

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Docket No: Q220395
Hikaru MIZUMURA et al.	Confirmation No.: 1531
Appln. No.: 14/650,767	Group Art Unit: 1656
Filed: June 9, 2015	Examiner: Steadman, David
For: NOVEL RECOMBINANT FACTOR C AND METHOD FOR PRODUCING THE SAME, AND METHOD FOR MEASURING ENDOTOXIN	

AMENDMENT UNDER 37 C.F.R. § 1.111

MAIL STOP AMENDMENT

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

Sir:

In response to the Office Action dated September 16, 2016, please amend the
above-identified Application as follows on the accompanying pages.

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AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Page 78, paragraph [0115] is amended as follows:

[0115]

The Factor C expression plasmid was incorporated into cultured HEK293 cells through transfection using polyethylenimine (product of Sigma) (Hashiguti *et al.*, Expression of Recombinant Protein Using Cultured Human Cells -Standard Protocol by 293-type cells-, PSSJ Archives, 1, e017 (2008), www.pssj.jp/archives/Protocol/Expression/293_01/293_01_01.html~~http://www.pssj.jp/archives/Protocol/Expression/293_01/293_01_01.html~~). HEK293 cells were statically cultured in a DMEM medium (containing 10% fetal bovine serum and 2mM L-glutamine) (product of Sigma) at 37°C under feeding of 5% CO₂ without replacement of the medium. Four days after transfection, the culture broth was recovered, and was centrifuged (3,300×g, 30 minutes, 4°C), to thereby recover a culture supernatant. The supernatant was filtered through a 0.22-μm filter, and the thus-recovered fraction (i.e. filtrate) was employed as recombinant Factor C (HEK).

AMENDMENTS TO THE CLAIMS

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

LISTING OF CLAIMS:

1-42. (Canceled).

43. (Currently Amended): A horseshoe crab Factor C having activity of Factor C₂ which contains (α-2,3)-linked terminal sialic acid in a greater amount, as compared with a native horseshoe crab Factor C and/or a horseshoe crab Factor C expressed using Sf9 as a host cell, wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*.

44-46. (Cancelled).

47. (Previously Presented): The Factor C according to claim 43, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

48. (Currently Amended): The Factor C according to claim 43, which has a molecular weight of 115 kDa to 140 kDa ~~or a molecular weight of 127 kDa ± 5 kDa~~, as measured through SDS-PAGE under a non-reducing condition.

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49. (Previously Presented): The Factor C according to claim 43, which is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

50. (Previously Presented): The Factor C according to claim 43, which is water-soluble.

51. (Withdrawn): A method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C in a mammalian cell as a host cell.

52. (Withdrawn): The method according to claim 51, wherein the mammalian cell is selected from the group consisting of:

- (A) a mammalian cell other than COS-1;
- (B) a cell of a mammal selected from the group consisting of a primate and a rodent, other than monkey;

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- (C) a Chinese hamster cell or a human cell;
- (D) CHO or HEK; and
- (E) CHO DG44 or HEK293.

53. (Withdrawn): The method according to claim 51, wherein the Factor C is a protein shown in the following (A), (B), (C), or (D):

- (A) a protein comprising the amino acid sequence shown in SEQ ID NO: 2;
- (B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;
- (C) a protein comprising the amino acid sequence shown in SEQ ID NO: 4;
- (D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

54. (Currently Amended): A horseshoe crab Factor C having activity of Factor C producible by ~~the method according to claim 51~~ a method for producing a horseshoe crab Factor C, the method comprising expressing the horseshoe crab Factor C using a mammalian cell as a host cell,

wherein the horseshoe crab is selected from the group consisting of *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas*,

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wherein the mammalian cell is selected from the group consisting of a Chinese hamster cell and a human cell.

55-56. (Cancelled).

57. (Currently Amended): An endotoxin assay kit comprising ~~the~~an endotoxin assay agent containing the Factor C according to claim ~~56~~43 and one or more elements.

58. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 56 and the test specimen, and a step of measuring the progress of a cascade reaction.

59. (Withdrawn): The method according to claim 58, wherein the test specimen contains at least one of:

- (A) an ion;
- (B) a cation;
- (C) a metal ion;
- (D) an alkali metal ion or an alkaline earth metal ion; and
- (E) one or more kinds of ions selected from the group consisting of sodium ion, potassium ion, calcium ion, and magnesium ion.

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60. (Withdrawn): The method according to claim 59, wherein the contained amount of the ion in the test specimen is such an amount that the concentration of the cation derived from the test specimen becomes 1 mM or higher, as a final concentration in the reaction system after mixing the test specimen with the assay agent.

61. (Withdrawn): The method according to claim 58, wherein the test specimen is an injection.

62. (Cancelled).

63. (Currently Amended): An endotoxin assay kit comprising ~~the~~an endotoxin assay agent containing the Factor C according to claim ~~62~~54 and one or more elements.

64. (Withdrawn): A method for measuring endotoxin in a test specimen, the method comprising a step of mixing the assay agent according to claim 62 and the test specimen, and a step of measuring the progress of a cascade reaction.

65. (New): The Factor C according to claim 48, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

66. (New): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

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67. (New): The Factor C according to claim 43, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

68. (New): The Factor C according to claim 43, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

69. (New): The Factor C according to claim 43, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

70. (New): The Factor C according to claim 54, which exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate.

71. (New): The Factor C according to claim 54, which exhibits a residual activity of 25% or higher in the presence of 52 mM sodium hydrogencarbonate.

72. (New): The Factor C according to claim 54, which exhibits a residual activity of 25% or higher in the presence of 214 mM sodium chloride.

73. (New): The Factor C according to claim 54, which exhibits a residual activity of 15% or higher in the presence of 16 mM magnesium sulfate.

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74. (New): The Factor C according to claim 54, which exhibits a residual activity of 35% or higher in the presence of 2.5 mM calcium chloride.

75. (New): The Factor C according to claim 54, which has a molecular weight of 115 kDa to 140 kDa as measured through SDS-PAGE under a non-reducing condition.

76. (New): The Factor C according to claim 75, which has a molecular weight of 127 kDa $\pm$ 5 kDa.

77. (New): The Factor C according to claim 54, which is a protein shown in the following (A), (B), (C), or (D):

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

(B) a protein comprising the amino acid sequence shown in SEQ ID NO:2 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C;

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

(D) a protein comprising the amino acid sequence shown in SEQ ID NO:4 but which includes substitution, deletion, insertion, or addition of one or several amino acid residues, and having activity of Factor C.

78. (New): The Factor C according to claim 54, which is water soluble.

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REMARKS

The specification has been amended to remove a hyperlink. Claims 43, 47-54, 57-61, 63-64 and 65-78 are all the claims pending in the application. Claims 43, 48, 54, 57 and 63 are amended; claims 1-42, 44-46, 55-56 and 62 are cancelled; claims 51-53, 58-61 and 64 are withdrawn; and claims 65-78 are newly added. Support for the amendments to claim 43 can be found, *inter alia*, in original claim 46 and at paragraph [0020] of the specification as filed. Claim 48 is amended to remove a preferred embodiment. Support for the amendments to claim 54 can be found, *inter alia*, in original claim 51 and at paragraphs [0020], [0016] and [0038] of the specification as filed. Support for the amendments to claim 57 can be found, *inter alia*, at paragraph [0077] of the specification as filed. Support for the amendments to claim 63 can be found, *inter alia*, in original claim 53 and at paragraph [0077] of the specification as filed. Support for new claims 65-78 can be found, *inter alia*, at claim 48; [1.2.3] in [0017]; [1.2.5] in [0017]; [1.2.7] in [0017]; [1.2.9] in [0017]; [3.3.1] in [0017]; [3.3.3] in [0017]; [3.3.5] in [0017]; [3.3.7] in [0017]; [3.3.9] in [0017]; [3.5.1] in [0017]; [3.5.5] in [0017]; [2.2.1] in [0017]; and [3.6] in [0017], respectively. No new matter is added. Entry and consideration of this Amendment are respectfully requested.

I. Specification

In paragraph 6, on page 3 of the Office Action, the disclosure is objected to because it contains an embedded hyperlink.

The hyperlink has been deleted.

II. Rejections under 35 U.S.C. §112

A. In paragraph 7, on page 4 of the Office Action, claims 43-50, 54-57, 62, and 63 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

According to the Examiner, the specification does not have a sufficient written description of all possible horseshoe crab factor C proteins that fall within the claims. In particular, the Examiner asserts that the claims do not require the factor C proteins to have any particular structure, and to the extent that specific factor C proteins are disclosed in the specification, there is insufficient information to define a common structure. To the extent other characteristics of the claimed factor C are specified (terminal sialic acid, residual activity in the presence of sodium citrate, molecular weight, amino acid sequence, solubility, and expression in a mammalian cell), the Examiner asserts that the characteristics are not sufficient to predict that a protein falling within the claim will have factor C activity.

Without agreeing with or acquiescing to the merits of the rejection, the rejection is overcome by amending independent claims 43 and 54 to recite that the horseshoe crab is *Tachypleus tridentatus*, *Limulus polyphemus*, *Carcinoscorpius rotundicauda*, or *Tachypleus gigas*. Furthermore, claim 43 is amended to recite that the Factor C has (α -2,3)-linked terminal sialic acid, and claim 54 is amended to recite that the mammalian cell is a Chinese hamster cell (superordinate concept of CHO) or a human cell (superordinate concept of HEK).

Accordingly, the Examiner is requested, respectfully, to reconsider and remove this rejection.

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B. In paragraph 8, on page 9 of the Office Action, claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §112, first paragraph, as lacking enablement in the specification. While the Examiner admits that the specification in enabling for factor C isolated from *Tachypleus tridentatus* or *Carcinoscorpius rotundicauda* hemocyte lysate and *Tachypleus tridentatus* factor C expressed in Sf9 insect cells, CHO cells, or HEK cells, the Examiner asserts that the specification does not reasonably provide enablement for all horseshoe crab factor C polypeptides as encompassed by the claims.

Without agreeing with or acquiescing to the merits of this rejection, Applicant submits that the amendments to claims 43 and 54, as discussed above, overcome this rejection.

Reconsideration is requested, respectfully.

III. Rejections under 35 U.S.C. §102/103(a)

A. In paragraph 9, on page 14 of the Office Action, claims 43, 44, 49, 50, 56 and 57 are rejected under 35 U.S.C. §102(b) as being anticipated by Mizumura et al. (WO 2012/118226; hereafter "Mizumura").

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Mizumura discloses Factor C derived from *Tachypleus tridentatus* "expressed using insect cells." In paragraph [0049], Mizumura teaches that the insect cells are preferably Sf9. Mizumura does not disclose any Factor C expressed using a mammalian cell, as recited in amended claim 54.

Paragraph [0154] of the present application states as follows: "The above analysis indicates that the N-type sugar chain of recombinant Factor C expressed in mammalian cells

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(CHO and HEK) contains (α -2,3)-linked terminal sialic acid, and that the N-type sugar chain of recombinant Factor C expressed in insect cells (Sf9) contains no (α -2,3)-linked terminal sialic acid."

Therefore, the Factor C in independent claim 43 is different from the Factor C disclosed in Mizumura, and is novel there over.

B. In paragraph 10, on page 16 of the Office Action, claims 54, 62, and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Koshiba et al. (J. Biol. Chem., 282:3962-3967 (2007); hereafter "Koshiba").

Regarding claim 54, the Examiner asserts that Koshiba teaches recombinant production of a *Tachypleus tridentatus* factor C polypeptide light chain and heavy chain using HEK293 cells.

Regarding claims 62 (now cancelled) and 63, the Examiner asserts that Koshiba teaches forming a complex of the factor C polypeptide heavy chain and LPS (page 3964, Figure 2; page 3966, paragraph bridging columns 1 and 2), which the Examiner considers to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Koshiba discloses recombinant production of a *Tachypleus tridentatus* Factor C polypeptide light chain and heavy chain using HEK293 cells (page 3963, paragraph bridging columns 1 and 2).

Paragraph [0005] of the present specification describes that "When endotoxin comes into contact with the lysate, Factor C, which is a serine protease zymogen present in the lysate, is

activated to thereby form activated Factor C." Accordingly, Factor C having activity of Factor C inevitably has both of endotoxin-binding domain and protease domain.

Koshiba describes several Factor C variants, all of which are a "truncated form of Factor C." (page 3963, *Cloning and Mutagenesis*). Koshiba discloses that protease domain of *Tachypleus tridentatus* Factor C consists of the amino acid residues from 738 to 994, which exists in its C-terminal (page 3963, *Cloning and Mutagenesis*). Koshiba discloses that endotoxin-binding domain of *Tachypleus tridentatus* Factor C exists in its N-terminal (page 3967, *Implications for Innate Immunity*). None of Factor C disclosed in Koshiba has both of an endotoxin-binding domain and a protease domain. Accordingly, Factor C disclosed in Koshiba does not have "activity of Factor C" as claimed.¹

Therefore, the Factor C in independent claim 54 is different from the Factor C disclosed in Koshiba, and is novel there over.

C. In paragraph 11, on page 17 of the Office Action, claims 43-46, 48-50, 56, and 57 are rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Nakamura et al. (J. Biochem., 99:847-857 (1986); hereafter "Nakamura") as evidenced by Mizumura (*supra*).

¹ Paragraph [0028] of the specification states that "the activity of Factor C refers to such an activity that the Factor C is activated in the presence of endotoxin to convert Factor B into activated Factor B." In other words, the activity of Factor C of the present invention is the "protease activity." Note that the "activity of Factor C" in the present invention is not equal to "the ability to bind LPS (Lipopolysaccharide, also known as endotoxin)" as defined by Koshiba (infra).

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According to the Examiner, Nakamura teaches an activated factor C polypeptide purified from the hemocyte lysate of *Tachypleus tridentatus*. With regard to claim 44 (now cancelled), the Examiner states that claim 44 is a product by process claim and, because the host cell is not specified, claim 44 does not further limit the factor C of claim 43. Regarding the remaining dependent claims, the Examiner asserts that because the factor C is purified from *Tachypleus tridentatus*, it has all of the characteristics of the proteins described in the present specification.

Mizumura is cited to allegedly show that the amino acid sequence of *Tachypleus tridentatus* factor C is identical to SEQ ID NO: 2.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Nakamura discloses purified "natural" Factor C of *Tachypleus tridentatus*, and does not disclose a recombinantly expressed Factor C of *Tachypleus tridentatus* using a mammalian cell as a host cell.

Paragraph [0153] of the present specification discloses that "MAM ((α -2,3)-linked terminal sialic acid-specific lectin) was strongly bonded to CHO and less strongly bonded to HEK. TAL and fetuin have almost the same weak reactivity, but did not react with Sf9." In paragraph [0018] in the specification, it is described that TAL is a native Factor C derived from *Tachypleus tridentatus*. Accordingly, a horseshoe crab Factor C expressed using a mammalian cell as a host cell contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with TAL, which is the same protein as the Factor C disclosed in Nakamura.

Therefore, the Factor C in independent claim 43 is different from the Factor C disclosed in Nakamura, and thereby novel and unobvious thereover.

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D. In paragraph 12, on page 20 of the Office Action claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Ding et al. (U.S. Patent 5,716,834; hereafter "Ding") as evidenced by Mizumura (*supra*).

Regarding claims 43, 44 (now cancelled), 54, and 55 (now cancelled), the Examiner asserts that Ding teaches a mammalian host cell transformed with a recombinant vector for expressing a factor C. The Examiner further asserts that Ding teaches that the host cell can be CHO cells and the factor C can be derived from *Tachypleus tridentatus*.

Regarding claim 45 (now cancelled), the Examiner admits that Ding does not teach the activated factor C polypeptide recombinantly expressed and recovered from CHO cells has (α -2,3)-linked terminal sialic acid. However, according to the Examiner, this characteristic would be inherent in any factor C from *T. tridentatus* expressed in CHO cells. The Examiner takes a similar position with regard to (now cancelled) claim 46 (the factor C has (α -2,3)-linked terminal sialic acid in a greater amount as compared with a horseshoe crab factor C expressed in Sf9 as a host cell), claim 47 (the factor C exhibits a residual activity of 10% or higher in the presence of 21 mM sodium citrate), and claim 48 (the factor C has a molecular weight of 128 kDa by SDS-PAGE under non-reducing conditions).

Regarding claim 49, The Examiner admits that Ding does not teach the amino acid sequence of *T. tridentatus* factor C has the amino acid sequence of SEQ ID NO: 2. However, Mizumura is cited as evidence to show that the amino acid sequence of *T. tridentatus* factor C is allegedly identical to SEQ ID NO: 2.

Regarding claim 50, the Examiner asserts that Ding teaches that the factor C can be secreted into the culture medium and thus the factor C is considered to be water-soluble.

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Regarding claims 56 (cancelled), 57, 62 (cancelled), and 63, the Examiner asserts that Ding teaches the recombinant factor C may be employed in a chromogenic assay, wherein upon activation by endotoxin, the factor C converts the substrate to a colored product, thereby detecting and quantifying endotoxin, which the Examiner considers to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Ding discloses a mammalian cell transformed with a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C. However, Ding neither discloses nor suggests any Factor C "having activity of Factor C," as claimed.

For example, Ding prepared a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C in working example 8. However, Ding does not practically prepare a recombinant cell having ability for expressing Factor C having activity of Factor C. Moreover, Ding does not show any scientific evidence for presuming that any Factor C in Ding has activity of Factor C, rather than endotoxin-binding activity. For example, in working example 12, endotoxin binding activity was evaluated. However, activity of Factor C was not evaluated.

Therefore, the Factor C in independent claims 43 and 54 is different from the Factor C disclosed in Ding, and claims 43 and 54 are novel there over.

E. In paragraph 13 on page 24 of the Office Action, claims 43-50, 54-57, 62 and 63 are rejected under 35 U.S.C. §102(b) as being anticipated by Ding et al. (U.S. Patent 5,858,706; hereafter "Ding2") as evidenced by Mizumura (*supra*).

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Regarding claims 43, 44 (cancelled), 54, and 55 (cancelled), the Examiner asserts that Ding2 teaches a mammalian host cell transformed with a recombinant vector for expressing a factor C. The Examiner further asserts that Ding teaches that the host cell can be CHO cells and the factor C can be derived from *Tachypleus tridentatus*.

Regarding claims 45 - 48 (45 and 46 are cancelled), the Examiner admits that Ding2 does not teach the additional recitations. However, according to the Examiner, these characteristics would be inherent in any factor C from *T. tridentatus* expressed in CHO cells.

Regarding claim 49, the Examiner relies on Mizumura as allegedly teaching the amino acid sequence of *T. tridentatus*.

Regarding claim 50, the Examiner asserts that Ding2 teaches solubilization of the factor C and the Examiner considers this to mean that the factor C is water-soluble.

Regarding claims 56 (cancelled), 57, 62 (cancelled) and 63, the Examiner asserts that Ding2 teaches the recombinant factor C for use in an assay for endotoxin detection and teaches elements for conducting such an assay, which is considered to be an endotoxin assay agent and an endotoxin assay kit.

For the following reasons, the rejection is overcome and/or traversed, respectfully.

Ding2 discloses a mammalian cell transformed with a recombinant vector comprising DNA encoding an amino acid sequence of *Carcinoscorpius rotundicauda* Factor C, as does Ding (supra). In Ding2, a recombinant Factor C "having endotoxin-binding activity" is disclosed. However, Ding 2 never discloses nor suggests a recombinant Factor C "having activity of Factor C," as claimed.

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In Ding2, cDNA of *Carcinoscorpius rotundicauda* Factor C (CrFC21) was cloned into mammalian expression vector. However, production of a recombinant Factor C was conducted using a yeast cell, and not conducted using a mammalian cell.

The subject of Ding 2 is to offer a Factor C "having endotoxin-binding activity." Thus, the subject of Ding 2 is not to offer a Factor C "having activity of Factor C."

That is, in Ding2, other substantial applications than endotoxin-binding are not disclosed as an application of recombinant Factor C. Moreover, Ding2 argues an advantage of his invention by comparison with a clotting reaction using LAL, which is equal to the cascade reaction in the present application. Furthermore, in paragraph 3 of the Detailed Description of the Invention in Ding2, it is described that the "rCrFC can be purified by affinity methods using gram-negative LPS as the affinity reagent."

In paragraph [0005] of the present specification, it is explained that "when endotoxin comes into contact with the lysate, Factor C, which is a serine protease zymogen present in the lysate, is activated to thereby form activated Factor C. The thus-formed activated Factor C activates Factor B present in the lysate, to thereby form activated Factor B." That is, Factor C is a protease zymogen which matures into activated Factor C when contact with endotoxin. Accordingly, if rCrFC in Ding2 was Factor C having activity of Factor C, rCrFC purified by affinity methods using gram-negative LPS as the affinity reagent should be activated Factor C, rather than Factor C (protease zymogen).

Activated Factor C already expresses activity for transforming Factor B into activated Factor B without depending on the presence or absence of endotoxin. Accordingly, activated Factor C cannot be used for endotoxin detection based on protease activity. That is, if rCrFC in

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Ding2 was Factor C having activity of Factor C, rCrFC could not be used for endotoxin detection based on protease activity. Therefore, at the time of the invention, one of ordinary skill in the art would readily recognize that a recombinant Factor C in Ding2 has endotoxin-binding activity, but does not have activity of Factor C.

In addition, a subsequent application of Ding (US 6,645,724) describes in paragraph 3 of the Related Art that "also, it was found that LPS could not activate the enzymatic activity of yeast rFC, thus, a direct enzyme-based LPS detection is not possible using rFC produced in yeast." That is, the inventor (Ding) himself admits that a recombinant Factor C disclosed in Ding2 does not have activity of Factor C, and thereby cannot be used for endotoxin detection based on enzymatic activity.

Therefore, in view of the description in Ding2 and also based on scientific facts, Factor C having activity of Factor C is not disclosed in Ding2. Moreover, specific means for producing Factor C having activity of Factor C is neither disclosed nor suggested in Ding2.

In conclusion, the Factor C in independent claims 43 and 54 is different from the Factor C disclosed in Ding2, and claims 43 and 54 are novel there over.

IV. Rejection under 35 U.S.C. §101

In paragraph 14 at page 27 of the Office Action, claims 43-46, 48-50, 56, and 57 are rejected under 35 U.S.C. §101 because the claimed invention allegedly is directed to a judicial exception without significantly more.

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According to the Examiner, given a broadest reasonable interpretation, the horseshoe crab factor C of claims 43-46 and 48-50 encompasses a naturally occurring horseshoe crab factor C.

Applicant submits that the amended claims 45 and 54 do not encompass naturally occurring Factor C. Claim 43 recites that the Factor C contains (α -2,3)-linked terminal sialic acid in a greater amount as compared with a native horseshoe crab Factor C and claim 54 recites a Factor C expressed in a mammalian host cell.

Accordingly, the Examiner is requested, respectfully, to reconsider and remove this rejection.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

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

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