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SUGHRUE MION, PLLC 2000 PENNSYLVANIA AVENUE, N.W. SUITE 9000 WASHINGTON, DC 20006			STEADMAN, DAVID J	
			ART UNIT	PAPER NUMBER
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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.

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sughrue@sughrue.com

Office Action Summary**Application No.**

18/346,420

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 --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 03 July 2023.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a) ☐ This action is **FINAL**.

2b) ☒ This action is non-final.

3) ☐ 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.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☒ Claim(s) 1-10 is/are pending in the application.

5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☒ Claim(s) 1-10 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, 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.

Application Papers

10) ☐ The specification is objected to by the Examiner.

11) ☒ The drawing(s) filed on 03 July 2023 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☒ 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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

3) ☐ Interview Summary (PTO-413)

Paper No(s)/Mail Date ____.

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)

4) ☐ Other: ____.

Paper No(s)/Mail Date ____.

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 1-10 are pending in the application.

Priority

This application is a continuation application of U.S. non-provisional application no. 17/554,128, filed on December 17, 2021, which is a continuation application of U.S. non-provisional application no. 16/399,386, filed on April 30, 2019, now U.S. Patent No. 11,236,318, which is a continuation application of U.S. non-provisional application no. 15/983,725, filed on May 18, 2018, now U.S. Patent No. 10,982,202, which is a continuation application of U.S. non-provisional application no. 14/650,767, filed on June 9, 2015, now U.S. Patent No. 10,144,923, which is filed under 35 U.S.C. 371 as a national stage of international application PCT/JP2013/083082, filed on December 10, 2013, which claims foreign priority under 35 U.S.C. 119(a)-(d) to Japanese application no. 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 July 3, 2023 and August 18, 2023 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the IDSs are being considered by the examiner.

Cite Nos. 20, 23, 24, 27, 29, and 80 of the IDS filed on July 3, 2023 have been lined through because, according to the applicant's "Submission of a Corrected Form PTO/SB/08" filed on August 18, 2023, these cites contain errors, which are corrected by the IDS filed on August 18, 2023.

Claim Objections

Claim 4 is objected to in the recitation of "*Tachypleus tridentatus*, and *Limulus polyphemus*" and in the interest of improving claim form, it is suggested that the comma be deleted from the noted phrase.

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.

Claim 9 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 9 is indefinite in the recitation of "wherein the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme are formulated separately", which is inconsistent with the recitation of "formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme" in claim 1. It is suggested that the applicant clarify the meaning of claim 9.

Claim Rejections - 35 USC § 112(d)

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

(d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim 8 is rejected under 35 U.S.C. 112(d) as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Given that claim 1 recites “formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme”, claim 8 does not further limit claim 1. Applicant may cancel the claim, amend the claim to place the claim in proper dependent form, rewrite the claim in independent form, or present a sufficient showing that the dependent claim complies with the statutory requirements.

Claim Rejections - 35 USC § 103

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 1-10 are rejected under 35 U.S.C. 103 as being unpatentable over Ding-1 et al. (US 5,858,706; cited on the IDS filed on July 3, 2023; hereafter “Ding-1”) in view of

Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on July 3, 2023; hereafter “Tamura”).

The claims are drawn to a method for producing an endotoxin assay agent comprising a horseshoe crab Factor C, a horseshoe crab Factor B, and a horseshoe crab Pro-clotting enzyme, the method comprising:

expressing the horseshoe crab Factor C in a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor C having activity of Factor C,

expressing the horseshoe crab Factor B in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Factor B having activity of Factor B,

expressing the horseshoe crab Pro-clotting enzyme in a mammalian cell as a host cell, and recovering a culture supernatant comprising the horseshoe crab Pro-clotting enzyme having activity of Pro-clotting enzyme, and

formulating the endotoxin assay agent comprising the horseshoe crab Factor C, the horseshoe crab Factor B, and the horseshoe crab Pro-clotting enzyme.

The specification does not appear to define the phrase “culture supernatant” and given a broadest reasonable interpretation, the phrase “culture supernatant” is interpreted as encompassing a supernatant recovered by centrifuging cultured and lysed host cells.

Regarding claim 1, the reference of Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to

express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant by centrifuging cultured and lysed host cells (column 10, lines 43-47).

Regarding claim 4, Ding-1 teaches the cDNA for expression encodes a *Tachypleus tridentatus* horseshoe crab Factor C (column 3, lines 14-20).

Regarding claim 5, Ding-1 does not teach 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 *T. tridentatus* 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 *T. tridentatus* factor C polypeptide

recombinantly expressed and recovered from CHO cells according to Ding-1 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 6, Ding-1 does not teach the Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

The differences between Ding-1 and the claimed invention are:

- 1) Ding-1 does not teach recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1; and
- 2) Ding-1 does not teach filtration of a supernatant as recited in claim 7.

Regarding difference 1), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-1 for an endotoxin assay reagent with Ding-1's recombinantly produced *Tachypleus tridentatus* Factor C. One would have been motivated to do this because Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme. One would have had a reasonable expectation of success to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-1 for an endotoxin assay reagent with Ding-1's recombinantly produced *Tachypleus tridentatus* Factor C because Tamura taught sequences of *Tachypleus tridentatus* Factor B and Pro-clotting proteins, and Ding-1 and Tamura taught methods for recombinant expression of a protein using a CHO cell.

Regarding difference 2), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Ding-1 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, the method for producing an endotoxin assay agent of claims 1-10 would have been obvious to one of ordinary skill in the art before the effective filing date.

Claims 1-10 are rejected under 35 U.S.C. 103 as being unpatentable over Ding et al. (US 5,716,834; cited on the IDS filed on July 3, 2023; hereafter “Ding-2”) in view of Tamura.

The specification does not appear to define the phrase “culture supernatant” and given a broadest reasonable interpretation, the phrase “culture supernatant” is interpreted as encompassing a supernatant obtained by recovering a culture broth from cultured host cells.

Regarding claims 1 and 4, Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster

Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Regarding claim 5, Ding-2 does not disclose the *T. tridentatus* 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 *T. tridentatus* 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 Factor C polypeptide recombinantly expressed and recovered from CHO cells according to Ding-1 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 6, Ding-2 does not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

The differences between Ding-2 and the claimed invention are:

- 1) Ding-2 does not teach recovering a culture supernatant as recited in claim 1;
- 2) Ding-2 does not teach recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1; and
- 3) Ding-2 does not teach filtration of a supernatant as recited in claim 7.

Regarding difference 1), Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to collect the culture medium following secretion of Factor C according to Ding. One would have been motivated to do this in order to isolate or harvest the Factor C following secretion. One would have had a reasonable expectation of success to do this because Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of

endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-2 for an endotoxin assay reagent with Ding-2's recombinantly produced *Tachypleus tridentatus* Factor C. One would have been motivated to do this because Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme. One would have had a reasonable expectation of success to recombinantly produce *Tachypleus tridentatus* Factor B and Pro-clotting proteins using the expression system of Ding-2 for an endotoxin assay reagent with Ding-2's recombinantly produced *Tachypleus tridentatus* Factor C because Tamura taught sequences of *Tachypleus tridentatus* Factor B and Pro-clotting proteins, and Ding-2 and Tamura taught methods for recombinant expression of a protein using a CHO cell.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Ding-2 and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, the method for producing an endotoxin assay agent of claims 1-10 would have been obvious to one of ordinary skill in the art before the effective filing date.

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(l)(1) - 706.02(l)(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 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 8 of U.S. Patent No. 10,982,202 B2 (Cite No. 9 of the IDS filed on July 3, 2023; hereafter “patent”) in view of Tamura.

The claim of the patent does not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application;
- 3) filtration of a supernatant as recited in claim 7 of this application; and
- 4) a method for measuring endotoxin as recited in claim 10 of this application.

Regarding difference 1), Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is considered to be “recovering a culture supernatant”.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects

and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (e.g., SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught collecting the culture medium of a protein that is secreted into a culture medium, Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins, and Tamura taught that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would

have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Regarding difference 4), Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to use the endotoxin assay agent of the claims of the patent, including recombinantly-produced Factor B, and recombinantly-produced Pro-clotting enzyme, to detect endotoxin. One would have been motivated to and would have had a reasonable expectation of success to do this because the claims of the reference application recite an endotoxin assay agent comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme, and Tamura taught detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claim 8 of the patent in view of Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 8, and 9 of U.S. Patent No. 10,144,923 B2 (Cite No. 2 of the IDS filed on July 3, 2023; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application; and
- 3) filtration of a supernatant as recited in claim 7 of this application.

Regarding difference 1), Ding-1 teaches recovery of the Factor C from mammalian cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47), *i.e.*, “recovering a culture supernatant”. Ding-2 teaches the alternative of secreting Factor C into a culture medium (column 3, lines 47-50; column 17, lines 24-28) and Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (*e.g.*, SEQ ID NO: 15 and 16 of Tamura) and Pro-clotting enzyme (*e.g.*, SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1, 3, 8, and 9 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 4, and 10-12 of U.S. Patent No. 11,236,318 B2 (Cite No. 10 of the IDS filed on July 3, 2023; hereafter “patent”) in view of in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) recovering a culture supernatant as recited in claim 1 of this application;
- 2) recombinant horseshoe crab Factor B and horseshoe crab Pro-clotting polypeptides as recited in claim 1 of this application; and
- 3) filtration of a supernatant as recited in claim 7 of this application.

Regarding difference 1), Ding-1 teaches recovery of the Factor C from mammalian cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47), *i.e.*, “recovering a culture supernatant”. Ding-2 teaches the alternative of secreting Factor C into a culture medium (column 3, lines 47-50; column 17, lines 24-28) and Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

Regarding difference 2), Tamura teaches that collecting hemolymph from horseshoe crabs as a source of enzymes for detection of endotoxin has negative effects and teaches that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins (paragraph [0031]). Tamura teaches the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme (paragraph [0040]). Tamura teaches the nucleic acid and amino acid sequences of *Tachypleus tridentatus* Factor B (*e.g.*, SEQ ID NO: 15 and 16 of Tamura)

and Pro-clotting enzyme (e.g., SEQ ID NO: 3 and 4 of Tamura). Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding difference 3), Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 2, 4, and 10-12 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1 and 3-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 of U.S. Patent No. 9,040,254 B2 (cited on the attached Form PTO-892; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

- 1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and
- 2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the

Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the patent do not recite the *T. tridentatus* 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 *T. tridentatus* 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 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 6, the claims of the patent do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1 and 3-10 of this application are unpatentable over claim 1 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-10 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1 and 7-9 of U.S. Patent No. 11,499,177 B2 (cited on the attached Form PTO-892; hereafter "patent") in view of Ding-1, Ding-2, and Tamura.

The claims of the patent do not recite:

1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and

2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin

testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1,

Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the patent do not recite the *T. tridentatus* 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 *T. tridentatus* 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 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 6, the claims of the patent do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128

kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1 and 7-9 of the patent in view of Ding-1, Ding-2, and Tamura.

Claims 1-6, 8, and 9 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application).

Regarding claims 1-4 and 8 of this application, claim 3 of the reference application recites an endotoxin assay agent comprising a horseshoe crab Factor C protein having an activity of Factor C, a horseshoe crab Factor B protein having an activity of Factor B, and a horseshoe crab Pro-clotting enzyme protein having an activity

of Pro- clotting enzyme, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus* and *Limulus polyphemus*,

wherein the Factor C protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell,

wherein the Factor B protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell,

wherein the Pro-clotting enzyme protein is produced through being recombinantly expressed from a Chinese Hamster Ovary cell or a Human Embryonic Kidney cell, and

wherein the endotoxin assay agent dose not comprise a native Factor C,

claim 15 of the reference application recites wherein the Factor C protein is secreted into a culture supernatant and recovered from the culture supernatant,

claim 16 of the reference application recites wherein the Factor B protein is secreted into a culture supernatant and recovered from the culture supernatant, and

claim 17 of the reference application recites wherein the Pro-clotting enzyme protein is secreted into a culture supernatant and recovered from the culture supernatant.

Regarding claim 5 of this application, claim 5 of the reference application recites wherein the Factor C protein contains (α -2,3)-linked terminal sialic acid in a greater amount, as compared with a corresponding Factor C protein recombinantly expressed using Sf9 as a host cell.

Regarding claim 6 of this application, claim 6 of the reference application recites wherein the Factor C protein has a molecular weight of 115 kDa to 130 kDa, as measured through SDS-PAGE under a non-reducing condition.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant provisional rejection.

Therefore, claims 1-6, 8, and 9 of this application are unpatentable over claims 2, 3, 5, and 15-17 of the reference application.

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

Claims 7 and 10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2, 3, 5, and 15-17 of co-pending application 17/554,128 (reference application) in view of Tamura.

The claims of the reference application do not recite:

- 1) filtration of a supernatant as recited in claim 7 of this application; and
- 2) a method for measuring endotoxin as recited in claim 10 of this application.

Regarding difference 1), Tamura teaches a recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly produce *Tachypleus tridentatus* Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Regarding difference 2), Tamura teaches detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme (Example 4).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to use the endotoxin assay agent of the claims of the reference application to detect endotoxin. One would have been motivated to and would have had a reasonable expectation of success to do this because the claims of the reference application recite an endotoxin assay agent comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme, and Tamura taught detection of endotoxin using a cascade reaction comprising recombinant Factor C, recombinant Factor B, and recombinant Pro-clotting enzyme.

Therefore, claims 7 and 10 of this application are unpatentable over claims 2, 3, 5, and 15-17 of the reference application in view of Tamura.

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

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3 of co-pending application 17/272,805 (reference application) in view of Ding-1, Ding-2, and Tamura.

The claims of the reference application do not recite:

1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and

2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2

discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2 discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the reference application do not recite the *T. tridentatus* 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 *T. tridentatus* 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 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 6, the claims of the reference application do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced Factor C, Factor B,

and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1-3 of the reference application in view of Ding-1, Ding-2, and Tamura.

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

Claims 1-10 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1 and 7-9 of co-pending application 18/045,705 (reference application) in view of Ding-1, Ding-2, and Tamura.

The claims of the reference application do not recite:

- 1) expressing a recombinant horseshoe crab Factor C, Factor B, and Pro-clotting polypeptides using a CHO cell and recovering a culture supernatant as recited in claim 1 of this application; and
- 2) the limitations of claims 5-7 of this application.

Regarding difference 1), Ding-1 teaches Factor C is a component of the Limulus amoebocyte lysate (LAL) assay that is presently the standard method for testing for endotoxin (column 1, lines 40-42). Ding-1 teaches that it is desirable to express recombinant Factor C which could be standardized for use in an assay for endotoxin

detection (column 1, lines 55-57). Ding-1 teaches a mammalian host cell transformed with a recombinant vector for expressing a cDNA encoding a Factor C (column 9, Example 1). Ding-1 teaches mammalian host cells include Chinese Hamster Ovary cells (column 9, Example 1). Ding-1 teaches a method for producing the Factor C by culturing the host cell and teaches recovery of the Factor C can be performed substantially as described for purification from yeast cells except for lysing the mammalian cells with detergents (column 9, Example 1). Ding-1 teaches recovery of the Factor C from yeast cells includes a step of preparing a supernatant of cultured and lysed host cells (column 10, lines 43-47).

Ding-2 discloses that there are four species of horseshoe crabs including *Tachypleus tridentatus*, which are sources of Limulus amoebocyte lysate for endotoxin testing (column 1, lines 17-36). According to Ding-2, the Japanese *Tachypleus tridentatus* has been pronounced an endangered species and is on its way to extinction (column 1, lines 48-49). Ding-2 discloses that the availability of a Factor C produced through recombinant DNA technology would alleviate problems of batch-variation, and also provide a standardized and continuous supply of material for endotoxin/pyrogen assays (column 1, lines 52-58). Ding-2 discloses a mammalian host cell transformed with a recombinant vector for expressing a Factor C (column 2, lines 46-56). Ding-2 discloses the mammalian host cell includes Chinese Hamster Ovary cells (column 19, lines 39-43). Ding-2 discloses a method for producing the Factor C by culturing the host cell and recovering the Factor C protein (column 2, lines 58-63). Ding-2 discloses the Factor C is secreted into the culture medium and discloses isolating or harvesting the Factor C following secretion (column 3, lines 47-50; column 17, lines 24-28). Ding-2

discloses the cDNA and corresponding amino acid sequence of *Tachypleus tridentatus* horseshoe crab Factor C (Figure 11). Ding-2 discloses the recombinant Factor C may be used in an assay for detecting endotoxin (column 2, lines 7-11).

Tamura teaches collecting the culture medium of a protein that is secreted into a culture medium (paragraph [0017]), which is also considered to be “recovering a culture supernatant”.

In view of Ding-1, Ding-2, and Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to recombinantly express and recover a culture supernatant of Factor C, Factor B, and Pro-clotting enzyme produced by a CHO DG44 cell for an endotoxin assay reagent. One would have been motivated to and would have had a reasonable expectation of success to do this because Ding-1, Ding-2, and Tamura taught recovery of Factor C and Tamura taught that all proteins of the coagulation cascade reaction for detection of endotoxin should be recombinant proteins and that the proteins involved in the cascade reaction can include only Factor C, Factor B, and Pro-clotting enzyme.

Regarding claim 5, the claims of the reference application do not recite the *T. tridentatus* 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 *T. tridentatus* 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 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 6, the claims of the reference application do not disclose the *T. tridentatus* Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, the specification discloses that Factor C polypeptide recombinantly expressed and recovered from CHO cells has a molecular weight of 128 kDa as measured by SDS-PAGE under a non-reducing condition (paragraph [0144] and Table 1).

Regarding claim 7, Tamura teaches the recombinantly-produced protein can be purified and discloses various purification steps, including ultrafiltration (paragraph [0119]).

In view of Tamura, it would have been obvious to one of ordinary skill in the art before the effective filing date to purify the recombinantly-produced Factor C, Factor B, and Pro-clotting proteins by ultrafiltration. One would have been motivated to and would have had a reasonable expectation of success to do this because Tamura taught that recombinantly-produced proteins can be purified and discloses ultrafiltration as a purification method.

Given the indefiniteness of claim 9 and in the interest of compact prosecution, claim 9 is included in the instant rejection.

Therefore, claims 1-10 of this application are unpatentable over claims 1 and 7-9 of the reference application in view of Ding-1, Ding-2, and Tamura.

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

Conclusion

Status of the claims:

Claims 1-10 are pending.

Claims 1-10 are rejected.

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 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.

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/David Steadman/
Primary Examiner, Art Unit 1656