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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/554,128	12/17/2021	Hikaru MIZUMURA	Q270767	8200

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EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1656	

NOTIFICATION DATE	DELIVERY MODE
03/20/2025	ELECTRONIC

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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CRL Exhibit 1042
CRL v. Seikagaku
PGR2025-00023

Office Action Summary	Application No. 17/554,128	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 28 January 2025.

☐ 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) 2-5,15-18 and 20-23 is/are pending in the application.

5a) Of the above claim(s) 4 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 2-3,5,15-18 and 20-23 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 _____ 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. _____.

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)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 01/28/2025.

3) ☑ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

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 2-5, 15-18, and 20-23 are pending in the application.

Applicant's amendment to the claims, filed on January 28, 2025, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

Applicant's amendment to the specification, filed on January 28, 2025, is acknowledged.

Applicant's remarks filed on January 28, 2025 in response to the non-final rejection mailed on December 20, 2024 are acknowledged and have been fully considered.

Election/Restriction

Claim 4 is withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected inventions, there being no allowable generic or linking claim. Election was made without traverse in the reply filed on March 2, 2023.

Claims 2, 3, 5, 15-18, and 20-23 are being examined on the merits.

Priority

This application is filed under 35 U.S.C. 120 as 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.

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, 365(c), or 386(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 or 365(c) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the priority application nos. US 14/650,767 and PCT/JP2013/083082 fail to provide adequate descriptive support and enablement in the manner provided by 35 U.S.C. 112(a) for one or more claims of this application for reasons that follow.

Claims 2, 3, 5, 15-18, and 20-23 recite *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme proteins that are produced through being recombinantly expressed from a HEK 293 cell. Recombinant expression of the recited *L. polyphemus* proteins from a HEK 293 cell necessarily requires cloning and expression of a polynucleotide encoding each of the *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme proteins.

Neither the instant application nor any of the priority applications discloses a working example of cloning and expression of *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme proteins from a HEK 293 cell.

The applicant's newly cited reference of McClymont, K. ("Structural studies of the endotoxin sensing protein Factor," Ph.D. Thesis, University of Glasgow, Institute of Molecular, Cell and Systems Biology College of Medical, Veterinary and Life Sciences, 2017; cited on the IDS filed on January 28, 2025; hereafter "McClymont"), which was published in March 2017, discloses "[t]he sequence of *Limulus polyphemus* Factor C is not publicly available. For this reason, it was necessary to assemble the lpFC gene from the raw data kindly provided from Washington University, St Louis, in order to synthesise full-length lpFC…Neither the amino acid, nor the genomic DNA or cDNA sequences for Factor C from *Limulus polyphemus* were available in any public databases. However, the McDonnell Genome Institute at Washington University, St Louis (WUSTL) granted pre-release access to their *Limulus polyphemus* genome data (project funded by the National Human Genome Research Institute (NHGRI)). This was mined in an attempt to assemble the Limulus polyphemus Factor C coding sequence and gene (p. 36, middle; paragraph bridging pp. 38-39).

The applicant's newly cited reference of Mizumura et al. (WO 2018/074498 A1; cited on the IDS filed on January 28, 2025; "Mizumura"), which was published in April 2018, discloses "[t]here have been no reports of a reagent using recombinant proteins derived from Limulus polyphemus, which is widely used as a lysate reagent. The reason is that in Limulus polyphemus, the proteins and their genes involved in the mechanism of coagulation caused by endotoxin have not been completely identified structurally or functionally…in Limulus polyphemus…complete identification of these proteins and genes in terms of both structure and function has not been achieved. This is because due to the various differences and diversity of biomolecules depending on the genus and species of horseshoe crab, it has not been possible to accurately determine the full-length sequence structure, expression, and function of these proteins and genes using conventional methods…The full-length sequences of all proteins and genes involved in the coagulation mechanism of Limulus polyphemus have not yet been elucidated. This fact also demonstrates the difficulty in accurately determining the full-length sequence structures of these proteins and genes in Limulus polyphemus" (paragraphs [0009], [0010], [0012], and [0013]). According to Mizumura et al., "[t]he present inventors have succeeded in obtaining full-length cDNAs encoding all of the protein factors involved in the coagulation mechanism of Limulus polyphemus, something that no one had succeeded in doing for many years…As a result, there was success for the first time in expressing and obtaining a protein from Limulus polyphemus that may have factor C activity (sequence number 2), a protein that may have factor B activity (sequence number 6), and a protein that may have pro-clotting enzyme activity (sequence number 14)…the expressed protein which may have factor C activity, the protein which may

have factor B activity, and the protein which may have pro-clotting enzyme activity have factor C activity, factor B activity, and pro-clotting enzyme activity, respectively, and this demonstrated for the first time that contact with endotoxin triggers a cascade reaction, It was also demonstrated for the first time that these recombinant proteins can be used to detect endotoxin (paragraphs [0017], [0180], and [0184]).

In view of the noted disclosures of McClymont and Mizumura, one of skill in the art would have recognized that the disclosures of priority application nos. U.S. 14/650,767 and PCT/JP2013/083082, which were filed prior to the publications of McClymont and Mizumura, fail to adequately describe and enable cloning and expression of a polynucleotide encoding each of the *L. polyphemus* Factor C, Factor B, and Pro-clotting enzyme proteins from a HEK 293 cell, and would have recognized that only *after* the publications of McClymont and Mizumura was the cloning and expression of a polynucleotide encoding each of the *L. polyphemus* Factor C, Factor B, and Pro-clotting enzyme proteins in a HEK 293 cell adequately described and enabled in the manner provided by 35 U.S.C. 112(a). As such, claims 2, 3, 5, 15-18, and 20-23 are not entitled to the benefit of the priority applications 14/650,767 and PCT/JP2013/083082 and the effective filing date of claims 2, 3, 5, 15-18, and 20-23 is considered to be the filing date of the priority application no. 15/983,725, which is May 18, 2018.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on January 28, 2025 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the IDS has been considered by the examiner.

Cite Nos. 138, 140, 152, and 160 have been lined through because there is no date as required by 37 CFR 1.98(b)(5).

Claim Objections

The objections to claims 3 and 22 for minor informalities are withdrawn in view of applicant's amendments to claims 3 and 22.

Applicant is advised that should claim 3 be found allowable, claim 23 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 608.01(m).

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.
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed 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 2, 3, 5, 15-18, and 20-23 are rejected under 35 U.S.C. 102(a)(1) or 35 U.S.C. 102(a)(2) as being anticipated by or, in the alternative, under 35 U.S.C. 103 as obvious over Mizumura (*supra*).

The applicant's attention is directed to 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.

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

wherein the horseshoe crab is *Limulus polyphemus*,

wherein the Factor C protein is produced through being recombinantly expressed from a HEK 293 cell,

wherein the Factor B protein is produced through being recombinantly expressed from a HEK 293 cell,

wherein the Pro-clotting enzyme protein is produced through being recombinantly expressed from a HEK 293 cell,

wherein the endotoxin assay agent detects an endotoxin through measuring the progress of a cascade reaction of the Factor C protein, the Factor B protein, the Pro-clotting enzyme protein, and the detection substrate, and

wherein the endotoxin assay agent is formulated into a liquid.

Regarding claims 3 and 23, Mizumura teaches producing recombinant *L. polyphemus* Factor C (also referred to by Mizumura as recombinant first protein), recombinant *L. polyphemus* Factor B (also referred to by Mizumura as recombinant second protein), and recombinant *L. polyphemus* Pro-Clotting Enzyme (also referred to by Mizumura as recombinant third protein) by expressing a polynucleotide in a host cell (p. 15, paragraph [0035] to p. 20, paragraph [0060] and p. 36, paragraph [0148]). Mizumura teaches the host cell is a HEK29 cell (p. 15, paragraph [0039]). Mizumura teaches an endotoxin detection agent comprising a recombinant first protein, a recombinant second protein, a recombinant third protein, and a detection substrate for the recombinant third protein (pp. 7-8, paragraph [1-3]; p. 25, paragraphs [0089] and [0090]; p. 29, paragraph [0111] to p. 30, paragraph [0119]). Mizumura teaches the endotoxin detection agent can be a mixture (p. 30, paragraph [0113]) and can be formulated as a liquid (p. 30, paragraph [0117]).

Regarding claim 2, Mizumura does not teach the recombinant *L. polyphemus* Factor C expressed from a HEK 293 cell has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, according to MPEP 2112.01.I, when the structure recited in the reference is substantially identical to that of the claims, claimed properties or functions are presumed to be inherent. In accordance with MPEP 2112.01.I, given that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura has the same structure as Factor C protein of claim 2, it is presumed that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura

inherently has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition.

Regarding claim 5, Mizumura does not teach the recombinant *L. polyphemus* Factor C expressed from a HEK29 cell 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. However, in accordance with MPEP 2112.0.I, given that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura has the same structure as Factor C protein of claim 5, it is presumed that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura inherently 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 claims 15-17, Mizumura teaches the expressed protein is in the culture supernatant and may be purified before use (p. 35, paragraph [0143]).

Regarding claim 18, Mizumura's method for producing the recombinant first protein (p. 15, paragraph [0036] to p. 17, paragraph [0046]) does not include attaching a peptide to the C-terminus. Thus, one of ordinary skill in the art would recognize that the recombinant first protein "has no peptide added to the C-terminus."

Regarding claims 20-22, Mizumura teaches examples of substances that are cleaved by the activity of the Pro-Clotting enzyme include synthetic substrates represented by the general formula X-Y-Z with X being a protecting group covalently bonded to Y, Y being a peptide residue, and Z being a signal substance bonded to Y by an amide bond (p. 25, paragraph [0091]). Mizumura teaches Y includes Leu-Gly-Arg

(LGR) and Ile-Glu-Gly-Arg (IEGR) (p. 26, paragraph [0093]) and Z includes para-nitroaniline, 7-methoxycoumarin-4-acetic acid, 2,4-dinitroaniline, and Dansyl dyes (paragraph [0094]).

Therefore, Mizumura anticipates claims 2, 3, 5, 15-18, and 20-23 as written.

Claim Rejections - 35 USC § 103

The rejection of claims 2, 3, 5, 15-18, and 20-22 under 35 U.S.C. 103 as being unpatentable over Tamura et al. (US 2009/0208995 A1; cited on the IDS filed on February 2, 2022) in view of Ding et al. (US 5,858,706; cited on the IDS filed on February 2, 2022) and Koshiba et al. (*J. Biol. Chem.* 282:3962-3967, 2007; cited on the IDS filed on February 2, 2022) is withdrawn in view of applicant's amendments to claim 3 to limit the endotoxin assay agent to a mixture of the recited proteins, and to limit the endotoxin assay agent to being formulated as a liquid. In view of these amendments, applicant's unexpected result is commensurate in scope with the claimed invention and sufficiently rebuts a *prima facie* case of obviousness.

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 § 2146 *et seq.* 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 filing of a terminal disclaimer by itself is not a complete reply to a nonstatutory double patenting (NSDP) rejection. A complete reply requires that the terminal disclaimer be accompanied by a reply requesting reconsideration of the prior Office action. Even where the NSDP rejection is provisional the reply must be complete. See MPEP § 804, subsection I.B.1. For a reply to a non-final Office action, see 37 CFR 1.111(a). For a reply to final Office action, see 37 CFR 1.113(c). A request for reconsideration while not provided for in 37 CFR 1.113(c) may be filed after final for consideration. See MPEP §§ 706.07(e) and 714.13.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The actual 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/apply/applying-online/eterminal-disclaimer.

The provisional rejection of claims 2, 3, 5, 15-18, and 20-22 on the ground of nonstatutory double patenting as being unpatentable over claims 10, 11, and 14 of co-pending application 17/272,805 (reference application) in view of Tamura, Ding, and Koshiba is withdrawn in view of applicant's amendments to claim 3 to limit the endotoxin assay agent to a mixture of the recited proteins, and to limit the endotoxin assay agent to being formulated as a liquid. In view of these amendments, applicant's unexpected result is commensurate in scope with the claimed invention and sufficiently rebuts a *prima facie* case of obviousness.

Claims 2, 3, 5, 15-18, and 20-23 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 10, 11, and 14 of co-pending application 17/272,805 (reference application) in view of Mizumura (*supra*).

Claims 10, 11, and 14 of the reference application recite (in relevant part) an endotoxin measuring reagent comprising a horseshoe crab factor C (claim 10),

wherein the horseshoe crab factor C is a recombinant protein, a recombinant protein of horseshoe crab factor B, a recombinant protein of horseshoe crab pro-clotting enzyme, and a compound for detection represented by general formula Y—X—Z, wherein in the above general formula, Y is a hydrogen atom or a protecting group, X is a peptide comprising the amino acid sequence, which is a substrate of the recombinant protein of pro-clotting enzyme, and Z is a labeling material which becomes optically detectable when being released from X (claim 11), and

wherein Z in the general formula is paranitroaniline (claim 14).

The differences between the instant claims and the claims of the reference application are as follows: the claims of the reference application do not recite the horseshoe crab is *Limulus polyphemus* as recited in claims 3 and 23, do not recite recombinant expression from a HEK cell as recited in claims 3 and 23, and do not recite the limitations of claims 2, 5, 15-18, and 20-22 of this application.

Regarding claims 3 and 23 of this application, Mizumura teaches producing recombinant *L. polyphemus* Factor C (also referred to by Mizumura as recombinant first protein), recombinant *L. polyphemus* Factor B (also referred to by Mizumura as recombinant second protein), and recombinant *L. polyphemus* Pro-Clotting Enzyme (also referred to by Mizumura as recombinant third protein) by expressing a

polynucleotide in a host cell (p. 15, paragraph [0035] to p. 20, paragraph [0060] and p. 36, paragraph [0148]). Mizumura teaches the host cell is a HEK29 cell (p. 15, paragraph [0039]). Mizumura teaches an endotoxin detection agent comprising a recombinant first protein, a recombinant second protein, a recombinant third protein, and a detection substrate for the recombinant third protein (pp. 7-8, paragraph [1-3]; p. 25, paragraphs [0089] and [0090]; p. 29, paragraph [0111] to p. 30, paragraph [0119]). Mizumura teaches the detection agent can be a mixture (p. 30, paragraph [0113]) and formulated as a liquid (p. 30, paragraph [0117]).

In view of the teachings of Mizumura, it would have been obvious to one of ordinary skill in the art for the recombinant horseshoe crab Factor C, Factor B, and Pro-clotting enzyme of the claims of the reference application to be recombinant *Limulus polyphemus* Factor C, Factor B, and Pro-clotting enzyme expressed from a HEK 293 cell. 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 recombinant horseshoe crab Factor C, Factor B, and Pro-clotting enzyme, and Mizumura teaches recombinant *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme and a mixture of these proteins in a liquid formulation as an endotoxin detection agent.

Regarding claim 2, Mizumura does not teach the recombinant *L. polyphemus* Factor C expressed from a HEK 293 cell has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition. However, given that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura has the same structure as Factor C protein of claim 2, it is presumed that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a

HEK 293 cell of Mizumura has a molecular weight of 115 kDa to 130 kDa as measured by SDS-PAGE under a non-reducing condition.

Regarding claim 5 of this application, Mizumura does not teach the recombinant *L. polyphemus* Factor C expressed from a HEK29 cell 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. However, given that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura has the same structure as Factor C protein of claim 3, it is presumed that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura 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 claims 15-17 of this application, Mizumura teaches the expressed protein is in the culture supernatant and may be purified before use (p. 35, paragraph [0143]).

Regarding claim 18 of this application, Mizumura's method for producing the recombinant first protein (p. 15, paragraph [0036] to p. 17, paragraph [0046]) does not include attaching a peptide to the C-terminus. Thus, one of ordinary skill in the art would recognize that the recombinant first protein "has no peptide added to the C-terminus."

Regarding claims 20-22 of this application, Mizumura teaches examples of substances that are cleaved by the activity of the Pro-Clotting enzyme include synthetic substrates represented by the general formula X-Y-Z with X being a protecting group covalently bonded to Y, Y being a peptide residue, and Z being a signal substance

bonded to Y by an amide bond (p. 25, paragraph [0091]). Mizumura teaches Y includes Leu-Gly-ArY (LGR) and Ile-Glu-Gly-Arg (IEGR) (p. 26, paragraph [0093]) and Z includes para-nitroaniline, 7-methoxycoumarin-4-acetic acid, 2,4-dinitroaniline, and Dansyl dyes (paragraph [0094]).

Therefore, claims 2, 3, 5, 15-18, and 20-23 of this application are unpatentable over claims 10, 11, and 14 of the reference application in view of Mizumura. This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 2, 3, 5, 15-18, and 20-23 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-13 of co-pending application 19/048,328 (reference application) in view of Mizumura (*supra*).

Claims 1-13 of the reference application recite (in relevant part) a cell culture supernatant comprising *Limulus polyphemus* Factor C protein having enzymatic activity of Factor C, wherein the Limulus polyphemus Factor C protein is produced from a Human Embryonic Kidney (HEK) 293 cell.

Regarding claim 2 of this application, claim 2 of the reference application recites the *L. polyphemus* Factor C protein has a molecular weight of 115 kDa to 140 kDa as measured by SDS-PAGE under a non-reducing condition.

The differences between the instant claims and the claims of the reference application are as follows: the claims of the reference application do not recite *Limulus polyphemus* Factor B and Pro-clotting enzyme proteins as recited in claims 3 and 23, and do not recite the limitations of claims 5, 15-18, and 20-22 of this application.

Regarding claims 3 and 23 of this application, Mizumura teaches producing recombinant *L. polyphemus* Factor C (also referred to by Mizumura as recombinant first protein), recombinant *L. polyphemus* Factor B (also referred to by Mizumura as recombinant second protein), and recombinant *L. polyphemus* Pro-Clotting Enzyme (also referred to by Mizumura as recombinant third protein) by expressing a polynucleotide in a host cell (p. 15, paragraph [0035] to p. 20, paragraph [0060] and p. 36, paragraph [0148]). Mizumura teaches the host cell is a HEK29 cell (p. 15, paragraph [0039]). Mizumura teaches an endotoxin detection agent comprising a recombinant first protein, a recombinant second protein, a recombinant third protein, and a detection substrate for the recombinant third protein (pp. 7-8, paragraph [1-3]; p. 25, paragraphs [0089] and [0090]; p. 29, paragraph [0111] to p. 30, paragraph [0119]). Mizumura teaches the detection agent can be a mixture (p. 30, paragraph [0113]) and formulated as a liquid (p. 30, paragraph [0117]).

In view of the teachings of Mizumura, it would have been obvious to one of ordinary skill in the art for the *Limulus polyphemus* Factor C of the claims of the reference application to be combined with recombinant *Limulus polyphemus* Factor B and Pro-clotting enzyme expressed from a HEK 293 cell for an endotoxin assay agent according to Mizumura. 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 recombinant *Limulus polyphemus* Factor C, and Mizumura teaches recombinant *Limulus polyphemus* Factor C, Factor B and Pro-clotting enzyme and a mixture of these proteins in a liquid formulation as an endotoxin detection agent.

Regarding claim 5 of this application, Mizumura does not teach the recombinant *L. polyphemus* Factor C expressed from a HEK29 cell 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. However, given that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura has the same structure as Factor C protein of claim 3, it is presumed that the *Limulus polyphemus* Factor C polypeptide recombinantly expressed from a HEK 293 cell of Mizumura 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 claims 15-17 of this application, Mizumura teaches the expressed protein is in the culture supernatant and may be purified before use (p. 35, paragraph [0143]).

Regarding claim 18 of this application, Mizumura's method for producing the recombinant first protein (p. 15, paragraph [0036] to p. 17, paragraph [0046]) does not include attaching a peptide to the C-terminus. Thus, one of ordinary skill in the art would recognize that the recombinant first protein "has no peptide added to the C-terminus."

Regarding claims 20-22 of this application, Mizumura teaches examples of substances that are cleaved by the activity of the Pro-Clotting enzyme include synthetic substrates represented by the general formula X-Y-Z with X being a protecting group covalently bonded to Y, Y being a peptide residue, and Z being a signal substance bonded to Y by an amide bond (p. 25, paragraph [0091]). Mizumura teaches Y includes Leu-Gly-ArY (LGR) and Ile-Glu-Gly-Arg (IEGR) (p. 26, paragraph [0093]) and Z includes

para-nitroaniline, 7-methoxycoumarin-4-acetic acid, 2,4-dinitroaniline, and Dansyl dyes (paragraph [0094]).

Therefore, claims 2, 3, 5, 15-18, and 20-23 of this application are unpatentable over claims 1-13 of the reference application in view of Mizumura. 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 2-5, 15-18, and 20-23 are pending.

Claim 4 is withdrawn from consideration.

Claims 2, 3, 5, 15-18, and 20-23 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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