] 8. By filing this certification and request, applicant acknowledges the following: • Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0. • The examiner will verify that the AFCP 2.0 submission is compliant, <i>i.e.</i>, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions: ○ The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, e.g., by mailing an advisory action. ○ If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview. • The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate. • If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.			
Signature	/Hui C. Wauters/	Date	April 3, 2020
Name	Hui C. Wauters	Practitioner Reg No.	57,426
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.			
☒ *Total of <u> 1 </u> forms are submitted.			

Electronic Acknowledgement Receipt	
EFS ID:	39054884
Application Number:	15983725
International Application Number:	
Confirmation Number:	5544
Title of Invention:	NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN
First Named Inventor/Applicant Name:	Hikaru Mizumura
Customer Number:	23373
Filer:	Hui Chen Wauters/Sharon Jones
Filer Authorized By:	Hui Chen Wauters
Attorney Docket Number:	Q233613
Receipt Date:	03-APR-2020
Filing Date:	18-MAY-2018
Time Stamp:	12:03:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no				
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Q233613Amendment116.pdf	156666	yes	13
			9772679dc1c84bae41c46441aac740deefc3556		

	Multipart Description/PDF files in .zip description								
	Document Description		Start	End					
	Applicant Arguments/Remarks Made in an Amendment		8	13					
	Claims		2	7					
	Response After Final Action		1	1					
Warnings:									
Information:									
2	After Final Consideration Program Request	Q233613AFCPRequest.pdf	211274	no	1				
			c02c39ae1f8ca98a6f79947d016b65564e2085c1						
Warnings:									
Information:									
Total Files Size (in bytes):			367940						
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.									

REMARKS

Claims 1 and 9 have been amended to delete the extra period and to incorporate the subject matter of claims 21 and 22, respectively, with further amendment to recite that the mammalian cell is a Chinese hamster ovary (CHO) cell or a human embryo kidney (HEK) cell. Claims 1 and 9 have also been amended to recite “recovering at least one of a culture broth and a culture supernatant of the mammalian cell containing the recombinant horseshoe crab Factor C secreted from the mammalian cell ...”. Support for the amendment is found, for example, in paragraphs [0042], [0105] and [0111] of the specification. Claims 21 and 22 have been cancelled accordingly. Claim 11 has been cancelled without prejudice or disclaimer.

No new matter has been added, and entry of the Amendment to place the present application in condition for allowance is respectfully requested.

Upon entry of the Amendment, claims 1-6, 8-9, 12-15 and 17-20 will be pending, of which claims 18-20 are withdrawn from consideration.

Response to Claim Objection

At page 3 of the Office Action, claims 1, 9, 21 and 22 are objected to because, per the Examiner, claims 1 and 9 end with two periods, and the recitation of “the mammalian cell is CHO or HEK” in claims 21 and 22 should be amended to recite, e.g., “the mammalian cell is a Chinese Hamster ovary (CHO) cell or a human embryonic kidney (HEK) cell”.

In response, claims 1 and 9 have been amended to delete the extra period.

Claims 21 and 22, now incorporated into claims 1 and 9, respectively, have been amended to recite “the mammalian cell is a Chinese hamster ovary (CHO) cell or a human embryo kidney (HEK) cell”, based on paragraph [0038] of the specification.

In view of the above, withdrawal of the claim objection is requested.

Response to § 112(b) Rejection

At page 3 of the Office Action, claim 11 is rejected under 35 U.S.C. § 112(b) as being indefinite.

Claim 11 has been cancelled, rendering this rejection moot.

Response to § 112(a) Rejection

At page 3 of the Office Action, claims 1-6, 8, 9, 11-15 and 17 are rejected under 35 U.S.C. § 112(a) as failing to comply with the written description requirement.

Claims 1 and 9 have been amended to incorporate the subject matter of claims 21 and 22. Claims 21 and 22 are not subject to this rejection. Accordingly, withdrawal of the § 112(a) rejection is requested.

Response to § 102/103 Rejection

At page 11 of the Office Action, claims 1-6, 8, 9, 11-15, 17, 21 and 22 are rejected under 35 U.S.C. § 102(a)(1) as being anticipated by or, in the alternative, under 35 U.S.C. § 103 as being obvious over Ding et al. (US 5,858,706; hereafter “Ding”) as evidenced by Mizumura et al. (WO 2012/118226 A1; hereafter “Mizumura”).

This rejection should be withdrawn because Ding does not disclose or render obvious the claimed invention.

In the Amendment filed December 12, 2019, Applicant explained that Ding does not teach or suggest the claimed feature of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” because Ding discloses the recombinantly produced Factor C is recovered from within the host cell as an insoluble protein, not from “a culture supernatant” as a secreted protein.

In response, the Examiner states that there are several instances in the specification of centrifugation of a cell culture for recovering a culture supernatant (e.g., p. 78, paragraph [0112]). In accordance with the specification, the Examiner interprets the limitation of “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” as meaning centrifugation of a culture of mammalian cells, wherein the mammalian cells comprise the horseshoe crab Factor C (i.e., the limitation “containing the horseshoe crab Factor C” refers to the mammalian cell, not the culture supernatant).

Given that Ding discloses recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells can be performed substantially as described for purification from yeast cells (column 9, Example 1) and that Ding discloses recovering the recombinant horseshoe crab Factor C from the yeast cells includes a step of centrifugation (column 10, lines 39 and 40; paragraph bridging columns 10 and 11), the Examiner considers that Ding is disclosing a method for recovery of the recombinant horseshoe crab Factor C produced by cultured mammalian cells, which includes a step of centrifugation.

On the other hand, the Examiner states that it appears Applicant intends for the claim limitation “recovering a culture supernatant of the mammalian cell containing the horseshoe crab Factor C” to be interpreted as meaning that the horseshoe crab Factor C is secreted by the

mammalian cell into and is soluble in a culture medium and the culture supernatant comprising the secreted, soluble horseshoe crab Factor C is isolated from the mammalian cell. However, per the Examiner, the claims do not recite a limitation that corresponds to Applicant's intended meaning.

In this regard, paragraph [0105] of the specification discloses that secretion of the recombinant Factor C protein in each culture supernatant was confirmed through western blotting by use of an anti-Factor C antibody. Paragraph [0111] of the specification discloses that the amount of Factor C protein secreted to the medium was confirmed through western blotting using a 2C12 antibody. Paragraph [0042] of the specification discloses that the expressed Factor C can be recovered as a solution containing Factor C, and the solution containing Factor C may be, for example, a culture broth, a culture supernatant, a disrupted cell extract, a mixture thereof, or the like.

Based on the above, claim 1 has been amended to recite “recovering at least one of a culture broth and a culture supernatant of the mammalian cell containing the recombinant horseshoe crab Factor C secreted from the mammalian cell, ...”. Claim 9 has been amended to recite “recovering at least one of a culture broth and a culture supernatant of the mammalian cell containing the recombinant horseshoe crab Factor C secreted from the mammalian cell as a component of the endotoxin assay agent, ...”.

Claims 1 and 9 as amended clearly indicate that the horseshoe crab Factor C is secreted by the mammalian cell into and is soluble in a culture medium and the culture broth or supernatant comprising the secreted, soluble horseshoe crab Factor C is isolated from the mammalian cell.

As the Examiner acknowledges, Ding does not teach or suggest the claimed feature.

In view of the above, the present claims define novel subject matter, are not obvious and are patentable over Ding. Reconsideration and withdrawal of the §102/103 rejection based on Ding are respectfully requested.

Response to Double Patenting Rejections

At page 24 of the Office Action, claims 1-6, 8, 9, 11-15, 17, 21 and 22 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 2, 5-7 and 14-17 of U.S. Patent No. 10,144,923 B2 in view of Ding.

At page 25 of the Office Action, claims 1-6, 8, 9, 11-15, 17, 21 and 22 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 6, 8, 12 and 13 of copending Application No. 16/399,386 [in view of Ding].

Without admitting that the above two double patenting rejections are proper, Applicant submits herewith an e-Terminal Disclaimer to obviate the rejections.

Allowance is respectfully requested. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

AMENDMENT UNDER 37 C.F.R. § 1.116
FILED UNDER THE AFCP 2.0 PROGRAM
Appln. No.: 15/983,725

Attorney Docket No.: Q233613

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/Hui C. Wauters/

SUGHRUE MION, PLLC
Telephone: 202.293.7060
Facsimile: 202.293.7860

Hui C. Wauters
Registration No. 57,426

WASHINGTON OFFICE

23373

CUSTOMER NUMBER

Date: April 3, 2020

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (currently amended): A method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell,

and

recovering at least one of a culture broth and a culture supernatant of the mammalian cell containing the recombinant horseshoe crab Factor C secreted from the mammalian cell,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,

wherein the mammalian cell is a Chinese hamster ovary (CHO) cell or a human embryo kidney (HEK) cell, and

wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:4 and having activity of Factor ~~C~~C.

2. (original): The method according to claim 1, wherein the salt ion is selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

3. (previously presented): The method according to claim 1, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

4. (previously presented): The method according to claim 1, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

5. (previously presented): The method according to claim 1, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

6. (previously presented): The method according to claim 1, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

7. (canceled).

8. (previously presented): The method according to claim 1, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*.

9. (currently amended): A method for producing an endotoxin assay agent comprising a water soluble horseshoe crab Factor C, the method comprising;

recombinantly expressing the horseshoe crab Factor C in a mammalian cell as a host cell, and

recovering at least one of a culture broth and a culture supernatant of the mammalian cell containing the recombinant horseshoe crab Factor C secreted from the mammalian cell as a component of the endotoxin assay agent,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using Sf9 as a host cell,

wherein the mammalian cell is a Chinese hamster ovary (CHO) cell or a human embryo kidney (HEK) cell, and

wherein the horseshoe crab Factor C expressed in the mammalian cell is a protein shown in the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;

(B) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:2 and having activity of Factor C;

(C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;

(D) a protein comprising an amino acid sequence having 90% or higher sequence identity to the amino acid sequence of SEQ ID NO:4 and having activity of Factor ~~C~~C.

10. (canceled).

11. (canceled).

12. (previously presented): The method according to claim 9, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

13. (previously presented): The method according to claim 9, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

14. (previously presented): The method according to claim 9, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

15. (previously presented): The method according to claim 9, wherein the horseshoe crab Factor C exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a corresponding horseshoe crab Factor C expressed in Sf9 as a host cell.

16. (canceled).

17. (original): The method according to claim 9, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, and *Carcinoscorpius rotundicauda*.

18. (withdrawn): A method for mitigating a reaction inhibition in endotoxin assay in the presence of a salt ion, the method comprising:

mixing a horseshoe crab Factor C recombinantly expressed in a mammalian cell as a host cell with a test specimen containing the salt ion,

wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a horseshoe crab Factor C expressed using Sf9 as a host cell.

19. (withdrawn): A method for measuring endotoxin in a test specimen, the method comprising:

a step of mixing an assay agent comprising a horseshoe crab Factor C recombinantly expressed in a mammalian cell as a host cell and the test specimen, and

a step of measuring response of a detection substrate,
wherein the horseshoe crab Factor C contains (α -2,3)-linked terminal sialic acid in a
greater amount as compared with a horseshoe crab Factor C expressed in Sf9 as a host cell, and
wherein the test specimen contains sodium ion, and
the contained amount of the sodium ion in the test specimen is such an amount that the
concentration of the sodium ion derived from the test specimen becomes 20 mM or higher, as a
final concentration in the reaction system after mixing the test specimen with the assay agent.

20. (withdrawn): A method for producing a water for medical use, a pharmaceutical
product, an infusion solution, a blood product, a medical apparatus, a medical instrument, a
biological specimen, a native or recombinant protein, a nucleic acid, or a saccharide which has
been subjected to endotoxin assay, comprising measuring endotoxin in the water for medical use,
the pharmaceutical product, the infusion solution, the blood product, the medical apparatus, the
medical instrument, the biological specimen, the native or recombinant protein, the nucleic acid,
or the saccharide using an endotoxin assay agent containing a water soluble horseshoe crab
Factor C produced by the method according to claim 9.

21. (canceled).

22. (canceled).



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NOTICE OF ALLOWANCE AND FEE(S) DUE

23373 7590 02/10/2021
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 900
WASHINGTON, DC 20006

EXAMINER

STEADMAN, DAVID J

ART UNIT

PAPER NUMBER

1656

DATE MAILED: 02/10/2021

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/983,725	05/18/2018	Hikaru Mizumura	Q233613	5544

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	05/10/2021

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

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P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

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23373 7590 02/10/2021
SUGHRUE MION, PLLC
2000 PENNSYLVANIA AVENUE, N.W.
SUITE 900
WASHINGTON, DC 20006

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/983,725	05/18/2018	Hikaru Mizumura	Q233613	5544

TITLE OF INVENTION: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING
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APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	05/10/2021

EXAMINER	ART UNIT	CLASS-SUBCLASS
STEADMAN, DAVID J	1656	435-219000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29☐ Applicant asserting small entity status. See 37 CFR 1.27☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/983,725	05/18/2018	Hikaru Mizumura	Q233613	5544
23373 7590 02/10/2021 SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 900 WASHINGTON, DC 20006			EXAMINER STEADMAN, DAVID J	
			ART UNIT	PAPER NUMBER
			1656	

DATE MAILED: 02/10/2021

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/983,725	Applicant(s) Mizumura et al.	
	Examiner DAVID J STEADMAN	Art Unit 1656	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the application papers filed on December 23, 2020 and January 8, 2021.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.

4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a) ☒ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☒ Certified copies of the priority documents have been received in Application No. 14/650,767.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☒ Notice of References Cited (PTO-892) 2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____. 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____. 4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.	5. ☒ Examiner's Amendment/Comment 6. ☒ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
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/David Steadman/ Primary Examiner, Art Unit 1656	
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Continuation of 3. The allowed claim(s) is/are: 1-6,8-10,12-15 and 17

NOTICE OF ALLOWABILITY

Status of the Application

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on November 30, 2020 has been entered.

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Applicant's amendment to the claims, filed on November 30, 2020, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's remarks filed on November 30, 2020 and January 8, 2021 and the applicant's declaration under 37 CFR 1.132, filed on January 8, 2021 in response to the advisory action mailed on December 11, 2020 and the final rejection mailed on August 28, 2020 have been fully considered.

Examiner's Amendment to the Claims

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Ms. Hui C. Wauters on January 22, 2021.

Please replace the claim set filed on November 30, 2020 with the following re-written claims.

1. (Currently Amended) A method for imparting a salt ion resistance for a horseshoe crab Factor C, the method comprising:

recombinantly expressing and secreting the horseshoe crab Factor C into a culture broth or a culture supernatant using ~~in~~ a mammalian cell as a host cell, and

recovering ~~at least one of a culture broth and a culture supernatant of the mammalian cell containing~~ the recombinant horseshoe crab Factor C secreted from the mammalian cell,

wherein the horseshoe crab Factor C recombinantly expressed and secreted using the mammalian cell contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding horseshoe crab Factor C expressed using a Sf9 insect cell as a host cell,

wherein the mammalian cell is a Chinese hamster ovary (CHO) cell or a human embryonic kidney (HEK) cell, and

wherein the horseshoe crab Factor C recombinantly expressed and secreted into a culture broth or a culture supernatant using ~~in~~ the mammalian cell is a protein selected from ~~shown in~~ the following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence of Factor C of *Tachypleus tridentatus*, wherein the protein has ~~having~~ no peptide added to the N-terminus of Factor C of *Tachypleus tridentatus*, and wherein the protein has ~~having~~ activity of Factor C;

(B) a protein comprising the amino acid sequence of Factor C of *Limulus polyphemus*,
wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Limulus polyphemus*, and wherein the protein has~~having~~ activity of Factor C;

(C) a protein comprising the amino acid sequence of Factor C of *Carcinoscorpius rotundicauda*, wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Carcinoscorpius rotundicauda*, and wherein the protein has~~having~~ activity of Factor C;

(D) a protein comprising the amino acid sequence of Factor C of *Tachypleus gigas*,
wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Tachypleus gigas*, and wherein the protein has~~having~~ activity of Factor C.

2. (Original) The method according to claim 1, wherein the salt ion is selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

3. (Currently Amended) The method according to claim 1, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

4. (Currently Amended) The method according to claim 1, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

5. (Currently Amended) The method according to claim 1, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.
6. (Currently Amended) The method according to claim 1, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.
7. (Canceled)
8. (Currently Amended) The method according to claim 1, wherein the protein comprises the amino acid sequence of Factor C ~~horseshoe crab is selected from the group consisting of~~ *Tachyplesus tridentatus*, *Limulus polyphemus*, ~~and~~ *Carcinoscorpius rotundicauda*.
9. (Currently Amended) A method for producing an endotoxin assay agent comprising a ~~water soluble~~ horseshoe crab Factor C, the method comprising:

recombinantly expressing and secreting the horseshoe crab Factor C into a culture broth or a culture supernatant using ~~in~~ a mammalian cell as a host cell, ~~and~~

recovering ~~at least one of a culture broth and a culture supernatant of the mammalian cell~~
~~containing~~ the recombinant horseshoe crab Factor C secreted from the mammalian cell ~~as a~~
~~component of the endotoxin assay agent, and~~

producing an endotoxin assay agent comprising the recombinant horseshoe crab Factor C,
wherein the horseshoe crab Factor C recombinantly expressed and secreted using the
mammalian cell contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared
with a corresponding horseshoe crab Factor C expressed using a Sf9 insect cell as a host cell,
wherein the mammalian cell is a Chinese hamster ovary (CHO) cell or a human
embryonic kidney (HEK) cell, and
wherein the horseshoe crab Factor C recombinantly expressed and secreted into a culture
broth or a culture supernatant using in the mammalian cell is a protein selected from~~shown in~~ the
following (A), (B), (C), or (D):

(A) a protein comprising the amino acid sequence of Factor C of *Tachypleus tridentatus*,
wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Tachypleus*
tridentatus, and wherein the protein has~~having~~ activity of Factor C;

(B) a protein comprising the amino acid sequence of Factor C of *Limulus polyphemus*,
wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Limulus*
polyphemus, and wherein the protein has~~having~~ activity of Factor C;

(C) a protein comprising the amino acid sequence of Factor C of *Carcinoscorpius*
rotundicauda, wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of
Carcinoscorpius rotundicauda, and wherein the protein has~~having~~ activity of Factor C;

(D) a protein comprising the amino acid sequence of Factor C of *Tachypleus gigas*,
wherein the protein has~~having~~ no peptide added to the N-terminus of Factor C of *Tachypleus*
gigas, and wherein the protein has~~having~~ activity of Factor C.

11. (Canceled)

12. (Currently Amended) The method according to claim 9, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 21 mM sodium citrate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

13. (Currently Amended) The method according to claim 9, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 52 mM sodium hydrogencarbonate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

14. (Currently Amended) The method according to claim 9, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 214 mM sodium chloride as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

15. (Currently Amended) The method according to claim 9, wherein the horseshoe crab Factor C recombinantly expressed and secreted using a mammalian cell exhibits a higher residual activity in the presence of 16 mM magnesium sulfate as compared with a corresponding horseshoe crab Factor C expressed in a Sf9 insect cell as a host cell.

16. (Canceled)

17. (Previously Presented) The method according to claim 9, wherein the protein comprises the amino acid sequence of Factor C ~~horseshoe crab is selected from the group consisting of~~ *Tachypleus tridentatus*, *Limulus polyphemus*, ~~or~~ and *Carcinoscorpius rotundicauda*.

18-22. (Canceled)

Election/Restrictions

The requirement for an election of species between the species of A) SEQ ID NO: 2 and B) SEQ ID NO: 4 as set forth at pp. 4-6 of the Office action mailed on August 7, 2019 is withdrawn. In view of the withdrawal of the requirement for an election of species, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Examiner's Statement of Reasons for Allowance

The following is an examiner's statement of reasons for allowance:

The independent claims 1 and 9 recite (in relevant part) "...recombinantly expressing and secreting the horseshoe crab Factor C into a culture broth or a culture supernatant using a mammalian cell as a host cell... wherein the horseshoe crab Factor

C recombinantly expressed and secreted into a culture broth or a culture supernatant... is a protein... wherein the protein has no peptide added to the N-terminus of Factor C” of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas* (underline added for emphasis).

Given that the recited protein “has no peptide added to the N-terminus of Factor C” of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*, the recombinantly expressed and secreted Factor C as recited in the claims cannot have an added signal sequence at the N-terminus and thus relies on the native signal sequence of the Factor C of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas* for secretion of the Factor C. However, before the effective filing date, the reference of Wang et al. (*Biotechnol. Lett.* 23:7176, 2001; cited on the IDS filed on May 18, 2018) disclosed recombinant Factor C (rFC) expressed using a COS-1 host cell (a mammalian cell) was not secreted and remained insoluble within the cell, indicating that the native signal sequence was not able to direct full length rFC out of the cell (p. 71, paragraph bridging columns 1 and 2). As such, one of ordinary skill in the art would have had no reasonable expectation for expressing and secreting recombinant Factor C of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas* from a mammalian cell without an added peptide at the N-terminus.

For at least these reasons, the methods of claims 1-6, 8-10, 12-15, and 17 are considered to be allowable over the prior art of record.

In the interest of clarity, it is noted that the specification defines the amino acid sequences of *Tachypleus tridentatus* Factor C and *Limulus Polyphemus* Factor C as

being SEQ ID NO: 2 and SEQ ID NO: 4, respectively (p. 29, middle) and the recitation of “the amino acid sequence of Factor C of *Carcinoscorpius rotundicauda*” and “the amino acid sequence of Factor C of *Tachypleus gigas*” in parts (C) and (D) of claims 1 and 9 is interpreted in accordance with MPEP 2111.01 as meaning the full-length amino acid sequences of *Carcinoscorpius rotundicauda* Factor C and *Tachypleus gigas* Factor C, which sequences were known in the prior art at the time of the invention (see, *e.g.*, UniProt Database Accession Number Q26423, November 2012, 2 pages and Miyata et al., *J. Biochem.* 95:1793-1801, 1984). Also, the recitation of “has activity of Factor C” is interpreted in accordance with the specification as referring to activation of Factor C in the presence of endotoxin to convert Factor B into activated Factor B (p. 37, first full paragraph).

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled “Comments on Statement of Reasons for Allowance.”

Information Regarding Quick Path IDS Program

For situations when the applicant needs to file an IDS *after the issue fee has been paid*, The United States Patent and Trademark Office (USPTO) has implemented a Quick Path Information Disclosure Statement (QPIDS) program intended to reduce pendency and applicant costs. This pilot program will permit an examiner to consider an IDS after payment of the issue fee without the need to reopen prosecution, effectively obviating the need to pursue a Request for Continued Examination (RCE).

Where the examiner determines that no item of information in the IDS necessitates reopening prosecution, the Office will issue a corrected notice of allowability. In addition to reducing pendency, this program will promote efficiency in the examination process. There will be no fee required to use this program, beyond existing fees, *e.g.*, fees for IDS submission. More information on this and other new initiatives at the Patent Office can be found at http://www.uspto.gov/patents/init_events/patapp-initiatives-timeline.jsp or by calling the examiner or his supervisor.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DAVID J STEADMAN whose telephone number is (571)272-0942. The examiner can normally be reached on Monday to Friday, 7:30 AM to 4:00 PM.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, MANJUNATH N. RAO can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David Steadman/
Primary Examiner, Art Unit 1656