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November 22, 2024

Opposition against European patent EP 3 530 670 (Application No. 17 862 926.7)

Title: Method for Producing Endotoxin Detecting Agent
Comprising Recombinant Limulus Factor C and Use
Thereof
Proprietor: Seikagaku Corporation
Opponent: Sandra Pohlman
Our Ref.: SP27447OP

NOTICE OF OPPOSITION

On behalf of **Sandra Pohlman**
df-mp Dörries Frank-Molnia & Pohlman
Patentanwälte Rechtsanwälte PartG mbB
Fünf Höfe, Theatinerstraße 16
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we herewith file **Opposition** pursuant to Article 99(1) EPC against:

European Patent No. EP 3 530 670 B1
(European Appl. No. 17 862 926.7)

Publication and mention of the grant: 28.02.2024.

Title of the patent: Method for Producing Endotoxin Detecting Agent
Comprising Recombinant Limulus Factor C and Use
Thereof

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CRL Exhibit 1048
CRL v. Seikagaku
IPR2025-00440

Proprietor: Seikagaku Corporation
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Japan.

We request that the opposition fee in the amount of EUR 880.00 be debited from our deposit account No. 28001000 (see also the corresponding debit order on the form accompanying the online submission of the present Notice of Opposition).

I. Extent of the Opposition and Requests

1. The opposition is directed against both claims 1 and 2 of the European patent EP 3 530 670 B1 (hereinafter “the opposed patent”). It is requested that the opposed patent be revoked in its entirety pursuant to Article 101(2) EPC.
2. Oral proceedings are requested in accordance with Article 116(1) EPC in the event that the Opposition Division does not consider the above request justified on the basis of our written submissions.

II. Grounds in Support of the Opposition

3. The opposition is based on the following grounds:
 - (a) the subject matter of the opposed patent extends beyond the content of the application as filed (Article 100(c) in connection with Article 123(2) EPC);
 - (b) the subject matter of the opposed patent is not based on an inventive step (Article 100(a) in connection with Article 56 EPC); and
 - (c) the subject matter of the opposed patent is insufficiently disclosed (Article 100(b) EPC in connection with Article 83 EPC).

III. Cited Documents

4. The following documents are relied upon and submitted herewith:
 - D1:** EP 2 930 241 A1;
 - D2:** Technical Bulletin for “*pCI-neo Mammalian Expression Vector*” from Promega, 2009;
 - D3:** Proprietor’s submission in US Application No. 14/650,767 dated Feb. 22, 2018;
 - D3a:** Inventor’s declaration accompanying the Proprietor’s submission;
 - D3b:** Claims accompanying the Proprietor’s submission;
 - D3c:** US 10,144,923 B2;

- D4:** Ding and Ho, *"Endotoxin Detection- from Limulus Amebocyte Lysate to Recombinant Factor C"*, C. Subcell. Biochem., 2010:53, 187-208;
- D5:** US 6,645,724;
- D6:** WO 2003/002976;
- D7:** A copy of the ESO;
- D8:** Declaration of Professor Frohman;
- D8a:** CV Professor Frohman;
- D9:** Sandle, T. *"FDA guidance on pyrogens and endotoxin testing"*, 2013, GMP Review 12(2): 7-9;
- D10:** Wimbish, L. *"Advantages of recombinant Factor C based endotoxin testing"*, 2015, European Pharmaceutical Manufacturer;
- D11:** Yeku and Frohman, *"Rapid Amplification of cDNA Ends (RACE)"*, Methods Mol. Biol., Chapter 8, 107-122, 2010;
- D12:** Frohman, M. et al., *"Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer"*, 1988, Proc. Natl. Acad. Sci. 85:8998-9002;
- D13:** Dorit and Ohara, *"cDNA Amplification Using One-Sided (Anchored) PCR"*, Curr. Protoc. Mol. Biol., 15.6.1-15.6.10, 2000;
- D14:** Frohman, M., *"On Beyond Classic RACE (Rapid Amplification of cDNA Ends)"*, PCR Methods Appl., 4(1):S40-S58, 1994;
- D15:** Partial screenshot of search results for "cDNA RACE kits" before Oct. 18, 2016;
- D16:** Manual accompanying the 5' RACE kit from Invitrogen, 2004;
- D17:** Manual accompanying the 3' RACE kit from Invitrogen, 2004;
- D18:** NCBI entry regarding the genome assembly of *Limulus polyphemus*-2.1.2, accessed from: https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000517525.1/
- D19:** Nossa, C. W. et al., *"Joint assembly and genetic mapping of the Atlantic horseshoe crab genome reveals ancient whole genome duplication"*, 2014, Gigascience 3(9): 1-21;
- D20:** NCBI entry for the predicted sequence of *Limulus polyphemus* clotting factor C-like mRNA (accession number XM_013937646.1);
- D21:** NCBI Release Information for the *Limulus polyphemus* Annotation Release, corresponding to **D20**;
- D22:** Tokunaga et al., *"Further Studies on Lipopolysaccharide-Sensitive Serine Protease Zymogen (Factor C): Its Isolation from Limulus polyphemus Hemocytes and*

Identification as an Intracellular Zymogen Activated by α -Chymotrypsin, Not by Trypsin,
J. Biochem. 109, 1991;

- D23:** US 5,176,834, Ding et al., Date of Patent: Feb 10, 1998
- D24:** Ding, JL and Navas, AA, *"Molecular cloning and sequence analysis of Factor C cDNA from the Singapore horseshoe crab, *Corcinoscorpius rotundicauda*"*, 1995, Mol. Mar. Biol. Biotechnol., 4(1): 90-103;
- D25:** Wikipedia page related to *"Tandem mass spectrometry"* captured by the WayBack Machine on August 11, 2016;
- D26:** Proprietor's response to the communication pursuant to Rules 70(2) and 70a(2) dated Oct. 16, 2020;
- D26a:** Annex to the Communication in response to the Proprietor's reply of Oct. 16, 2020, dated Oct 21, 2022;
- D27:** Miura, Y. et al., *"Preparation and Properties of Monoclonal Antibodies against Lipopolysaccharide-Sensitive Serine Protease Zymogen, Factor C, from Horseshoe Crab (*Tachypleus tridentatus*) Hemocytes"*, 1992, J. Biochem. 112: 476-481;
- D28:** WO 2012/118226;
- D29:** A copy of the Annex to the Communication pursuant to Rule 71(3) EPC;
- D30:** Chong, S., *"Overview of Cell-Free Protein Synthesis: Historic Landmarks, Commercial Systems, and Expanding Applications"*, 2014, Curr. Protoc. Mol. Biol. 180(16.30): 1-16;
- D31:** Dwarakanath, RS et al., *"Recombinant COS-1 cells express *Carcinoscorpius rotundicauda* Factor C"*, 1997, Biotechnol. Lett. 19: 357-362;
- D32:** Alwis & Milton, *"Recombinant factor C assay for measuring endotoxin in house dust: Comparison with LAL, and (1 $\rightarrow$ 3)- β -D-glucans"*, Am. J. Ind. Med., 2006 Apr;49(4):296-30; and

Annex I: DNA Sequence Alignments

Annex II: Protein Sequence Alignment

References cited in the Declaration of Professor Frohman (D8):

Appendix A

Appendix B

Appendix C

Appendix D

5. Documents **D1**, **D4**, and **D22** were cited as **D3**, **D4**, and **D2** during prosecution of the opposed patent, respectively. None of the other documents were previously considered.

IV. The Opposed Patent and the Claimed Subject Matter

6. The European Patent EP 3 530 670 B1 is based on PCT application PCT/JP2017/037629 filed on October 18, 2017 and published as WO 2018/074498 on April 26, 2018 (hereinafter “the application as filed”). The opposed patent claims priority from JP 20160204729 filed on October 18, 2016. The date of publication and mention of the grant of the opposed patent was February 28, 2024.
7. The opposed patent has been granted with 2 claims, each of which is formulated as an independent method claim.
8. **Independent claim 1** is reproduced below, with points (i)-(iii.a) itemizing features of the claim:
 - (i) **A method for producing an endotoxin detecting agent**, comprising
 - (ii) a step of **artificially expressing a protein**
 - (ii.a) **using a cDNA**, and
 - (iii) **collecting the expressed recombinant protein as a solution fraction** containing the expressed recombinant protein,
 - (ii.b) wherein **the cDNA encodes any one of the following**:
 - (A) a recombinant protein expressed as containing the amino acid sequence represented by SEQ ID NO: 2 or 4;
 - (B) a recombinant protein expressed as containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and has factor C activity; and
 - (C) a recombinant protein that is encoded by a DNA containing a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and has factor C activity, and
 - (iii.a) wherein **the solution fraction** containing the expressed recombinant protein **is a culture solution, or a culture supernatant**.
9. **Independent claim 2** is reproduced below, with points (i)-(iii) itemizing features of the claim:
 - (i) **A method for detecting an endotoxin** in a sample requiring detection of endotoxin
 - (ii) using **the endotoxin detecting agent produced according to** the method according to **claim 1**, comprising
 - (iii) a step of **bringing the recombinant protein into contact with the sample** requiring detection of endotoxin.

V. Added Matter (Article 100(c) EPC in connection with Article 123(2) EPC)

10. As will be explained below, claim 1 of the opposed patent extends beyond the content of the original application as filed, contrary to Article 123(2) EPC.
11. We submit that the claimed combination of features cannot be clearly and unambiguously derived from the application as filed. On the contrary, to arrive at the subject matter of claim 1, a selection from at least two lists is required. In particular, (i) a three-fold selection must be made to arrive at the sequences in items (A)-(C); and (ii) a two-fold selection must be made to arrive at the “solution fraction containing the expressed recombinant protein” which is “a culture solution or a culture supernatant”.
12. Regarding the first selection, the application as filed describes a method for producing endotoxin detecting agent beginning on page 59, par. [0139]. Here, the present invention is said to provide “a method for producing an endotoxin detecting agent including a step of artificially expressing a protein using the first cDNA” (par. [0139], emphasis added).
13. According to the application as filed, “the first cDNA” is “a cDNA which is any one of the following:
 (A) a cDNA that contains the base sequence represented by SEQ ID NO: 1 or 3;
 (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and encodes a protein having factor C activity;
 (C) a cDNA that hybridizes to a DNA composed of a complementary sequence to the base sequence represented by SEQ ID NO: 1 or 3 under stringent conditions, and encodes a protein having factor C activity;
 (D) a cDNA that encodes a protein containing the amino acid sequence represented by SEQ ID NO: 2 or 4; and
 (E) a cDNA that encodes a protein containing an amino acid sequence having a 95% or more identity with the amino acid sequence represented by SEQ ID NO: 2 or 4, and having factor C activity” (page 53, par. [0121]).
14. To arrive at “the first cDNA” as recited in items (A)-(C) of granted claim 1, a selection of (D), (E) and (B) must be made from list of options recited above, respectively. However, there is no indication that the three options recited in the claim would be preferred.
15. According to the application as filed, “the most preferred is the above-mentioned cDNA (A)” (page 53, par. [0123]). Thus, the only option singled out by the disclosure is the full cDNA sequence represented by SEQ ID NO: 1 or 3, which cannot serve as a pointer for the three-fold selection of items (D), (E) and (B) made from the list on page 53.

16. As far as the originally filed claims are concerned, we note that these were directed to “a recombinant protein”. To the extent that the “recombinant protein” according to claim 1 as originally filed may be equated with the presently claimed *cDNA* (which we deny), claim 1 as granted would still require a selection of three options from this original claim, for which the necessary pointers are lacking.
17. Accordingly, items (A)-(C) are the first selection(s) from a list.
18. Regarding the second selection, the application as filed states that “*The expressed recombinant first protein is collected as a solution fraction*” which “*can be, for example, a culture solution, a culture supernatant, a cell homogenate extract, a mixture thereof, or the like*” (page 27, par. [0043]-[0044]).
19. To arrive at the “solution fraction” as recited in claim 1, the “*culture solution or culture supernatant*” must be selected from the list of options recited in par. [0044]. However, all of the options on page 27 for the solution fraction are presented on equal footing. Accordingly, there is no pointer to select the options which are recited in granted claim 1. Thus, the “*culture solution or culture supernatant*” represent further selections from a list.
20. In view of the above, it is apparent that the application as filed does not clearly and unambiguously disclose a method for producing an endotoxin detecting agent comprising a step of artificially expressing a protein using a *cDNA* which can encode any of the options recited in (A)-(C) and collecting the expressed recombinant protein as a solution fraction which is a culture solution or a culture supernatant.
21. In view of the above, it is apparent that the claimed combination of features in claim 1 cannot be derived in a direct and unambiguous manner from the application as filed.
22. For completion, we note that the method of claim 2 requires the use of the endotoxin detecting agent produced according to claim 1. Therefore, the arguments outlined above equally apply to claim 2.
23. We thus submit the claimed subject matter does not meet the requirements of Article 123(2) EPC already for these reasons.
24. We additionally object to the wording of feature (C) in granted claim 1 under Art. 123(2) EPC: According to the original description the *cDNA* used in the claimed method is required to be identical (see par. [0128] on page 55, “the Same”) with the “first *cDNA*”, compare page 59, par. [0139]:

*“the present invention also provides a method for producing an endotoxin detecting agent including a step of artificially expressing a protein **using the first cDNA**”*

25. The “first cDNA” is defined in par. [0121] on page 59 as follows, with feature (C) of granted claim corresponding most to feature (B) of par. [0121]:

“The first cDNA is a cDNA which is any of the following:

... (B) a cDNA that contains a base sequence having a 95% or more identity with the base sequence represented by SEQ ID NO: 1 or 3, and encodes a protein having factor C activity”

26. In contrast thereto, granted claim 1 (C) no longer requires that a cDNA identical to a first cDNA be used. In conjunction with feature (C), granted claim 1 refers in more general terms to a cDNA encoding a recombinant protein encoded by a DNA, and thereby, granted claim 1 omits the mandatory requirement that the cDNA used in the method **is the first cDNA** “which has a base sequence with 95% or more identity with the base sequence represented by SEQ ID NO:1 or 3, and encodes a protein having factor C activity”.
27. In other words, while the reference point in feature (B) in the original application is the cDNA, it is now the protein. Accordingly, the cDNA is no longer directly defined in relation to SEQ ID NO: 1 or 3, but merely in an indirect fashion. Due to the degeneracy of the genetic code, such a cDNA does not necessarily need to share 95% or more identity with the base sequence represented by SEQ ID NO:1 or 3. Such a broad definition of the cDNA is nowhere disclosed in the application, such that feature (C) in particular does not meet the requirements of Article 123(2) EPC.

VI. Inventive Step (Article 100(a) EPC in connection with Article 56 EPC)

VI.1 Overview

28. Claim 1 generally relates to a method for producing an endotoxin detecting agent by artificially expressing a protein using a cDNA encoding the Factor C derived from *Limulus polyphemus* and collecting the expressed protein as a culture solution or culture supernatant.
29. But, as further explained below, neither the use of Factor C derived from *Limulus polyphemus* as such, nor the claimed method steps for producing a recombinant Factor C are new. Accordingly, it would have been obvious to use the claimed method to recombinantly produce this protein for the following reasons.
30. In particular, each one of **D1**, **D5**, and **D6** discloses methods for recombinantly producing Factor C, comprising a step of artificially expressing the protein and collecting the expressed recombinant protein as a solution fraction which is a culture solution or culture supernatant; and directly points the skilled person to carry out this method with a Factor C derived from *Limulus polyphemus* species. Each of these documents could equally serve as closest prior art.

31. Regardless of which document is taken as the starting point, the skilled person merely had to determine the sequence of the *Limulus* Factor C protein, or cDNA sequence encoding the same, to carry out its recombinant production. However, and as noted by the Examiner during prosecution, this was a well-known, publicly available protein, the specific amino acid sequence (as defined in items (A)-(C)) “*being an inherent property of the protein*” (cf., item 1.2.1 of the ESO, submitted herewith as **D7**). Accordingly, it would have merely been a matter of routine analysis to obtain the sequences recited in feature (ii.b) of claim 1. Thus, to the extent that reciting the claimed sequences can even be considered novel (we note that the Examiner handling the prosecution did not seem to think so¹), it is certainly not inventive.
32. The fact that the sequence determination necessary to arrive at claim 1 requires nothing more than “routine analysis” has been confirmed in the expert declaration of Professor Frohman, submitted herewith as **D8**.
33. Professor Frohman is the father of RACE and an expert with **38** years of experience in molecular cloning. He is certainly qualified to provide an opinion as to what a person of ordinary skill in the art would have known and concluded as of the claimed priority date of the opposed patent and how such person would elucidate the cDNA sequence encoding a known protein, or its amino acid sequence. As Professor Frohman explains, the sequences recited in claim 1 could have been derived in a “*straightforward manner*” using techniques which were routinely used by the person skilled in the art, such as RACE or molecular cloning (see **D8**).
34. According to the opposed patent, the sequence of the *Limulus* Factor C had yet to be determined, allegedly “*because accurate determination of the full-length sequence structures and elucidation of the expression and functions of these proteins and genes could not be achieved by general techniques due to various differences in biomolecules, diversities, etc. because of differences in genera or species of horseshoe crabs*” (par. [0012]) (emphasis added).
35. However, this statement is very generic and entirely unsupported². Indeed, and as Professor Frohman has confirmed, the example in the opposed patent merely demonstrates the use of standard RACE techniques, which were available well before the priority date, to determine the claimed Factor C sequences (**D8**, section VII).
36. Accordingly (and as demonstrated by the opposed patent), the skilled person merely had to carry out the teaching of, e.g., **D1**, **D5**, or **D6** using standard techniques to arrive at the claimed subject matter. Therefore, to the extent that any technical effect may be attributed to the *Limulus* Factor

¹ See item 1.2.1 of the ESO (**D7**)

² In fact, Alwis & Milton (Am. J. Ind. Med., 2006 Apr; 49(4):296-30, submitted herewith as **D32**) discloses on page 299, right col., first par. that the factor C sequence of *Carcinoscorpius rotundicauda* was already determined by 2006 by the inventor named on **D6**, Lin Chen (although not published, perhaps for commercial reasons) and compared with the factor C sequence of *Limulus polyphemus*. **D32** indicates a precise homology of 89.3% and therefore suggests that the entire sequence was accurately determined. This further supports our position that the patent's claim that the sequence could not previously be determined is factually incorrect and that there were **no** unforeseen technical difficulties in determining the sequence.

C sequence (which we deny), it could at best be considered a “bonus” effect, which cannot indicate the presence of an inventive step.

*“The board in T 936/96 held that, once a realistic technical problem had been defined and once it had been established that a particular solution to such a problem would have been envisaged by a skilled person in the light of the relevant state of the art, that solution could not be said to involve an inventive step, and this assessment was not altered by the fact that the claimed invention inherently also solved further technical problems. In the case in point **the claimed surprising effect could not be regarded as an indication of the presence of an inventive step**” (page 300, section I.D.10.8 of the CLB, 2022; emphasis added).*

37. Thus, irrespective of which path is taken, one arrives at a complete lack of inventive character in the subject matter of claim 1.
38. Regarding claim 2, it is noted that the Factor C derived from *Limulus polyphemus* has been used as the first component in the LAL assay for decades (D4, abstract and page 198, 1st par.). This well-established assay was commonly known as *“the compendial test for the examination of bacterial endotoxin in pharmaceutical products, as well as intermediates, medical devices and in water”* (D9, Sandle, 2013, abstract) at the time of priority. It was further common general knowledge that recombinant versions of Factor C could be used as an alternative that is *“comparable to the natural equivalent”* in this assay (D10, Wimbish, 2015, page 2, 1st par. under the Figure caption). Accordingly, it would have been entirely obvious to use a *Limulus* Factor C which has been recombinantly produced according to the method of claim 1 for measuring endotoxin. Therefore, irrespective of how one derives the obviousness of the sequences recited in claim 1, a lack of inventive step for claim 2 immediately follows.

VI.2 Lack of inventive step in view of D1 (EP 2 930 241) in combination with (i) the common general knowledge (represented by e.g., D11, D13-D18, and D20) and optionally D22; or (ii) D23 (‘834), or D24 (Ding, 1995)

39. D1 relates to the provision of a recombinant horseshoe crab Factor C and is therefore aimed at the same purpose as the opposed patent (*cf.*, D1, par. [0013] and [0015]). D1 discloses all features of claim 1 and claim 2, except the sequences in items (A)-(C) of claim 1 and may thus be taken as closest prior art.

VI.2.1 Claim 1

40. D1 *“relates to a novel recombinant Factor C, to a method for producing the recombinant Factor C, and to a method for measuring endotoxin”* (par. [0001]). According to D1, it is common general knowledge that Factor C is used to detect endotoxin (par. [0003]-[0008]). Therefore, *“The Factor C of the present invention may be used in an endotoxin assay. That is, the present invention*

provides an endotoxin assay containing the Factor C..." (par. [0174]). The recombinant Factor C according to **D1** is thus an **endotoxin detecting agent** (see also **D1**, par. [0201] and claim 29). Accordingly, this disclosure provides a method for producing an endotoxin detecting agent, as required by feature (i) of claim 1.

41. The method of **D1** comprises *"expressing the horseshoe crab factor C in a mammalian cell as a host cell"* (claim 12 and par. [0168]). According to the disclosure, the method for producing the Factor C involves *"introducing the gene encoding Factor C into a mammalian cell"* by means of, e.g., the pCI-neo vector from Promega (*cf.*, par. [0170]). We note that the pCI-neo vector was indeed used in the examples to introduce the Factor C coding sequence into a CHO-DG44 cell (i.e., a mammalian cell) for expression (*cf.*, par. [0241]).
42. It is known that the pCI-neo vector provides for the introduction of a coding sequence as a "*cDNA insert*". In that regard, we refer to the technical information for the "pCI-neo Mammalian Expression Vector" from Promega, submitted herewith as **D2** (*cf.*, page 2, section 3.B). In view of this common general knowledge, the skilled person immediately understands that the example in **D1** involved *"artificially expressing"* the Factor C protein *"using a cDNA"*. Accordingly, features (ii) and (ii.a) of claim 1 are clearly and unambiguously derivable from **D1**.
43. Further, **D1** discloses that *"[t]he expressed Factor C can be recovered as a solution containing Factor C, and can be used as a component of the endotoxin assay agent of the present invention. The solution containing Factor C may be, for example, a culture broth, a culture supernatant, a disrupted cell extract, a mixture thereof, or the like."* (par. [0173]; emphasis added).
44. Most remarkably, in Example (8) of the opposed patent an experimental procedure *identical* with that of par. [241] of **D1** is being employed for obtaining a solution fraction containing the expressed recombinant factor C protein. Example (8) at par. [0187] explicitly refers to *"Example 1. (2) in the same literature [i.e., WO2014/092079 which is the WO publication underlying D1]"* (explanation added), and Example 1. (2) of WO2014/092079 is identical with Example 1. (2) of **D1**. Hence, all of the steps of the claimed method for obtaining recombinant factor C in a culture supernatant were disclosed in the prior art, at par. [241] of **D1**.
45. Accordingly, a step of *"collecting the expressed recombinant protein as a solution fraction...[which] is a culture solution, or a culture supernatant"* according to features (iii) and (iii.a) of claim 1 is disclosed in **D1**.
46. Therefore, **the only difference** between claim 1 and the disclosure of **D1** is the recitation of the sequences in items (A)-(C) (feature ii.b).

VI.2.1.1. The technical problem in view of D1

47. Although there does not appear to be any credible effect associated with the claimed *Limulus* Factor C sequence (see our discussion in section VI.5 below), the presence of any alleged effect would nevertheless be irrelevant for the assessment of inventive step in the present case.
48. In particular, the opposed patent merely carries out the direct teaching of **D1** regarding the *Limulus* Factor C. Indeed, the use of a Factor C derived from *Limulus polyphemus* is explicitly disclosed as one of the specific “Modes for Carrying Out the Invention” of **D1** (page 15, line 17 and par. [0150]-[0151]).
49. In line with this, **D1** teaches that Factor C can have an identity of 80% or more, preferably 90% or more with respect to SEQ ID NO: 2 (*Tachypleus tridentatus*) or SEQ ID NO: 4 (*Carcinoscorpius rotundicauda*) and have Factor C activity (cf., par. [0161]). Substitutions, deletions, insertions, or additions may be made, with conservative variations (such as substitutions) being preferred, i.e., the function of the protein is appropriately maintained (cf., par. [0160]). **D1** explicitly teaches that the aforementioned substitution, deletion, insertion, addition or the like of an amino acid residue(s) includes naturally occurring variations, such as those based on individual differences between horseshoe crabs and the strain and species of horseshoe crabs from which the gene is derived (cf., par. [0160]). The identities of the sequences SEQ ID NO: 2 and 4 of EP 241 compared to the claimed amino acid sequences of the opposed patent are shown in the table below:

	Claim 1 SEQ ID NO: 2	Claim 1 SEQ ID NO: 4
EP 241 SEQ ID NO: 2	91,95%	91,85%
EP 241 SEQ ID NO: 4	90,87%	90,78%

50. In particular, the Proprietor has submitted to the USPTO that the skilled person reading the description of **D1** would not only be able to produce a *Limulus* Factor C without undue burden, but that they would further expect the invention of **D1** to “also work when Factor C is the Factor C of *Limulus Polyphemus*” (**D3**, pages 13-14, bridging par.; and **D3a**, page 4, penultimate par.).
51. In that regard, we refer to US Application No. 14/650,767, which corresponds to **D1** and contains the same description as this disclosure. In the US case, the Proprietor sought to obtain a claim directed to a recombinant Factor C which is a protein having an identity of 80% or higher with respect to the *Tachypleus tridentatus* Factor C, or the *Carcinoscorpius rotundicauda* Factor C (therein SEQ ID NO 2 and 4, respectively) (see claim 77 corresponding to the remarks submitted in **D3**, submitted herewith as **D3b**). It is noted that this percent identity encompasses the Factor C derived from *Limulus polyphemus*, which was indeed included in the claims as granted (see **D3c**, claim 1).

52. Regarding the patentability of these claims, the Proprietor submitted the following in their response to the USPTO (**D3**; emphasis added):

*“Furthermore, with regard to the Examiner’s assertion that the specification discloses a single working example of a Factor C in SEQ ID NO. 2, Applicant notes that the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation (see MPEP 2164.02; In re Borkowski, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970)). In this regard, **Applicant respectfully notes that the specification provides ample guidance regarding the kind of amino acid modifications which would allow the function of Factor C to be maintained**, i.e., conservative substitutions. The specification also discloses several examples of conservative substitutions. See paragraph [0029] of the specification” (page 10, 2nd par.).*

Applicant submits that, in view of the guidance in the specification; the high level of skill in the art; and the existing knowledge in the art, a person skilled in the art would readily be able to generate and use the polypeptides that are encompassed by the claims” (page 11, 2nd par.).

***Applicant respectfully submits that the presently amended claims are adequately described** for the following reasons. Initially, Applicant notes that an examination of whether a person of ordinary skill in the art would recognize that Applicant was in possession of the claimed invention should include a consideration of not only the application disclosure as a whole, but also the method of making the claimed invention and the level of skill and knowledge in the art, among other factors. See Written Description Guidelines, March 2008. In this regard, as described above in the context of addressing the enablement rejection, **Applicant submits that the method of making the claimed Factor C is well-established, in view of the existing level of skill and knowledge in the art**” (page 12, 2nd par.).*

*Further, the **Declaration** submitted herewith, which is executed by one of the inventors of the Application, **Mr. Mizumura**, shows the alignment of the Factor C of *Tachypleus tridentatus* (TtFC) and the Factor C of *Limulus polyphemus* (LpFC). Consensus sequences which can undergo N-type sugar chain (N-linked sugar chain) modification are underlined. See pages 2-4 of the Declaration. The similarity between the amino acid sequence of TtFC and that of [L]pFC is 99.5%. Further, the amino acid residues which can undergo N-type sugar chain modification are conserved. **The Declarant, who is a person skilled in the art, states that in view of the as-filed disclosure, a person skilled in the art would readily recognize that the present invention could also work when Factor C is the Factor C of Limulus Polyphemus**” (pages 13-14, bridging par.).*

*In sum, Applicant submits that, in view of the as-filed disclosure, **a person skilled in the art would recognize Applicant to be in possession of the full scope of the claims**, as amended herewith. Accordingly, it is submitted that claims 49 and 77 are adequately described” (page 14, 2nd par.).*

53. In view of the above submissions made by the Proprietor to the USPTO, it is apparent that they believe, “*in view of the guidance in the specification; the high level of skill in the art; and the existing knowledge in the art a person skilled in the art would readily be able to generate and use the polypeptides that are encompassed by the claims*”, including a Limulus Factor C.
54. Moreover, and also by the Proprietor’s own admission, the skilled person reading the disclosure of **D1** had a clear incentive and expectation of success for carrying out this specific mode of **D1**. In that regard, we further refer to the declaration by Mr. Mizumura mentioned in the above quote from **D3** (submitted herewith as **D3a**), where the inventor declared the following:

*“...in view of the as-filed disclosure [i.e., the disclosure of **D1**], **a person skilled in the art would readily recognize that the presently claimed invention could also work when the Factor C is the Factor C of Limulus polyphemus. In addition...the Factor C of Limulus polyphemus would also have [the] unexpectedly superior property of being less susceptible to inhibition by ions, as exhibited by Factor C of Tachypleus tridentatus**” (**D3a**, page 4, penultimate par. and pages 4-5, bridging par.; emphasis added).*

55. In view of the above declaration from the inventor, the Proprietor apparently agrees that the skilled person reading **D1** would have been motivated to obtain *Limulus polyphemus* by expressing this Factor C in mammalian cells using the method of **D1** and that the skilled person could also have expected the alleged “superior property” for this Factor C.
56. Moreover, it was common general knowledge that the Limulus Factor C had been used for decades as the first component of the LAL assay for testing pharmaceuticals, owing to its extreme sensitivity to bacterial endotoxin. In that regard, we refer to the following disclosure from **D4**:

“The American horseshoe crab, Limulus polyphemus amoebocyte lysate (LAL) is well known and its coagulation cascade has been fully characterized and shown to defend the animal against the invasion of Gram-negative bacteria (Armstrong and Rickles, 1982). Owing to its extreme sensitivity to the bacterial endotoxin, LAL has been widely marketed for decades as a tool for detecting LPS in pharmaceuticals, parenterals and surgical implants, water and food (Levin et al., 1970; Novitsky, 1994). The LAL can detect femtogramme levels of LPS (Ho, 1983)” (page 198, 1st par.; emphasis added).

57. Accordingly, any additional alleged effect observed for the *Limulus* Factor C would merely be a “bonus” effect, which the skilled person would achieve by carrying out the direct teaching of **D1**

³ Cf., Figure 7 of **D1**.

using routine measures. It is established case law at the EPO that this type of “bonus” effect cannot be relied upon for supporting the presence of an inventive step. In that regard, we refer to the following exemplary Decisions:

“An effect which may be said to be unexpected can be regarded as an indication of inventive step (T 181/82, OJ 1984, 401). However, certain preconditions have to be met. In T 21/81 (OJ 1983, 15) the board considered that if, having regard to the state of the art, it would already have been obvious for a skilled person to arrive at something falling within the terms of a claim, because an advantageous effect could be expected to result from the combination of the teachings of the prior art documents, such claim lacked inventive step, irrespective of the circumstance that an extra effect (possibly unforeseen) was obtained (see T 365/86, T 350/87, T 226/88, T 1317/13).”

“The board also pointed out in T 506/92 that an additional effect achieved inevitably by the skilled person on the basis of an obvious measure without any effort on his part simply represented a bonus under EPO case law which could not substantiate inventive step, even as a surprising effect (see also T 431/93, T 681/94, T 985/98, T 794/01, T 688/13, T 179/18).”

(page 300, section I.D.10.8 of the CLB, 2022).

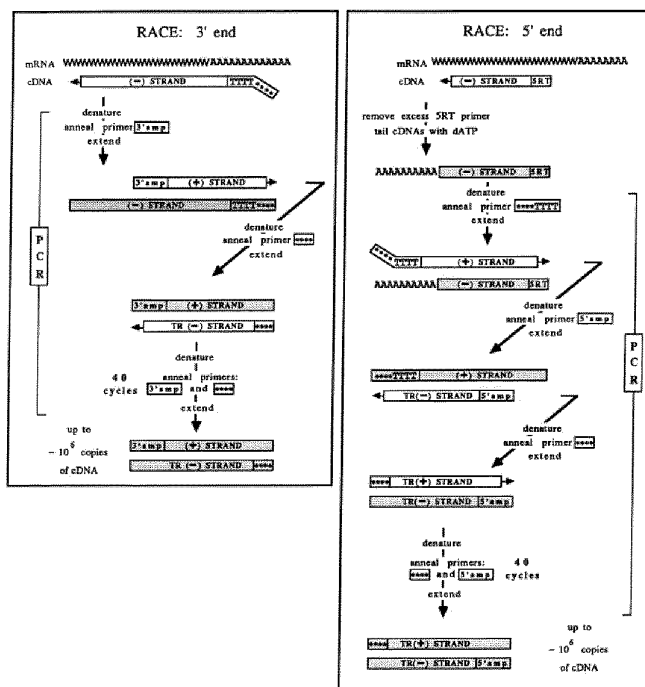
58. In the absence of any recognizable technical effect, **the problem** in view of D1 may thus be formulated as the determination of the full-length *Limulus* Factor C sequence (cf., par. [0017] of the opposed patent).
59. Deriving the solution to this problem (i.e., one of the sequences recited in claim 1) does not require any inventive skill. As we have already noted above, it is a matter of routine practice for a person skilled in the art to determine the sequence of a known protein.
60. We emphasize again that the Proprietor has even submitted this themselves to the USPTO stating that, “*in view of the guidance in the specification; the high level of skill in the art; and the existing knowledge in the art a person skilled in the art would readily be able to generate and use the polypeptides that are encompassed by the claims*”, including a *Limulus* Factor C (see par. 50-52 above).
61. Accordingly, and as will be explained below, the skilled person would have determined the claimed sequences in an obvious manner.

VI.2.1.2. The solution is obvious in view of the common general knowledge (represented by e.g., D11, D13-D18, and D20) and optionally D22

62. By 2016 (the year of priority), it was well-established that the cDNA sequence encoding a protein of interest could be readily determined using Rapid Amplification of cDNA Ends (RACE), if a

partial sequence of the mRNA sequence encoding the protein was known (or if a partial sequence of the protein's amino acid sequence was known).

63. In particular, it was common general knowledge that RACE (sometimes called one-sided PCR or anchored PCR)⁴ “provides **an inexpensive and powerful tool to quickly obtain full-length cDNA when the sequence is only partially known**. Starting with an mRNA mixture, gene-specific primers generated from the known regions of the gene and non-specific anchors, **full-length sequences can be identified in as little as 3 days**. RACE can also be used to identify alternative transcripts of a gene when the partial or complete sequence of only one transcript is known” (D11, abstract) (emphasis added).
64. As may be taken from, e.g., D11, the “initial description of RACE” was first published in **1988** by Professor Frohman in the disclosure provided herewith as D12⁵ (cf., D11, page 108, 1st sentence).
65. According to D12, RACE was developed by Professor Frohman in an effort to overcome the difficulties in obtaining full-length cDNA clones of low abundance mRNAs and merely requires the knowledge of “a single short stretch of sequence within the mRNA to be cloned” (D12, Frohman, 1988, abstract) (emphasis added). The basic scheme of the original protocol is shown in Figure 1 of D12, which is reproduced below:



⁴ see D16, page 2, 1st par

⁵ Note that D12 corresponds to reference number (7) in D11.

66. Since the initial publication of RACE, there have been various modifications to the protocol in an effort to optimize its efficiency and convenience, but the basic idea of the scheme depicted in the above figure remains the same.
67. In particular, 3' RACE exploits the ubiquitous poly-A tail of mRNA to reverse transcribe an mRNA using a primer comprising a poly-T tail and a unique "adapter" sequence at the end, thus generating a first (-) cDNA. A gene-specific primer ("gsp") (which is designed based on the "single short stretch" of mRNA that is known) is then annealed to the first (-) strand cDNA and extended by PCR to generate a second (+) strand. Re-iterative PCR using the gsp and a primer which anneals to the unique "adapter" sequence amplifies the mRNA of interest. 5' RACE is carried out in a similar manner using a gene specific primer to perform the initial reverse transcription step; the resulting strand is extended and re-iterative PCR amplifies the product of interest. The amplified products are sequenced and cloned and the full-length cDNA can then be obtained by, e.g., amplifying reverse transcribed mRNA using 5' and 3' primers, which can be designed based on the analysis above (D12, page 8999, right col., final par-page 9000, left col., 3rd par.).
68. This elegant approach can be used to obtain a full-length cDNA sequence within a matter of days (cf., D13, Dorit, 2000, page 15.6.9, right col., "Time Considerations"; and D11, Yeku, 2010, page 107, abstract) and thus effectively replaced the more time-intensive, traditional method of screening cDNA libraries in instances where partial sequence information is available. Indeed, and as may be taken from e.g., D14 (Frohman, 1994), it was common general knowledge that the use of RACE for determining full-length cDNA sequences was *"advantageous for several reasons"*. In particular,
- "...it takes weeks to screen cDNA libraries, obtain individual cDNA clones, and analyze the clones to determine whether the missing sequence is present. Using PCR, such information can be generated within a few days. As a result, it becomes practical to modify RNA preparation and/or reverse transcription conditions until full-length cDNAs are generated and observed. In addition, essentially unlimited numbers of independent clones can be generated using RACE, unlike library screens in which generally a single to a few cDNA clones are recovered. The availability of large numbers of clones provides confirmation of nucleotide sequence and allows the isolation of unusual transcripts that are alternately spliced or that begin at infrequently used promoters" (D14, page S40, Overview; emphasis added).*
- "RACE is cheaper, much faster, requires very small amounts of primary material, and provides rapid feedback on the generation of the desired product."** (D14, page S57, 1st par.; emphasis added).*
69. By 2016, the *"initial description of RACE"* (i.e., D12) had been cited more than 5,000 times and had become a cornerstone of the skilled person's common general knowledge (cf., D11, D13 and D14). Moreover, it was known that numerous kits were commercially available for carrying out 5'

and/or 3' RACE (see **D11**, page 120, "Notes", item 1; **D14**, page S40, 1st par.; the *partial* screenshot of our google search result for "cDNA RACE kits" before Oct. 18, 2016, submitted herewith as **D15**; and exemplary manuals for the 5' and 3' RACE kits from Invitrogen dated 2004, submitted herewith as **D16** and **D17**, respectively).

70. Furthermore, there was ample information available regarding the sequence of *Limulus* Factor C for the skilled person to design "*a single short*" primer for carrying out RACE in 2016. In particular, we refer to the following disclosures which provide such information, two of which constituted common general knowledge before 2016⁶:

(i) Genomic data for *Limulus polyphemus*, which was publicly available in the NCBI database as of January 3, 2014, as evidenced by **D18** (NCBI entry) and **D19** (Nossa, 2014) (see also par. [0013] of the opposed patent)

(ii) An mRNA sequence encoding part of the *Limulus polyphemus* Factor C, which had been predicted by automated computational analysis in the NCBI database. This predicted sequence was part of the *Limulus polyphemus* Annotation Release 100, which was submitted to the public database on September 10, 2015 (*cf.*, **D20** and **D21**)⁷

(iii) Partial amino acid sequences of the *Limulus* Factor C protein, which had been published already in **1991** (see **D22**, page 157, Figure 7S)

71. Accordingly, the skilled person knew that: (a) partial sequence information was available for the *Limulus* Factor C protein; and (b) this information could be used to readily generate the full-length cDNA sequence within a matter of days using a commercially available RACE kit. Thus, it would have been entirely obvious to the skilled person to use RACE to derive the cDNA sequence encoding *Limulus* Factor C, based on the known sequence information outlined above.

72. In particular, and according to Professor Frohman, "any of the sources of information listed in (i)-(iii) above could have been used to design RACE primers", as explained in detail in section IV of his declaration (**D8**) (emphasis added).

⁶ It is established case law that information that can be retrieved from publicly available databases (such as sequence information from the NCBI database) forms part of the skilled person's common general knowledge. In that regard, we refer to the following exemplary decision, which may be found on page 86, section I.C. 2.8.4 of the CLB, 2022:

"In T 890/02 the board concluded that whilst not being stricto sensu encyclopaedias or handbooks, databases (a) which are known to the skilled person as an adequate source for obtaining the required information, (b) from which this information may be retrieved without undue burden and (c) which provide it in a straightforward and unambiguous manner without any need for supplementary searches, represent the common general knowledge of the skilled person as defined in the case law" (emphasis added)

⁷ Of note, **D20/D21** also confirm that sequence information relating to *Limulus* factor C was comprised in the genomic sequence data in **D18** and available before the priority date. **D21** explicitly references "assembly accession" "GCF_000517525.1" as the source sequence for the computational annotation, which is the **D18** "RefSeq assembly" (*cf.*, 1st page of **D18**, no revision since Jan 3, 2014).

73. In his opinion, ***“it would have been entirely straightforward for the skilled person to design RACE primers based on the available Limulus sequence information...and then use any one of the available RACE-PCR kits to obtain the full-length cDNA sequence”*** (D8, page 3, par. 10) (emphasis added).
74. Indeed, this is exactly what the Proprietor did in the example of the opposed patent (*cf.*, par. [0151]-[0174]). In particular, and according to Professor Frohman’s assessment of this disclosure, the example merely describes *“standard 3’ and 5’ RACE protocols, followed by standard cloning to obtain the full-length cDNA sequence”*, and offers *“no sign that any of it was hard to do or required modifications of the RACE protocol”* (D8, page 10, par. 41-42).
75. Accordingly, and as we have already noted above, the mere statement in par. [0012] of the opposed patent, which alleges that the determination of this sequence *“could not be achieved by general techniques”* is entirely unsupported.
76. On the contrary, and as Professor Frohman has confirmed, there was *“more than enough”* sequence information available in 2016 to design RACE primers (D8, page 4, par. 17). Therefore, all the skilled person needed to derive the claimed sequences was a standard kit to carry out a common general knowledge technique, which had been around since 1988.
77. Accordingly, and as we have already noted above, the determination of the sequences recited in items (A)-(C) of claim 1 is merely a matter of routine analysis, which does not require any inventive skill.
78. Therefore, we submit that claim 1 lacks an inventive step in view of D1 in combination with the common general knowledge represented by D11, D13-D18, and D20, and optionally D22.

VI.2.1.3. The solution is obvious in view of D23 (US 5,716,834) or D24 (Ding, 1995)

79. Alternatively, the solution (the claimed sequences) can be derived from D23 or D24 in an obvious manner.
80. Each one of D23 and D24 describe cloning the *Carcinoscorpius rotundicauda* Factor C cDNA (*cf.*, D23, examples 1-3 and D24, Title). Each of these disclosures thus addresses the problem of determining the cDNA sequence and corresponding amino acid sequence of a horseshoe crab Factor C. Accordingly, the skilled person seeking to determine the cDNA sequence of the *Limulus polyphemus* Factor C cDNA sequence would have considered these disclosures.
81. In D23 and D24, mRNA extracted from horseshoe crab blood is used to generate cDNA. Adapter sequences were added to the cDNAs to generate restriction sites at the ends, and then the cDNAs were cloned into LambdaGT22. The resulting recombinant phage DNAs were then transduced into *E. coli* to produce a library (*cf.*, D23, col. 9, example 1- col. 10, line 11; or D24, page 100, left col., final par. - right col. par. 1-3).

82. The cDNA library was then screened using the known *T. tridentatus* Factor C cDNA⁸, whereby a selection of presumptive clones was identified, isolated and further studied. In particular, the sizes of their inserts were determined; further subcloned and transformed into *E. coli* and then characterized by restriction mapping and DNA sequencing (D23, col. 10, line 12-col. 13, line 22; and D24, page 100, right col., final par.-page 102, left col. final par.).
83. As may be taken from Figure 5 of D24; or, e.g., Figure 8 of D23, the authors were able to determine the complete cDNA sequence and deduce the amino acid sequence of *Carcinoscorpius rotundicauda* Factor C using the protocol described above.
84. According to Professor Frohman, who has 38 years of experience in molecular cloning, the approach described in each of these disclosures was successful *“because the cDNA sequences encoding the Factor C coding regions of T. tridentatus and C. rotundicauda were similar enough for hybridization approaches to be usable”* (D8, page 3, par. 13).
85. In particular, the cDNA encoding the Factor C derived from *T. tridentatus* (SEQ ID NO 1 of D1) is 97.1% identical to the cDNA encoding the Factor C derived from *C. rotundicauda* (SEQ ID NO 3 of D1) (see alignment results submitted herewith as Annex I). Therefore, the *T. tridentatus* sequence was able to act as a good hybridization probe for the *Carcinoscorpius* Factor C.
86. We submit that the skilled person merely had to repeat the example described in D23 or D24 with a cDNA library obtained from *Limulus* mRNA to arrive at the sequences recited in items (A)-(C) of claim 1. Although this approach may have required more time than the RACE protocol described above, it would have nevertheless merely involved routine work and could have equally been used to determine the *Limulus* Factor C sequence.
87. According to Professor Frohman, the approach described in D23 or D24 would have been **“very straightforward”** and the skilled person would have had **“every reason to believe that this approach would have been successful to obtain the Limulus Factor C cDNA. Given the success of obtaining the cDNA sequence of the other, closely related species, there would be no reason to expect any technical difficulties for repeating this protocol for Limulus”** (D8, page 3, par. 14) (emphasis added).
88. Indeed, based on the high level of identity between the cDNA encoding the *Limulus* Factor C and the cDNA sequence encoding *T. tridentatus*, it is immediately apparent that, had the skilled person used the protocol described in D23 or D24, they would have arrived at the sequences recited in items (A-C) without any technical difficulty.
89. In particular, the cDNA encoding the Factor C derived from *Limulus* (according to SEQ ID NO 1 of the opposed patent) is 90.1% identical to that of *T. tridentatus*; and 89.3% identical to that of

⁸ Each disclosure points the skilled person to the necessary reference to obtain the *T.t.* Factor C probe sequence (D23, col. 10, lines 13-16; and D24, page 100, right col., final par.)

C. rotundicauda (see alignment results in **Annex I**)⁹. Each of these prior art cDNA sequences provide a high enough identity to act as a good hybridization probe for *Limulus* Factor C. Thus, “either the *T. tridentatus* or *C. rotundicauda* cDNA sequence could have been used as a hybridization probe to screen the *Limulus* cDNA library and would have been expected to work” (**D8**, page 3, par. 15).

90. In view of the above, we submit that **D23** (or **D24**) provides a straightforward roadmap that the skilled person could have followed with a reasonable expectation of success to arrive at the claimed sequences. Accordingly, the derivation of the sequences recited in items (A)-(C) of claim 1 must be found devoid of an inventive step in view of this disclosure.
91. In particular, we note that the Boards of Appeal have consistently denied an inventive step in cases where the identification of a (c)DNA sequence merely comes down to a matter of routine cloning. In that regard, we refer to the following decisions:

*“In the light of the closest prior art the board saw the technical problem to be solved in T 886/91 in the exact identification and characterisation of DNA sequences of HVB genome subtype adyw. The board pointed out that the situation in **T 886/91** could not be compared with the one in **T 223/92** and **T 500/91**, where production of a partially known protein in a recombinant-DNA system was achieved and considered inventive on the basis of the fact that, in the specific circumstances of those cases, there was no realistic expectation of success. In the case in point the closest prior art had already disclosed the cloning and expression of the HBV genome subtype adyw. The identification and characterisation of the claimed specific sequences of the same genome involved for the skilled person **nothing more than the performance of experimental work by routine means** in connection with the normal practice of filling gaps in knowledge by the application of existing knowledge”* (pages 223-224, section I.D.7.2 of the CLB, 2022; emphasis added).

*“In **T 386/94** (OJ 1996, 658), again citing **T 816/90**, the board ruled that, in gene technology, **inventive step could not be acknowledged if, at the priority date, a skilled person could expect to perform the cloning and expression of a gene in a fairly straightforward manner**, and the cloning, although requiring much work, did not pose such problems as to prove that the expectation of success was ill-founded”* (page 222, section I.D.7.1 of the CLB, 2022; emphasis added).

92. Accordingly, we submit that claim 1 further lacks an inventive step in view of **D1** in combination with **D23** or **D24**.

⁹ See also page 299, right col., first par. of **D32**.

VI.2.2 Claim 2

93. As we have explained in our Overview above (section VI.1), the use of the Factor C produced according to claim 1 in a method for measuring endotoxin would have been entirely obvious in view of the common general knowledge.
94. At any rate, this method is explicitly taught in **D1**, which further discloses *“a method for measuring endotoxin present in a test specimen, the method comprising a step of mixing the endotoxin assay agent of the present invention and the test specimen, and a step of measuring the progress of a cascade reaction”* (par. [0211] and claim 33).
95. A specific embodiment of the method of **D1** includes *“a method for measuring endotoxin present in a test specimen, the method comprising a step of mixing the Factor C of the present invention with the test specimen and a step of detecting activated Factor C”* (par. [0219]).
96. As explained above, the “endotoxin assay agent” of the invention of **D1** includes a Factor C which has been produced according to a method as recited in claim 1.
97. Further, we submit that a step of *“mixing the endotoxin assay agent...and the test specimen”* is equivalent to *“bringing the recombinant protein into contact with the sample”*. Indeed, endotoxin can only be detected if it comes into contact with Factor C, as explained in par. [0005] of **D1**.
98. The only difference between claim 2 and the disclosure of **D1** is the recitation of the sequences in items (A)-(C) of claim 1.
99. However, and as we have explained above, this would have been derived by the skilled person without the need for any inventive skill.
100. Accordingly, claim 2 lacks an inventive step for the same reasons as claim 1.

VI.3 Lack of inventive step in view of D5 (US 6,645,724) in combination with (i) the common general knowledge (represented by e.g., D11, D13-D18, and D20) and optionally D22; or (ii) D23 ('834), or D24 (Ding, 1995), optionally D5 combined with D1 in view of the common general knowledge

101. **D5** relates to the provision of a recombinant horseshoe crab Factor C and is therefore aimed at the same purpose as the opposed patent (*cf.*, **D5**, col. 1, “field of the invention”). **D5** also discloses all features of claim 1 and claim 2, except the sequences in items (A)-(C) of claim 1. This disclosure may thus equally be taken as the closest prior art.

VI.3.1 Claim 1

102. **D5** relates to a “recombinant Factor C (rFC) of a horseshoe crab, produced in an insect cell system” (col. 1, lines 14-15). According to **D5** the Factor C may be used “to detect endotoxins in a sample” (col. 1, lines 18-19). Thus, this protein may be considered an “endotoxin detecting agent”.
103. The Examples of **D5** carry out methods for producing this endotoxin detecting agent by introducing a cDNA encoding the Factor C derived from *Carcinoscorpius rotundicauda* into a baculovirus-infected Sf9 cell, from which the Factor C protein is expressed (see Examples 1 and 2).
104. It is noted that claim 1 does not require the use of any specific type of expression system. In particular, a step of “artificially expressing” the Factor C according to claim 1 broadly encompasses the use of “a heterologous expression system or a cell-free protein synthesis system” (par. [0037] of the opposed patent). *If* a host cell is used to express Factor C, the opposed patent teaches that this host may be any cell other than a horseshoe crab (par. [0036], [0038]).
105. Therefore, the use of the baculovirus-infected Sf9 cell as an expression host in **D5** qualifies as “artificially expressing” the Factor C protein, in the sense of the patent.
106. Further, example 2 of **D5** shows that, after Factor C has been artificially expressed by the insect cell, the protein is then collected from the culture supernatant¹⁰. In particular, a step of collecting the “culture supernatant” is described on col. 10, lines 13-15, where it is disclosed that “the cell medium was collected and centrifuged.”
107. Finally, **D5** discloses that “cDNAs appropriate for expression in the presently described system can be cDNAs encoding Factor C of any horseshoe crab” (col. 8, lines 6-8). In particular, it is disclosed that the invention may be carried out using “a Factor C enzyme of a horseshoe crab, preferably of the genus *Carcinoscorpius*, *Tachyplesus* or *Limulus*” (col. 3, lines 22-25, emphasis added).
108. Accordingly, **D5** demonstrates a method for producing an endotoxin detecting agent (feature i), comprising a step of artificially expressing a protein (feature ii) using a cDNA (feature ii.a), and collecting the expressed recombinant protein as a solution fraction (feature iii), which is a culture supernatant (feature (iii.a), wherein the cDNA may encode for a Factor C from any horseshoe crab, including *Limulus*.

¹⁰ Interestingly, Example 5 in col. 13, lines 12-16 describes that “[a] comparison of the amount of total proteins present in the cell lysate (Lysate: 50 µg) and culture Supernatant (Sup: 5 µg) illustrates that the culture supernatant from 72 h p.i. contained rPC that is >5-10 fold more effective in LPS detection”, thus disclosing that using Factor C collected from the supernatant is particularly advantageous.

109. In view of the above, it is apparent that the only feature which is not explicitly disclosed in **D5** is a *Limulus* Factor C sequence according to items (A)-(C) (feature ii.b).
110. Thus, **the only difference** between the claimed subject-matter and the disclosure of **D5** is the recitation of the amino acid sequence of the *Limulus* Factor C, or a cDNA sequence encoding this protein.

VI.3.1.1. The technical problem in view of D5

111. To the extent that any alleged effect may be associated with said difference (which we deny, for the reasons outlined in section VI.5 below), it would nevertheless be irrelevant for the assessment of inventive step in view of **D5**.
112. In particular, in the examples of **D5**, a recombinant Factor C (rFC) derived from *Carcinoscorpius rotundicauda* is produced and shown to be capable of detecting endotoxin (*cf.*, Figure 5). According to **D5**, the use of a recombinant Factor C is advantageous in that it “*unequivocally exhibits full biological functionality*” and “*obviates the need for routine harvesting of the horseshoe crab for procurement of their amoebocyte lysate, and therefore, conserves this endangered living fossil*” (col. 2, lines 22-50).
113. Moreover, as we have pointed out before, it was common general knowledge that the *Limulus* Factor C had been used for decades as the first component of the LAL assay for testing pharmaceuticals owing to its extreme sensitivity to bacterial endotoxin. In that regard, we refer to the following disclosure from **D4**:

*“The American horseshoe crab, Limulus polyphemus amoebocyte lysate (LAL) is well known and its coagulation cascade has been fully characterized and shown to defend the animal against the invasion of Gram-negative bacteria (Armstrong and Rickles, 1982). **Owing to its extreme sensitivity to the bacterial endotoxin, LAL has been widely marketed for decades as a tool for detecting LPS in pharmaceuticals, parenterals and surgical implants, water and food** (Levin et al., 1970; Novitsky, 1994). The LAL can **detect femtogramme levels of LPS** (Ho, 1983)”* (page 198, 1st par.; emphasis added).

114. Accordingly, the skilled person would have carried out the teaching of **D5** as it regards *Limulus polyphemus* because this Factor C would be expected to exhibit the “full biological activity” of this “extremely sensitive” and well-known endotoxin detecting agent. Therefore, any additional effect observed for the *Limulus* Factor C would merely be a “bonus” effect, inevitably obtained by carrying out the direct teaching of **D5**. As we have already noted above, this type of effect cannot support the presence of an inventive step (*cf.*, page 300, section I.D.10.8 of the CLB, 2022).
115. In the absence of any recognizable technical effect, **the problem** in view of **D5** may thus be formulated as the determination of the full-length *Limulus* Factor C sequence (*cf.*, par. [0017] of the opposed patent).

116. Deriving the solution to this problem (the sequences recited in claim 1) does not require any inventive skill. Indeed, and as we have already explained above, deriving the claimed *Limulus* sequence was merely a matter of routine analysis.

VI.3.1.2. The solution is obvious in view of the common general knowledge regarding RACE, and optionally D22; or in view of D23 or D24

117. As we have explained above, the skilled person would have readily derived the *Limulus* sequence using standard RACE protocols or cloning. In that regard, we submit that the arguments outlined in sections VI.2.1.2 and VI.2.1.3 above equally apply when starting from **D5** as the closest prior art.
118. Therefore, the claimed subject matter further lacks an inventive step in view of **D5** in combination with the common general knowledge (represented by **D11**, **D13-D18**, and **D20**) and optionally **D22**, or in combination with **D23** or **D24** for the same reasons outlined above when starting from **D1**.
119. Furthermore, if necessary, **D5** can be combined with **D1** and the above-cited common general knowledge relating to sequencing methods. As discussed in detail above, **D1** discloses methods for recombinantly producing Factor C in mammalian cells, comprising a step of artificially expressing the protein from a eukaryotic expression vector (see par. [0170] of **D1**) and collecting the expressed recombinant protein as a solution fraction which is a culture solution or culture supernatant (see [0173] of **D1**); and directly points the skilled person to carry out this method with a Factor C derived from *Limulus polyphemus* species (see par. [0150]/[151] of **D1**).

VI.3.2 Claim 2

120. As we have explained in our Overview above (section VI.1), the use of the Factor C produced according to claim 1 in a method for measuring endotoxin would have been entirely obvious in view of the common general knowledge.
121. At any rate, this method is explicitly taught in **D5**, which further discloses “methods for using the recombinant Factor C to detect endotoxins in a sample” (col. 1, lines 17-19).
122. In particular, Example 5 of **D5** demonstrates the use of “*Fluorimetric and Colorimetric Assays for LPS-activated rFC Enzyme Activity*”. An exemplary assay is disclosed as follows:

“Using rFC obtained from a 72 h p.i. culture supernatant, 10 µg total protein in a volume of 0.1 ml was mixed with 1.9 ml of 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl and 0.05 M CaCl₂. The mixtures were preincubated with 0.01 to 100 µg of LPS (E. coli 055:B5, Sigma) at 37° C. for 1 h before addition of 15 µl of 2 mM fluorimetric substrate, Boc-Val Pro-Arg-MCA (Sigma). Incubation was continued for 30 min and the reaction was terminated with 0.1 ml glacial acetic acid. The product AMC was read in Fluorescence Units (FU) at EX-so

(slit 10 nm) and Emo (slit 5 nm) using a Perkin Elmer Luminescence Spectrophotometer (LS-50B)” (col. 12, lines 41-53; emphasis added).

123. As may be taken from the above disclosure, the method exemplified in **D5** comprises a step of bringing the recombinant Factor C into contact with a sample requiring detection of endotoxin.
124. The Factor C used in this example was produced according to the method of example 2, which, as we have explained above, differs only from the method of claim 1 in its recitation of the sequences in items (A)-(C).
125. However, and as we have explained above, the skilled person would have derived this information without the need for any inventive skill.
126. Accordingly, claim 2 lacks an inventive step for the same reasons as claim 1.

VI.4 Lack of inventive step in view of D6 (WO 03/002976) in combination with (i) D5 or (ii) D1 (or in combination with the common general knowledge regarding RACE or D23 or D24)

127. **D6** also relates to the provision of a recombinant horseshoe crab Factor C and is therefore aimed at the same purpose as the opposed patent (*cf.*, **D6**, claim 1). **D6** discloses all features of claim 1 and claim 2, except the use of a cDNA encoding one of the sequences recited in claim 1. Therefore, **D6** may further be considered suitable closest prior art.

VI.4.1 Claim 1

128. **D6** discloses “a reagent for detecting endotoxin, comprising a purified horseshoe crab Factor C protein; and a surfactant” (claim 1), “wherein the horseshoe crab is *Limulus polyphemus*” (claim 3). According to the description of **D6**, the term “purified horseshoe crab Factor C” “specifically includes a cell culture supernatant which comprises recombinant Factor C protein” (page 6, par. [22]).
129. According to this disclosure, “Methods of producing proteins recombinantly are well known in the art and generally involve culturing a host cell comprising an expression vector encoding the Factor C protein in a supernatant under conditions such that the protein is expressed. The expressed protein can be recovered or, preferably, the supernatant comprising the expressed protein is used directly as the source of recombinant Factor C” (page 7, par. [26]).
130. Exemplary methods for producing the Factor C in **D6** are shown in examples 2-9, which involve “transfection of bacmid DNA-Factor C into Sf9 cells” (example 2) and “harvest [of] culture supernatant”, “which contains recombinant Factor C” (example 5).
131. Accordingly, **D6** discloses a method for producing an endotoxin detecting agent (feature i), comprising a step of artificially expressing a protein (feature ii), and collecting the expressed

recombinant protein as a solution fraction (feature iii), which is a culture supernatant (feature (iii.a), wherein the DNA may encode for a Factor C from any horseshoe crab, including *Limulus*.

132. Thus, **the only difference(s)** between the claimed subject-matter and the disclosure of **D6** is the use of a cDNA (feature ii.a) which encodes one of the sequences recited in items (A)-(C) (feature ii.b).

VI.4.1.1. The technical problem in view of D6

133. To the extent that any alleged effect may be associated with the *Limulus* sequence (which we deny, for the reasons outlined in section VI.5 below), it would nevertheless be irrelevant for the assessment of inventive step in view of **D6**.
134. In particular, in the examples of **D6**, a recombinant Factor C (rFC) derived from *Carcinoscorpius rotundicauda* is produced and shown to be capable of detecting endotoxin. Further, **D6** shows that the activity of rFC can be enhanced by the addition of a surfactant to the endotoxin assay (*cf.*, Figures 1-3 and 7). Figure 6 shows that the same enhanced activity is observed for the *Limulus* lysate. Accordingly, the skilled person would have carried out the teaching of **D6** as it regards *Limulus polyphemus* because this Factor C could be expected to exhibit the same advantageous properties that were demonstrated for the *Carcinoscorpius* rFC.
135. Therefore, any additional effect observed for the *Limulus* Factor C would merely be a “bonus” effect, inevitably obtained by carrying out the direct teaching of **D6** and could not be relied upon to support the presence of an inventive step (*cf.*, section I.D.10.8 of the CBL, 2022).
136. Accordingly, the only alleged “problem” that has been solved by the application is the provision of a molecule encoding the full-length *Limulus* Factor C sequence (*cf.*, par. [0017] of the opposed patent).

VI.4.1.2. The solution is obvious in view of D6 in combination with the common general knowledge (represented by D25) and D5

137. We submit that the solution to this problem (the use of a cDNA encoding one of the sequences recited in items (A)-(C) of claim 1) is obvious in view of **D6** in combination with the common general knowledge and **D5**.
138. In particular, **D6** already provides the skilled person with a means for determining the *Limulus* Factor C sequence in Example 11, which discloses the following:

“We isolated the factor C from Limulus and generated antisera against the protein. The Limulus lysate was first separated with a S-100 gel filtration followed by cation-ion exchange column. The fractions were assayed for factor C activity using the N-t-BOC-Val-Pro-Arg 7-Amido-4-methyl coumarin substrate. The Limulus factor C was purified 73 fold

with 80% homogeneity as determined by enzyme assay and SDS-PAGE. The molecular weight of Limulus factor C was 117 kDa by SDS-PAGE under non-reducing conditions. Reduced SDS-PAGE showed two major bands of 79 kDa and 40 kDa, corresponding to the heavy and light chain. Tryptic peptide sequencing revealed that the Limulus factor C sequence closely matched the Asian species, with > 90% sequence identity... (page 27, par. [93]; emphasis added).

139. Although **D6** does not disclose the *Limulus* Factor C amino acid sequence, it is nevertheless apparent in view of the above example that the authors had sequenced a sufficient level of coverage of this protein in order to determine that it shared “>90% sequence identity” with the Asian species.
140. It would thus be immediately apparent to the skilled person that the *Limulus* sequence could be directly obtained by performing tryptic peptide sequencing. Deriving the complete sequence would merely have been a matter of analyzing the purified protein with tandem mass spectrometry (MS/MS). Indeed, it was common general knowledge at the time of priority that MS/MS “can be used for protein sequencing” (**D25**, page 7, “Applications”, “Peptides”, 1st sentence).
141. Finally, **D5** clearly teaches that a Factor C sequence may be encoded as a cDNA, which can be used to express the protein in a baculovirus-infected Sf9 cell (see discussion in section VI.3.1.1 above). Since **D6** uses the same expression system as **D5** (compare, e.g., Example 2 of **D6** and Examples 1 and 2 of **D5**), it would have been obvious to use a cDNA encoding the derived sequence to express Factor C.
142. Thus, claim 1 lacks an inventive step in view of **D6** in combination with **D5** and the common general knowledge of **D25**.

VI.4.1.3. The solution is obvious in view of the common general knowledge regarding RACE, and optionally D22; or in view of D23 or D24; or optionally, D6 combined with D1 in view of the common general knowledge

143. Alternatively, the solution would have been an obvious measure in view of the common general knowledge regarding RACE, or **D23/D24**. In particular, and as we have explained above, the claimed sequences could have been readily determined using standard RACE protocols or cloning. In that regard, we submit that the arguments outlined in sections VI.2.1.2 and VI.2.1.3 above equally apply when starting from **D6** as the closest prior art. Additionally, since the molecule one obtains by carrying out RACE or by following the disclosure of **D23** (or **D24**) is a cDNA, the use of this molecule to encode the Factor C sequence would have further been an obvious measure.
144. At this juncture, we reiterate what has been explained in detail above: at the priority date the LAL assay based on the natural *Limulus* Factor C was commonly known for decades, and **D1** had

already disclosed to express a recombinant *Limulus* Factor C (in the culture supernatant) in mammalian cells for use in the endotoxin assay.

145. Accordingly, a lack of inventive step can equally be derived from a combination of **D6** with the common general knowledge regarding RACE (**D13-D18**, and **D20**) and optionally **D22**; or in combination with **D23** or **D24** for the same reasons outlined above when starting from **D1**.
146. Furthermore, if necessary, **D6** can be combined with **D1** and the above-cited common general knowledge relating to sequencing methods. As discussed in detail above, **D1** discloses methods for recombinantly producing Factor C in mammalian cells, comprising a step of artificially expressing the protein from a eukaryotic expression vector (see par. [0170] of **D1**) and collecting the expressed recombinant protein as a solution fraction which is a culture solution or culture supernatant (see par. [0173] of **D1**); and directly point the skilled person to carry out this method with a Factor C derived from *Limulus polyphemus* species (see par. [0150]/[151] of **D1**).

VI.4.2 Claim 2

147. As we have explained in our Overview above (section VI.1), the use of the Factor C produced according to claim 1 in a method for measuring endotoxin would have been entirely obvious in view of the common general knowledge.
148. At any rate, this method is also explicitly taught in **D6**, which further discloses (emphasis added):

“A method of detecting endotoxin in a test sample, comprising the steps of:

contacting a test sample with (1) a reagent comprising (a) a purified horseshoe crab Factor C protein and (b) a surfactant and (2) a Factor C substrate, wherein cleavage of the Factor C substrate generates a detectable signal to form a contacted test sample to form a test sample-reagent-substrate mixture; and

assaying the contacted test sample for the presence or absence of the detectable signal, wherein an amount of the detectable signal that is increased relative to a control sample that does not contain endotoxin indicates a presence of endotoxin in the test sample” (claim 11).

*“...wherein the horseshoe crab is *Limulus polyphemus*”* (claim 12).

149. The only difference(s) between claim 2 and the disclosure of **D6** is that the endotoxin detecting agent is produced according to a method which uses of a cDNA encoding one of the sequences recited in items (A)-(C) of claim 1.
150. However, and as we have explained above, this would have been derived by the skilled person without the need for inventive skill.

151. Accordingly, claim 2 lacks an inventive step in view of **D6**, for the same reasons as claim 1.

VI.5 No technical effect associated with the claimed sequences

152. We maintain that any alleged effect observed for the *Limulus* Factor C would merely be the inevitable result of carrying out the direct teaching of any one of **D1**, **D5**, or **D6**. As explained above, to the extent that any technical effect may be attributed to the *Limulus* Factor C sequence, it could at best be considered a “bonus” effect, which cannot indicate the presence of an inventive step (see e.g. **T 936/96**).

153. However, and for the sake of completion, we deny the presence of any effect associated with the claimed sequences. We will explain in detail below why the alleged effect recited in par. [0192] of the opposed patent is not credible.

154. During prosecution of the opposed patent, it was argued that par. [0192] discloses “*that a culture supernatant containing a recombinant Factor C protein derived from Limulus polyphemus (LFC) showed an activity about 2.2 times higher than recombinant Factor C protein derived from Tachypleus tridentatus (TFC)*” and that this allegedly “*excellent effect*” could not have been expected from the prior art (**D26**, page 5, 2nd par.).

155. However, this technical effect should be disregarded because it is not based on the true difference. **D1** already discloses “*a recombinant Factor C protein derived from Limulus polyphemus (LFC)*”. The only difference compared to this disclosure is the explicit sequence of LFC, but not recombinant LFC itself. In that regard it should also be noted that the applicant argued on page 5 of **D26** that it was surprising that the recombinant *Limulus* Factor C in the supernatant had higher activity than Factor C from *Tachypleus*, whereas the native enzymes isolated from amoebocytes displayed opposite reactivities. This reasoning already shows that the difference in activity cannot, by the Patentee’s own standard, reside in the use of the *Limulus* Factor C itself, let alone its specific sequence. Thus, for all of these reasons, the comparison of the activity of recombinant LFC to TFC is **irrelevant** for the assessment of inventive step.

156. Importantly, as demonstrated by Example (8) at par. [0188] of the opposed patent, the claimed method employs the experimental steps provided in Example 1. (2) of **D1** for obtaining a culture supernatant fraction containing the expressed recombinant Factor C (in par. [0188], the patent refers to Example 1. (2) of WO2014/092079 underlying **D1**)¹¹.

157. Moreover, even when taken into consideration, the alleged effect relied upon by the Proprietor during prosecution of the opposed patent has not been supported by any data. The passage in par. [0192] is a mere statement that an effect had been achieved. In this type of situation, an effect cannot be acknowledged. In that regard, it is established case law that:

¹¹ See also our discussion under par. 44 above.

*“alleged advantages to which the patent proprietor/applicant merely refers, **without offering sufficient evidence** to support the comparison with the closest prior art, cannot be taken into consideration in determining the problem underlying the invention and therefore in assessing inventive step (see e.g. T 20/81, OJ 1982, 217; T 181/82, OJ 1984, 401; T 1051/97; T 632/03; T 1211/07; T 736/12; T 2400/12; T 825/18; T 2210/19). In T 1027/08 the board added that there was no reason to deviate from this case law as it was based on the understandable rule that a patent could only properly be granted for a solution claimed as non-obvious if it actually achieved the alleged effect” (page 206, section I.D.4.3.1 of the CLB, 2022; emphasis added).*

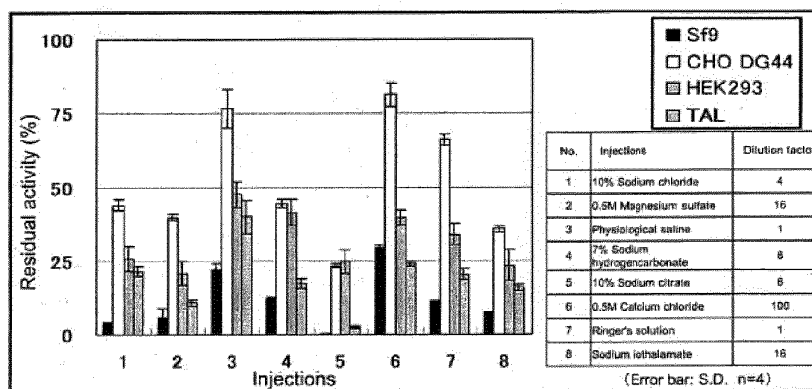
*“If the patent proprietor/applicant alleges the fact that the claimed invention improves a technical effect, then the burden of proof for that fact rests upon him (T 355/97, T 1213/03, T 1097/09, T 2418/10, T 1487/16). **In the absence of any data confirming the alleged improvement, such an effect cannot be taken into account in the formulation of the technical problem (T 2044/09)**” (page 206, section I.D.4.3.1 of the CLB, 2022; emphasis added).*

*“**A mere statement** that a certain effect arises (under conditions that were not reflected by the technical features of the claim), in the absence of any supporting circumstances, did not render the achievement of the effect plausible” (T 1322/17, page 210, section I.D.4.3.3 of the CLB, 2022; emphasis added).*

158. Not only has the opposed patent failed to provide any data which may be used to confirm the statement in par. [0192], but there are further serious reasons to doubt that the alleged effect has actually been achieved, particularly over the entire scope of the claim.
159. As a first point, we note that the relative activities of the *Limulus* and *Tachypleus* Factor C proteins was determined based on a “concentration measurement” using a Western blot, in which the two proteins were stained using the 2C12 monoclonal antibody (par. [0189]). However, according to the reference cited in par. [0189] (Miura, 1992, submitted herewith as **D27**), this antibody was developed by immunizing mice with purified Factor C from *Tachypleus tridentatus* (cf., **D27**, page 477, left col., par. 1-2; see also document title) and specifically binds to an epitope in the H chain of this protein (cf., **D27**, abstract; page 480, Fig. 9; and page 481, left col., discussion).
160. Accordingly, the antibody which was used for the Western blot staining in the example of the opposed patent is known to be specific for the H chain of the *Tachypleus tridentatus* Factor C. Since the *Limulus* sequence is not 100% identical to that of *Tachypleus* (see alignment results submitted herewith as **Annex II**), there are serious reasons to doubt that this antibody would bind *Limulus* Factor C with the same affinity, and thus result in staining of an *equal* intensity, as a Factor C derived from *Tachypleus*. In other words, there are serious reasons to doubt that the Western blot data which is mentioned (but conveniently not shown) in par. [0189] could be used for an accurate determination of the relative concentrations of these two proteins.

161. Given the use of a sub-optimal antibody for staining the *Limulus* Factor C, it is questionable whether its concentration was indeed lower than the Factor C derived from *Tachypleus* (as reported in par. [0189]), or if it was just stained to a lesser extent. We submit that the uncertainty in this “concentration measurement” significantly calls into question whether the experiment in example (8) can be used to reliably compare the activities of these two proteins.
162. It is further noted that the experiment in example (8) was carried out using a very specific cascade system comprising a *Tachypleus* Factor B and a *Tachypleus* proclotting enzyme. However, the only “endotoxin detecting agent” required by claims 1 and 2 is the *Limulus* Factor C. The Proprietor has not shown that the alleged effect is independent of the interaction of the three proteins¹² and thus, if said effect can be achieved if the *Limulus* Factor C is used without these other cascade components for detecting endotoxin. Accordingly, example (8) cannot possibly demonstrate the alleged effect over the scope of the claim.
163. Further, claim 1 does not require the use of a specific expression system. Indeed, it does not even require the use of a host cell to express Factor C. However, it is known that the type of expression host which is used to express Factor C can greatly influence its activity. In that regard, we refer to Figure 7 of D1, which is reproduced below:

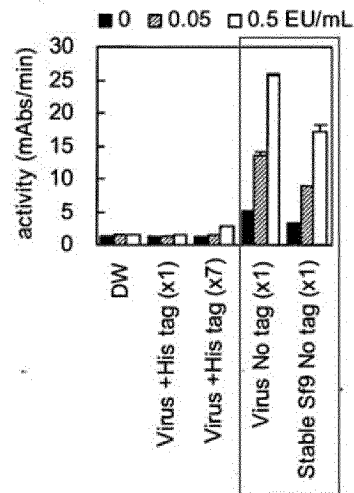
[Fig. 7]



164. As may be taken from the above Figure, it is apparent that Factor C behaves quite differently depending on which host cell is used to express it.
165. Indeed, even the method by which the Factor C coding sequence is introduced into a given host cell can influence its activity. In that regard, we refer to Figure 4 of D28, which is reproduced below with a red box highlighting data of interest:

¹² We note that, as a consequence of the possibly different concentrations of Factor C from *Tachypleus* and *Limulus* as described in par. [0189], both systems may not be comparable, as the measured output is a function of the stoichiometric relationship of all components.

[Fig. 4]



166. The red box in figure 4 above highlights the relative activities of a *Tachypleus* Factor C which has been expressed by a virus-infected Sf9; and a stably-expressing Sf9 cell (cf., page 40, par. [0087] and page 46, par. [0103]). As may be taken from these results, the activity of Factor C can even differ when expressed using the same host cell, depending on how the coding sequence is introduced into the cell (by viral infection, or stable transfection).
167. In view of the above, we submit that it is simply not credible that a *Limulus* Factor C which is “artificially expressed” using any system of one’s choosing would exhibit a higher activity than a *Tachypleus* Factor C, which has been “artificially expressed” with any other system of one’s choosing.
168. Additionally, and as may be taken from Figure 7 of **D1**, Factor C behaves quite differently depending on which salts are present in solution. However, it is known that different expression hosts often require different salt compositions in their culture solutions. Therefore, it is further not credible that a *Limulus* Factor C which is collected in any culture solution of one’s choosing would exhibit a higher activity than a *Tachypleus* Factor C, which has been collected in any other culture solution of one’s choosing.
169. It is noted that the description of example (8) does not clearly and unambiguously disclose which culture solutions were used. Par. [0188] merely states that “as a vector for protein expression, pCI-neo (Promega) was used for both” the *Tachypleus* and *Limulus* factor C. Hence, the comparative data are very limited. Thereby, the application documents do not provide a basis for alleging a technical effect (higher activity of the recombinant LFC) for a culture solution or supernatant fraction in general and irrespective of the particular type of host cell or cultivation procedure employed. Due to the limited disclosure in example (8) it is impossible to (i) convincingly show that the allegedly higher activity of the *Limulus* Factor C was not influenced by these variables (i.e., expression host and/or solution composition discussed above) and (ii) verify

the results of the experiment. The present claim is not limited at all as regards the type of host cell or expression system (cell culture process, cell culture medium, expression vector used and type of promoter, etc.), and thereby, includes a solution fraction containing the expressed recombinant protein from insect cells (compare **D5**, **D6**) and for which no experiments have been disclosed in the application. The patent's example (8) clearly fails to prove that the alleged technical effect occurs over the entire scope of the claim.

170. Finally, it is noted that the “comparative example” in the application only compares a *Limulus* Factor C to a Factor C derived from *Tachypleus tridentatus*. However, **D5** and **D6** each demonstrate the use of a recombinant Factor C derived from *Carcinoscorpius rotundicauda*. The *Tachypleus* and *Carcinoscorpius* Factor C sequences are not 100% identical (see alignment results in **Annex II**). If small changes to the sequence can lead to very different activities, as the Proprietor alleges in par. [0192], then it is immediately apparent that the comparative data using *Tachypleus* cannot demonstrate an effect over the other equally known Factor C from *Carcinoscorpius* (i.e., no improvement whatsoever is demonstrated vis-à-vis **D5** and **D6**).
171. The patent itself acknowledges that the recombinant version of the *Carcinoscorpius rotundicauda* Factor C was well known before the priority date (cf., par. [0008]-[0009])¹³. It is not apparent why a comparison to this Factor C has not been attempted. However, it is clear that no effect has been demonstrated that could serve to distinguish the claimed sequences over the equally suitable closest prior art disclosures of **D5** and **D6**.
172. In view of the above, there does not appear to be any effect attributable to the sequences in claim 1, least of all one that is achieved over the whole scope of the claim.

VI.6 No technical effect associated with the culture solution or culture supernatant

173. During prosecution of the opposed patent, the Proprietor argued that the claimed subject matter was distinguished from the prior art because it was “defined by the feature ‘a culture solution or a culture supernatant’”, which feature was alleged to provide a surprising technical effect (**D26**, page 5, 1st par.); and the patent was granted based on this allegedly surprising technical effect (cf., **D26a**, item 4 and **D29**, items 1.3 and 2).
174. However, this technical effect must be disregarded, since as explained above, that recombinant *Limulus* Factor C is contained in a culture supernatant or culture solution is already disclosed in **D1**, **D5** and **D6**.
175. In any event, as will be explained in detail below, this feature has not been convincingly shown to be causally linked to any effect and should thus be ignored for the assessment of inventive step.

¹³ For example, the PyroGene™ assay (Lonza) mentioned in par. [0008] uses *C. rotundicauda* Factor C, which is also known as *Tachypleus rotundicauda*, cf., par. [0004].

176. First, we submit that there are serious reasons to doubt that a *Limulus* Factor C which is contained in a culture supernatant or culture solution would exhibit a higher activity than the purified form of the protein. In that regard, we refer to the evidence of **D5**, which clearly shows that a step of purifying a Factor C which has been collected from the supernatant leads to a higher activity.
177. In particular, **D5** discloses in col. 10, lines 13-26, that fractions of the rFC which had been collected as a supernatant were subjected to further purification steps. For example, *"The medium was then concentrated 10-fold by centrifugation through a BIOMAX™-50 kDa cutoff ultrafree membrane (Millipore) at 2000xg for 20 min or more...Partial purification of rFC was carried out... by gel filtration chromatography through SEPHADEX G-100."* The relative activities of these rFCs were then compared in Example 5, which discloses the following:

*"A comparison of the amount of total proteins present in the cell lysate (Lysate: 50 µg) and culture supernatant (Sup: 5 µg) illustrates that the culture supernatant from 72 h p.i. contained rFC that is >5-10 fold more effective in LPS detection. Twenty µg of BIOMAX™-purified rFC was able to detect 0.01 ng LPS. Using 40 to 80 µg of this protein, the detection limit could be easily extended to LPS levels below 0.01 pg or 0.001 ng/ml (FIG. 4). Purification of rFC by chromatography through SEPHADEX™ G-100 yielded enzymatic activity of even higher sensitivity to LPS (FIG. 5). **It is envisaged that more elaborate purification of rFC following the methods covered in reference 24 would vastly improve the efficacy of the rFC for endotoxin detection**" (col. 13, lines 12-25; emphasis added).*

178. As may be taken from Figure 5 of **D5**, *"In both the fluorimetric and calorimetric assays, further purification of BIOMAX™-purified rFC by SEPHADEX™ G-100 dramatically improved the sensitivity of rFC to LPS" (col. 5, lines 12-15; emphasis added).*
179. Accordingly, it has been shown in **D5** that the purified form of Factor C exhibits a *"dramatically improved"* sensitivity to LPS than the protein which is contained in the culture supernatant.
180. In view of these results, there are clearly serious reasons to doubt that the activity of the *Limulus* Factor C is improved when it is contained in a culture solution or culture supernatant.
181. Further, there is simply no comparative data whatsoever in the opposed patent which shows that the activity of Factor C is improved when it is contained in a culture solution or culture supernatant. Therefore, the Proprietor has not discharged their onus of proof that the alleged effect has its origin in the distinguishing feature, contrary to established case law:

"According to the established case law, an unexpected effect (advantageous effect or feature) demonstrated in a comparative test can be taken as an indication of inventive step (T 181/82, OJ 1984, 401). In T 197/86 (OJ 1989, 371) the board supplemented the principles laid down in T 181/82, according to which, where comparative tests were

submitted as evidence of an unexpected effect, there had to be the closest possible structural approximation in a comparable type of use to the subject-matter claimed. It stated that if comparative tests are chosen to demonstrate an inventive step on the basis of an improved effect over a claimed area, the nature of the comparison with the closest state of the art must be such that the alleged advantage or effect is convincingly shown to have its origin in the distinguishing feature of the invention compared with the closest state of the art (see also T 234/03, T 568/11, T 1457/13, T 1521/13; T 1401/14; T 710/16, T 990/17, T 2406/18, T 816/16)" (page 207, Section I.D.4.3.2 of the CLB, 2022; emphasis added).

182. First, the "comparative experiments" performed in example (8) were carried out using equal volumes of culture supernatant. According to the opposed patent, *"the same volume of each of the recombinant factor C (LCF or TFC)"* was added to equivalent reaction vessels comprising further components for carrying out the clotting cascade (par. [0191]). Accordingly, any volume of culture supernatant or culture solution in the comparative reaction vessels was nominally the same. Since the presence of culture supernatant or culture solution was never tested as a variable, these experiments cannot possibly convincingly show that it is the origin of the alleged effect.

183. Second, we submit that the details of example (8) are so vague that the results of this experiment cannot possibly be verified. However, it is established case law that any comparative test relied upon for the basis of inventive step must be reproducible such that the results of such test can be directly verifiable. In that regard, we refer to the following passage from the CLB:

"To be of relevance in demonstrating that a technical improvement is achieved in comparison with the closest state of the art, any comparative test presented must be reproducible on the basis of the information thus provided, thereby rendering the results of such tests directly verifiable (T 494/99, T 234/03, T 236/09, T 1962/12, T 383/13). This requirement implies, in particular, that the procedure for performing the test relies on quantitative information enabling the person skilled in the art to reproduce it reliably and validly (T 234/03, T 236/09, T 383/13, T 1962/12, T 532/14, T 795/14). Vague and imprecise operating instructions render the test inappropriate and thus irrelevant (T 234/03, T 236/09, T 383/13, T 1962/12, T 795/14)" (page 207 Section I.D.4.3.2 of the CLB, 2022; emphasis added).

184. The description of example (8) clearly does not pass the bar of reproducibility according to the case law outlined above.

185. Not only is this disclosure completely silent regarding the actual concentrations of Factor C used, but it further fails to disclose, e.g., the relative ratios of the different factors used in the cascade, which expression hosts were used to produce each recombinant protein, which culture solutions were used and the amount of culture solution or culture supernatant in each reaction vessel (i.e., it does not disclose the final solution composition in each reaction vessel).

186. Of particular interest, we would emphasize again that the opposed patent does not clearly and unambiguously disclose which culture solutions were used for the expression either LFC or TFC in example (8). To further complicate matters, the description merely states that the LFC and TFC were “*obtained as a culture supernatant fraction*” (par. [0188]), without specifying how the supernatant was collected (e.g., which centrifuge speed was used, for how long and at which temperature?). Accordingly, not only is it completely unclear whether the “comparative example” was carried out using the same starting culture solutions for LFC and TFC, but it is also unclear to what extent the contents of those solutions were ultimately concentrated while obtaining each supernatant. Therefore, even if the same “culture solution” was initially used for the expression of LFC and TFC, the final composition of each solution may have varied significantly in terms of salt concentration, which is known to affect Factor C activity (*cf.*, **D1**, Figure 7).
187. Regarding the amount of culture solution or culture supernatant used in each reaction vessel, we note the following. According to par. [0190], the recombinant Factor B and Proclotting enzyme that were used in the example were obtained “using the method described in...Example 2 [of WO 2014/092079]”, which corresponds to **D1** (*cf.*, par. [0190] of the opposed patent). However, according to Example 2 of **D1**, the method for producing a recombinant Factor B and Proclotting enzyme involves a step of **filtering** the supernatants containing each of the proteins. In that regard, we refer to par. [0252] of **D1**, which discloses the following:

“Through the same method as employed in the case of Factor C, the expression plasmids were each incorporated into Sf9 cells, high-expression clones were selected, and culture supernatants thereof were prepared. The culture supernatants were individually filtered through a 0.22-mm filter, and the obtained fractions (i.e. filtrates) were employed.”
(emphasis added)

188. In view of the above, it must be assumed that filtrates of recombinant Factor B and Proclotting enzyme were used in example (8) of the opposed patent. In this case, any amount of culture solution or supernatant in each reaction vessel could only come from the addition of Factor C to the solution, which may have been significantly diluted upon addition to the other components in the solution¹⁴. Accordingly, it is entirely unclear what amount of culture solution or supernatant was actually present in the solution when the activity was measured. To add further confusion to this variable, the description states that the activity in example (8) “was measured by the method described in the above (7)” (par. [0191]). However, this method described in (7) discloses the use of a 50 mM tris buffer (*cf.*, par. [0182]). Was this component also present in (8)? If so, then the experiment in example (8) was clearly not carried out “in a culture solution or culture supernatant”.
189. In summary, the Proprietor has argued that the claimed subject matter is distinguished from the prior art in terms of its solution composition (i.e., by the requirement that it is contained in a culture

¹⁴ Since the volumes of each component are not disclosed, it is not possible to determine a dilution factor for the supernatant

solution or culture supernatant). However, this is not a difference compared to **D1** (**D5** or **D6**), so any alleged effect based on this difference should be disregarded.

190. This is confirmed in the recent decision **T 1343/19**. When faced with evidence of an effect that was demonstrated over a piece of prior art *not* representing the closest prior art, the Board clearly held that such effect is not to be taken into account:

*[...] it is the established case law of the boards in the context of the problem and solution approach that the only effects taken into account for the formulation of the problem are those demonstrated to be caused by features by which the claimed invention and the prior art, **which is used as the starting point** in accordance with the problem and solution approach, **differ** (see also CLBA, section I.D.2). Hence, since the current analysis starts with the dosage regimen of GA 40 mg QOD, **evidence comparing the claimed dosage regimen with regimens other than this dosage regimen is not relevant.**" (Reasons 43; emphasis added)*

191. In any case, the experiments are unreliable. Not only have they failed to carry out the appropriate comparative experiments to convincingly show that any alleged effect has its origin in this feature, but they have further failed to disclose the necessary experimental details to verify their alleged effect. Moreover, we have shown that there are serious reasons to doubt that containing a Factor C in a culture solution or culture supernatant would lead to the alleged technical effect.
192. Accordingly, we submit that the Proprietor's arguments which allege that the "culture solution or culture supernatant" leads to a surprising technical effect should be disregarded entirely.

VI.7 Summary

193. In summary, claim 1 merely describes a method for recombinantly producing a well-known protein, whose steps also formed part of the state of the art (as acknowledged by the Proprietor in their submission to the USPTO). Given the strong incentive to recombinantly produce this known protein, we are of the view that any one of **D1**, **D5**, or **D6** could equally serve as closest prior art. Irrespective of which closest prior art is selected, the problem would merely amount to deriving the full-length sequences recited in items (A)-(C). To the extent that reciting the inherent sequence of a known protein may even be considered novel, it certainly does not involve an inventive step, since these sequences can readily be determined by the skilled person using common general knowledge techniques.
194. Further, and as we have explained above, the Limulus cascade was "*the compendial test for the examination of bacterial endotoxin*" for decades and it was common general knowledge that recombinant versions of Factor C could be used as an alternative "*comparable to the natural equivalent*" in this assay (**D10**, page 2, 1st par. under the Figure caption). Thus, the method of claim 2 is entirely obvious in view of the common general knowledge.

195. The alleged technical advantage proposed by the Proprietor should be ignored in the inventive step analysis. Firstly, arriving at the claimed solution (providing the *Limulus* FC sequence) is so straightforward based on the prior art that any alleged effect is a mere bonus effect, which cannot impart an inventive step. Secondly, the alleged advantage compared to the closest prior art is not adequately proven.
196. Taken together, we simply see no basis for acknowledging an inventive step for the claimed subject matter.

VII. Sufficiency of Disclosure (Article 100(b) EPC in connection with Article 83 EPC)

197. We submit that the skilled person cannot carry out the claimed method over the scope of the claim, contrary to Article 83 EPC.
198. As we have already noted above, the method of claim 1 merely requires a step of “artificially expressing” the Factor C protein. The breadth of this claim is such that it does not even require the use of a host cell, but also encompasses cell-free expression systems. In particular, the patent specifically teaches the following:

“The recombinant first protein can be produced by, for example, utilizing a heterologous expression system or a cell-free protein synthesis system” (par. [0037]; emphasis added).

199. However, it is common general knowledge that protein expression in cell-free systems is typically carried out in extracts and lysate solutions. In that regard, we refer to the following evidence presented in **D30** (Chong, 2014):

“Commercial cell-free systems are now available from a variety of material sources, ranging from “traditional” E. coli, rabbit reticulocyte lysate and wheat germ extracts to recent insect and human cell extracts to defined systems reconstituted from purified recombinant components” (abstract; emphasis added).

200. Since cell-free expression is carried out in an extract or lysate solution, without the use of cells, it is clear that the expressed protein cannot be collected from a culture solution or culture supernatant, if this type of system is used to “artificially express” it.
201. Moreover, even if a host cell is to be used to “artificially express” the protein, there is further no limitation as to which type of cell may be used. According to the opposed patent, this optional method for expressing the protein can be carried out using any host cell *“other than a horseshoe crab”* (par. [0036], [0038]). Further guidance is given in par. [0040], which states the following:

“The host cell other than a horseshoe crab is not particularly limited as long as it can functionally express the protein from the first cDNA. Examples of the host cell other than a

*horseshoe crab include mammalian cells and insect cells. Examples of mammals include rodents and primates...The primates are not particularly limited, however, examples thereof include humans, **monkeys**, and chimpanzees*" (emphasis added).

202. However, it has been shown that if Factor C is produced using monkey cells, such as COS-1 cells, the expressed protein is "associated with the cellular debris" and is not secreted into the culture medium (D31, Dwarakanath, page 360, right col., 2nd par.). Thus, it has been shown that the expressed protein cannot be collected from a culture solution or culture supernatant, if this type of host cell is used to "artificially express" the Factor C.
203. In view of the above, we submit that the method of claim 1 has not been enabled over its scope and thus does not meet the requirements of Article 83 EPC.

VIII. Conclusion

204. In view of arguments outlined above, it is apparent the claimed subject matter does not meet the requirements of the EPC. Our request to revoke the patent in its entirety on any one of the grounds according to Article 100(a), 100(b) or 100(c) is entirely justified.



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Enclosures

Cited documents **D1 to D32, Annex I-II and Appendices A-D**