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November 21, 2025

Opposition against European Patent EP 3 530 670
(Application No. 17862926.7)

Title:	METHOD FOR PRODUCING ENDOTOXIN DETECTING AGENT COMPRISING RECOMBINANT LIMULUS FACTOR C AND USE THEREOF
Proprietor:	Seikagaku Corporation
Opponent:	Sandra Pohlman
Our Ref.:	SP27447OP

In Response to the Summons to Attend Oral Proceedings pursuant to Rule 115(1) EPC dated August 29, 2025

1. This is in response to the Summons to Oral Proceedings and the Preliminary Opinion (PO) of the Opposition Division (OD) of August 29, 2025.

2. We hereby confirm that the undersigned representatives will attend the Oral Proceedings scheduled for January 21, 2025.

3. At the Oral Proceedings, we expect to be accompanied by Dr. Tom Schwarzer, a European patent attorney of the df-mp firm. Any submission that we will make at the Oral Proceedings will be in English. Assuming that the Patentee will make their submissions in English, no translation from English will be required.

4. In the following we will provide our comments on the OD's PO, in particular on added matter and on the proper selection of the starting point for the assessment of inventive step. We additionally provide our comments as to why we do not agree with the OD's preliminary finding that the alleged technical effect is not a mere bonus effect., since there is a sufficiently clear pointer to Limulus Factor C, which the skilled person is expected to follow. Also included

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are comments on some of the Proprietor's recent arguments provided in their latest submission of October 23, 2025.

5. For the avoidance of doubt, we explicitly maintain all our previous arguments raised in our Notice of Opposition and in **our Reply** to the Proprietor's Observations **dated August 25, 2025** (hereinafter "Reply"), including our objections to the alleged technical effect. Since it appears that the OD did not have the opportunity to adequately consider the detailed arguments therein when drafting the PO, these arguments are, for the most part, consolidated herein for the convenience of the OD.

I. New documents submitted herewith

6. We herewith submit documents **D48** (Yamashita et al, 2023) and **D49** (Masakazu, 2020). These documents are submitted in direct response to the Proprietor's incorrect assertions under item 45 of their latest submission and contain data demonstrating that factor C protein (FC) is not the only-rate-determining enzyme in the experimental set-up (i.e., a three-enzyme cascade reaction) chosen by the Proprietor for showing an allegedly greater activity of LFC over TFC. The results obtained in **D48** and the discussion in **D49** reveal that the signal generated by the cascade reaction is not linearly dependent on FC activity alone and are thereby *highly relevant* for interpreting the results in the opposed patent, in **D33** and **D47**. Moreover, the Proprietor can be expected to be already familiar with the data relied upon, given that the work reported in **D48** was supported by the joint research fund of the Seikagaku Corporation (i.e., the Proprietor) and the explanations in **D49** are a direct result of the known catalytic efficiencies already reported in **D48**.

7. Also submitted herewith are US 6,569,484 B1 (**D50**) and Cheong et al., 2023 (**D51**), which are filed in direct reply to the Proprietor's assertion that the meaning of the term "soluble fraction" is irrelevant because it does not reflect the wording of the claim, whereas we will explain below, *inter alia* by reference to **D50** and **D51**, that there is no difference in meaning.

II. The new claim requests ARs 12-29 should not be admitted

8. With their latest submission, the Proprietor filed 18 new claim requests, ARs 12-29. These requests are late-filed. Impermissibly, these requests are not based on granted claims but instead bring in language from the description at a very late stage, potentially necessitating a new search. They raise numerous new issues (in addition to the issues we have pointed out in section III.2 of

our Reply with regard to ARs 4-7, 10 and 11, on which the new ARs are based) and should therefore not be admitted.

9. Claim 1 of new **ARs 12-17** additionally recites that the cDNA may contain the base sequence represented by SEQ ID NO: 1 or 3, or a base sequence having 99% or more identity with the base sequence represented by SEQ ID NO: 1 or 3. This definition is closely related to options (A) and (B) of paragraph [0121] of the application as filed, which is a DNA-level definition. In view of the definition of the claimed cDNA sequence on a different structural level (i.e., on the DNA level rather than on the protein level), this definition clearly represents an *aliud*, compared to the subject matter encompassed by the granted claims and the amendment is thus in breach of Art. 123(3) EPC. For this reason alone, these ARs should not be held inadmissible.

10. Claim 1 of new **ARs 18-23** additionally recites that the recombinant protein of feature (B) contains a conservative substitution of one or several amino acids. It is highly unclear whether some or all of the possible amino acid substitutions must be conservative. The amendment thus does not comply with Art. 84 EPC and should be held inadmissible for this reason alone. It also follows that this amendment does not clearly delimit the claimed subject matter if only one of the substitutions is conservative, so it cannot be regarded as a serious attempt to overcome a Ground of Opposition, contrary to Rule 80 EPC. As regards basis, the Proprietor indicated paragraphs [029], [0123] and [0124] of the application as originally filed. But, paragraphs [0123] and [0124] refer to variants of the cDNA and hence, refer to identity of 95% or more at the cDNA level, and do not mention conservative amino acid substitutions at all. These passages cannot support the amendment made. Paragraph [029] explicitly refers to protein variants *per se* and thus cannot lend basis to the amendment which requires a cDNA encoding it, contrary to Art. 123(2) EPC. Said paragraphs do not clearly and unambiguously disclose that a recombinant protein "*expressed as containing an amino acid sequence having 99% or more identity with the amino acid sequence of SEQ ID NO: 2 or 4*" is additionally required to contain a conservative substitution of one or several amino acids. There is no basis under Art. 123(2) EPC in [029] for this combination of features.

11. Claim 1(B) of new **ARs 24-29** additionally recites a variant of (A) that has a conservative substitution that maintains the function of the protein normal at 1 to 5 sites in SEQ ID NO: 2 or 4. What has been said before regarding lack of basis in paragraphs [123] and [124] of ARs 18-23 equally applies with respect to ARs 24-29. There is also no direct and unambiguous basis for the feature "*at 1 to 5 sites*" in the claim's context. At the very least, the amendment requires at least one further selection of conservative mutation that maintains the function of the protein normal in combination with yet another selection from the equal options of "1 to 20", "1 to 10", "1 to 5", or "1 to 3" recited in [029], thus contravening Article 123(2) EPC.

12. In addition, ARs 24-29 are even more unclear than ARs 18-23 because of the introduced ambiguity as to whether the function of the protein should remain normal with respect to 1-5 sites or if it should remain normal despite a substitution at 1-5 sites. Additionally, the amino acid substitution remains undefined in the claim with respect to whether the same amino acid substitution is introduced at 1 to 5 sites, or whether different and even multiple amino acid substitutions may be introduced at each site, such that the feature lacks clarity. Worse still, claim 1(B) of new ARs 24-29 has the requirement in the granted claims for 95% or more identity deleted, and thus, it more broadly refers to a variant of (A) having (i.e., comprising) a conservative amino acid substitution that maintains the function of the protein normal at 1 to 5 sites in SEQ ID NO: 2 or 4 but which does not exclude the presence of further mutations: E.g., due to the "having" language, the protein may comprise an unlimited number of additional insertions, deletions or substitutions, resulting in a protein that no longer fulfills the requirement for at least 95% identity to SEQ ID NO: 2 or SEQ ID NO: 4. These ARs thus further contravene Article 123(3) EPC.

III. Documents D36-D43 filed with our previous Reply should be admitted

13. The Proprietor argues that documents **D36-D43** filed with our Reply should not be admitted because they are late-filed and not *prima facie* relevant.

14. First of all, we do not believe these documents are late-filed. They were filed well before the Rule 116 EPC deadline and in direct reply to the Proprietor. Proprietor has apparently had sufficient time to provide detailed comments on these documents.

15. **D36** and **D37** were filed in reaction to the Proprietor's new claims requests filed on May 9, 2025 and in particular to show that these new claims contain a two-list selection. The Proprietor claims that documents **D36** and **D37** are not prima facie relevant because these documents were cited under Art 123(2) but do not provide an assessment of the application as filed. However, as we have already explained in our Reply, the Board's PO in **T 0012/23-3.3.08** (**D36**) is highly relevant for the present claims. **D36** relates to another patent from the Proprietor and discusses the same feature (i.e., the culture supernatant which corresponds in essence to the solution fraction feature) as present in the claims of the MR. Moreover, the relevant passages in the corresponding application are highly comparable to the ones in the application corresponding to the opposed patent. It is our position that the minor difference in wording in the claims assessed in **T 0012/23-3.3.08** and the present MR does not overcome the added matter problem addressed by the Board in **D36**. Thus, the Board's assessment expressed in **D36** is immediately relevant also to the present claims of the MR and even more so to AR2 and subsequent ARs including the same amendment.

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16. **D38** was filed in reaction to the Proprietor's new claims requests filed on May 9, 2025. **D38** is similarly criticized for being not *prima facie* relevant, because the term "solution fraction" is not used in the application as originally filed or the granted claims. However, it is apparent that the somewhat unusual term "solution fraction" is merely the result of the awkward English translation filed on March 14, 2019 with entry into the EPO regional phase by the Proprietor, but that there is no essential difference in meaning of the terms "solution fraction" and "soluble fraction" (*cf.*, paragraph [0044]). This is consistent with the use of the term "solution fraction" in the very few technical documents actually using this term in a similar context, see, e.g., US 6,569,484 B1 (submitted herewith as **D50**[1]) or Cheong et al., 2023 (submitted herewith as **D51**[2]). What the Proprietor moreover apparently missed is that the gold standard requires that an amendment is directly and unambiguously derivable, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the application documents as filed. **D38** is evidence of the common general knowledge and informs the skilled person's understanding of the term soluble/solution fraction. It is thus highly relevant for the question of whether there has been an undue selection of a group of soluble/solution fractions disclosed in paragraph [0044].

17. Finally, the Proprietor requests that documents **D39-D43** should not be admitted. However, these documents were clearly filed in reply to the introduction of ARs 1 and 8 into the proceedings, in order to show that the feature in AR1 and AR8 of "mammalian cell" or "CHO or HEK cell" taken from the description is unsuited to establish an inventive step, as we have explained in section OV.6 of our Reply. As such, these documents could not have been filed any earlier. In any event, they are *prima facie* relevant to the assessment of inventive step of the new features relating to mammalian cells.

IV. The Main Request contains Added Matter (Art. 100(c) and Art. 123(2) EPC)

18. The OD is of the preliminary opinion that the Main Request (MR) meets the requirements of Article 123(2) EPC, stating that the deletion of option (C) and the resulting definition of the cDNA according to options (D) and (E) from paragraph [0121] of the application as filed does not represent a singling out of options from a list of non-converging alternatives, but rather a combination of two nearly identical polypeptide sequences SEQ ID NO:2 and 4 with another sequence definition, i.e. a broader definition allowing 95% or greater identity (*cf.*, items 8 and 11-12 of the OD's PO). The OD further considers the definition of the solution fraction as either "*a*

[1] The 4th paragraph of Col. 3 of **D50** discloses that "[t]he soybean solution mixture is then subjected to centrifugation at about 5,000 to 10,000 rpm for 10 to 20 min. to separate a **protein solution fraction** from a soybean residue fraction" (emphasis added), i.e., that a solution fraction is a fraction in which particulate matter has been removed.

[2] Along the same lines, the 1st sentence of the results on page 4 of **D51** discloses that "*protein was synthesized in an E. coli expression system and purified from the **solution fraction** of the cell lysates using immobilized metal affinity chromatography*"; it is further apparent from the methods section on "Protein synthesis and purification" on page 3 that the solution fraction corresponds to the "clarified supernatant containing the soluble proteins", which has been obtained by centrifuging at 10,000 xg for 10 min.

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culture solution or culture supernatant" to be a mere deletion of alternatives from a list (*cf.*, item 12 of the OD's PO).

19. We do, however, maintain that claim 1 of the MR contains added matter due to impermissible selections from the original disclosure, contrary to Article 123(2) EPC. The OD's PO apparently did not take into account our detailed reasoning provided with our reply to the Proprietor's observations dated August 25, 2025, as to why the subject matter of claim 1 of the MR is even more problematic in terms of added matter than claim 1 as granted.

20. As stated therein, compared with the original disclosure of the cDNA definition, current claim 1 defines the cDNA only on the basis of the encoded amino acid sequences, namely SEQ ID NO: 2 or 4, or sequences having 95% or more identity thereto. As correctly noted by the OD, these definitions correspond to options (D) and (E) of paragraph [0121] of the application as filed. However, paragraph [0121] presents five distinct options (A)-(E) for "the first cDNA", defining it in alternative ways and on different structural levels. Features (A) and (B) define the cDNA by way of its base sequence, whereas feature (C) defines the first cDNA by its ability to hybridize to a reference DNA, and features (D) and (E) define the cDNA only on the level of the encoded amino acid sequence.

21. Claim 1, as amended, retains only the amino acid sequence-based definition. This is not a mere deletion of alternatives from a non-converging list; rather, it constitutes an impermissible selection from three *different structural categories* for defining cDNA. Importantly, the application as filed explicitly states that "*the most preferred is the above-mentioned cDNA (A)*" (*cf.* paragraph [0123]), which refers to cDNA containing the base sequence represented by SEQ ID NO: 1 or 3 (i.e., a DNA-level definition). This explicit preference for a *different type* of definition of the sequence further highlights that the current selection, focusing solely on amino acid sequence identity, is not particularly preferred but the result of a selection from a list of several equal (non-preferred) alternatives for the definition of the first cDNA sequence. Such selection lacks direct and unambiguous basis in the application as filed, thereby violating Article 123(2) EPC (as we have explained in greater detail under items 8-11 and 19-22 of our Reply).

22. Secondly, the claim combines this first selection with a further selection, namely that of the "solution fraction". The present definition of solution fraction recited in the claim cannot be viewed merely as the shrinking of a generic group.

23. Rather, for the definition of the solution fraction feature of the "wherein clause", paragraph [0044] of the application as filed provides a longer list of different alternative types of solution fractions, including "*a cell homogenate extract, a mixture thereof, or the like*". The original application presents all these options on equal footing, with no pointer to "*a culture solution, or a culture supernatant*" over the other alternatives. The OD's preliminary assessment that a "*mere deletion*

of alternatives" (*cf.*, item 12 of the OD's PO) is required to arrive at this definition fails to take into account the different nature of the options recited in paragraph [0044].

24. As we have explained in greater detail under items 15-16 of our Reply, the limitation to a "*a culture solution, or a culture supernatant*" singles out solution fractions (also commonly known as the soluble fraction) in which the recombinant Factor C has been secreted into the extracellular space and which are free from intracellular components. The exclusion of other (especially the non-secreted forms of) solution fractions by way of selecting "*a culture solution or culture supernatant*" is not a simple quantitative reduction of options but requires a specific technical choice for secreted proteins, which has consequences in the downstream processing of the solution fraction obtained (e.g., for protein isolation and purity), and thus, singles out a particular combination of a specific fraction sub-class which introduces new information that is not directly and unambiguously derivable from the original application (compare **T 50/97**, which identified this as objectionable selection of "*a hitherto not specifically mentioned sub-class of compounds*", see also CLBA11 II.E.1.6.3).

25. On the question of whether or not it suffices that the remaining subject matter (in respect of both the definition of the cDNA and the solution fraction) still relates to a generically defined group, the Board in **T 795/21** observed that

> *"in accordance with the established jurisprudence regarding the deletion of meanings from multiple lists defining variables in a generic formula (see Case Law of the Boards of Appeal, 10th Edition, II.E.1.6.3 with reference to T 615/95, T 859/94, T 50/97, T 783/09, T 948/02 and T 801/02) it is not sufficient that the remaining subject-matter still relates to a generically defined group of compounds. In order to comply with Article 123(2) the deletion* ***must not result in a particular combination of specific meanings*** *which was not originally disclosed and which thereby generates another invention."* (Reasons 4.1.2; emphasis added)

26. Our position is further supported by Board of Appeal decision **T 0012/23-3.3.08** (**D36**), which, in a highly similar case involving EP 3 441 466 A1 of the Proprietor (**D37**), concluded that a disclosure analogous to paragraph [0044] of the present application as filed contained no pointer to "culture supernatant", and thus its selection constituted added matter. The Board's reasoning is directly applicable to present claim 1 of the MR, as we have explained in more detail under items 17-18 of our reply to the Proprietor's observations.

27. The Proprietor now cites **T 1713/19**, arguing that a specific level of sequence identity in combination with a specific sequence does not single out a particular molecule. However, this Decision (as well as Decision **T 2134/10** referred to by the Board in this respect) only finds that a given sequence identity (or similarity) in combination with the corresponding base sequence *alone*

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do not result in the selection of features from two (or three) independent lists. In the present case, however, the situation is different in that a certain way of defining the sequence identity in relation to a base sequence (i.e., on a particular structural level) is combined with an entirely different property selected from another list (i.e., certain solution fractions). These properties are not linked but are fully independent of one another (*cf.*, **T 1259/16**, Reasons 37). Therefore, **T 1713/19** does not apply to the present case. Moreover, **T 1713/19** is not generally followed, and other recent decisions still support that the selection of a particular level of sequence identity can be a selection from a list (see, e.g., **T 1767/18**, Reasons 31).

28. Therefore, the combination of these two independent selections from distinct lists, without any direct and unambiguous disclosure or pointer in the application as filed, results in subject matter extending beyond the content of the application as filed, thereby violating Article 123(2) EPC.

V. The Main Request lacks an Inventive Step (Art. 100(a) and Art. 56 EPC)

29. The OD is of the preliminary opinion that the MR meets the requirements of inventive step. The OD acknowledges that any of **D1**, **D5**, or **D6** could serve as closest prior art, but identifies the distinguishing feature as "*the use of the L. polyphemus Factor C sequence for production of recombinant Factor C*" and the effect of this difference as "*an increase in enzymatic activity*". Consequently, the objective technical problem is defined as "providing an *improved* recombinant Factor C," with the solution being the use of cDNA encoding *L. polyphemus* Factor C. The OD concludes that this solution would be inventive because the prior art does not suggest that this recombinant protein would exhibit enhanced Factor C activity (*cf.*, items 20-22 of the OD's PO).

30. The Opponent respectfully disagrees with the OD's preliminary opinion and maintains that the subject matter of claim 1 lacks an inventive step.

31. The closest prior art **D1** essentially teaches all of the features of claim 1: Paragraph [0150] of **D1** teaches that "[t]*he factor C of the present invention is a Factor C derived from a horseshoe crab*". The same paragraph also explains that "*the Factor C of the present invention is a recombinant protein*" and that a "*recombinant protein*" refers to a "*protein obtained through introduction of a* ***gene encoding the protein*** *into a host cell and* ***heterologous expression of the gene***" (emphasis added). In paragraph [0151], *Limulus polyphemus* is then disclosed as one of only four examples of *the* horseshoe crab. 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). Remarkably, the procedure for obtaining recombinant Factor C in

Example (8) of the opposed patent is identical to the one already taught in paragraph [0241] of Example 1.(2) of **D1** (*cf.*, paragraph 44 of our Notice of Opposition), which uses a culture supernatant. The skilled person thus only had to obtain the cDNA sequence of the Limulus Factor C in order to carry out the express teaching of **D1** and thus arrive at a method according to claim 1.

32. **D1** (or alternatively **D5** or **D6**) clearly suggests that obtaining a recombinant *Limulus polyphemus* Factor C is desired and once the cDNA sequence is obtained it will necessarily have its inherent properties when compared to any other recombinant Factor C cDNA sequence. Therefore, the alleged technical effect is a mere bonus effect and is to be disregarded when formulating the technical problem. In any event, we maintain that the technical effect is not sufficiently linked to the difference of utilizing the cDNA sequences of *Limulus polyphemus* Factor C (see section IV.3.2 of our Reply). The alleged technical effect is additionally not commensurate with the scope of the claims (see section IV.3.3 of our Reply).

33. Notably, at paragraph 22 of the PO, the OD held that "[i]*f the objective technical problem had merely been to provide an alternative Factor C sequence from a known species of horseshoe crab, the skilled person could have obtained the L. polyphemus Factor C cDNA using routine methods*". It is our position that, in light of OD's conclusion and in view of the clear and express teaching in **D1**, the skilled person not only could, but also **would** (Guidelines, G-VII, 5.3) have obtained recombinant *Limulus polyphemus* Factor C as a solution fraction containing the expressed recombinant protein based on expression from cDNA.

V.1 <u>The prior art contains a sufficient pointer to Limulus polyphemus Factor C, which is a suitable starting point for obtaining the cDNA sequence</u>

34. We agree that **D1** is a suitable closest prior art document. However, we disagree with the OD's implicit suggestion that the prior art does not sufficiently guide the skilled person to <u>*L. polyphemus*</u> Factor C (PO, item 20) so that this is *not* a suitable starting point. **D1** explicitly points to *Limulus polyphemus* Factor C, such that the skilled person is indeed guided to Limulus Factor C as a suitable starting point for obtaining the cDNA sequence (compare in this regard section IV.1 of our Reply, in particular paragraph 62). In particular, **D1**, in its "Modes for Carrying Out the Invention," explicitly discloses *Limulus polyphemus* Factor C as one specific option for the "*Factor C of the present invention*", which is a recombinant protein (**D1**, paragraphs [0150]-[0151]). The "invention" of **D1** is to provide a recombinant horseshoe crab Factor C having Factor C activity (see e.g. claims 1 and 2). Notably, [0150] of **D1** explains that "recombinant protein" refers to a protein obtained through introduction of a **gene encoding the protein** into a host cell and **heterologous expression of the gene** (emphasis added), and SEQ ID NO: 1 *(Tachypleus*

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tridentatus) and SEQ ID NO:3 (*Carcinoscorpius rotundicauda*) of [151] of **D1** represent Factor C **cDNA** ("gene") **sequences**. Considering the disclosure of **D1** as a whole, and not just those passages referring to the allegedly preferred embodiments, the skilled person learns from **D1** that *Limulus polyphemus* Factor C is a suitable endotoxin detection agent (see paragraphs 62-68 of our Reply for further details).[3]

35. Given the explicit reference in **D1**, the skilled person would have had a clear incentive to consider *Limulus polyphemus* Factor C as the starting point for developing the claimed invention, and next, to obtain the cDNA sequence thereof, which the OD concludes is possible using routine methods. Another reason to do so is that the *Carcinoscorpius* (CFC) and *Tachypleus* (TFC) cDNA sequences had already been provided by the prior art. Thus, the skilled person had a strong motivation to obtain the sequence of *Limulus polyphemus* Factor C as a next step. Notably, the skilled person was aware from **D1** (*cf.*, paragraph [0271]) that the recombinant expression of Factor C may positively affect the catalytic properties of FC and would have been highly motivated to reproduce this effect with *Limulus* Factor C.

36. It is also established EPO case law (**T 2443/18** and **T 787/17**) that the selection of TFC or CFC as a "more preferred" starting point is not decisive for the problem-solution approach. (see also **T 824/05**) (see paragraphs 81-85 of our Reply for further details). To the contrary, the indication of a preference in the prior art is plainly irrelevant to the issue of whether an embodiment should be selected for further development under inventive step (see **T 1654/22**, Reasons 1.1.5):

> *"There is no requirement in the case law that an embodiment of the state of the art must be exemplified for it to be considered in the assessment of inventive step. There is likewise* ***no requirement*** *that the prior art contains any pointer, suggestion or incentive to select a particular embodiment for further development for this embodiment to qualify as a starting point in the problem solution approach." (Emphasis added).*

37. Finally, there is no teaching in the prior art that would lead the skilled person away from using *Limulus* for recombinant Factor C production.

38. Accordingly, we submit that the prior art guides the skilled person to a *Limulus* Factor C as a suitable starting point for recombinant Factor C production.

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[3] We also maintain that **D5** or **D6** can serve as suitable closest prior art documents. Like **D1**, these documents contain explicit pointers towards using Limulus Factor C. **D5** discloses that cDNAs encoding Factor C of "any horseshoe crab", "***preferably*** *of the genus* ***Carcinoscorpius, Tachypleus or Limulus"*** (**D5**, col. 8, lines 6-8; col. 3, lines 22-25), are appropriate for expression, and even individualizes *Limulus* Factor C in claims 11-13. **D6**, in its description of "Purified Factor C," lists purified native Factor C of *Limulus polyphemus* as an example that can be used (**D6**, page 6, paragraph [21]), and also individualizes *Limulus* Factor C in claim 12.

39. The Proprietor cited **T 2759/17**, alleging that the case law requires a "realistic" starting point. We do not deny that a realistic starting point is in line with the problem-solution-approach (and no decision from another court, such as **D44**, is necessary to demonstrate this). However, we do not agree with the Proprietor's reasoning that Limulus Factor C would not be a reasonable starting point, as shown above. **T 2759/17** certainly does not support this notion. To the contrary, the Board in that case explains that

> *"the skilled person was normally confronted with a certain purpose or effect to be achieved in a certain technical field. With this in mind, the skilled person would then look for a prior-art disclosure that was in the* ***same technical field and aimed at the same or a similar purpose or effect****. This, in the board's view, was what was meant by the 'most promising springboard'" (Reasons 5.5; emphasis added).*

40. Moreover, the Proprietor took the quote from **T 2759/17** out of context to support their overly narrow view of what should be the closest prior art, whereas the Board in that case explicitly elaborated on the relevant criteria to assess if the skilled person realistically started from a disclosure within a prior art document. In full, the relevant passage quoted by the Proprietor reads as follows:

> *"Hence, a disclosure within a prior-art document can only be considered to represent a suitable starting point for assessing inventive step if the skilled person would have realistically started from it. An important consideration in this assessment is generally whether this disclosure* ***aims at the same or a similar purpose or effect*** *as that underlying the patent in question". (Reasons 5.6; emphasis added)*

41. In view of, e.g., paragraphs [0150]-[0151] of **D1** disclosing *Limulus polyphemus* Factor C as one "*mode for carrying out the invention*", there can be no doubt that Limulus Factor C is a realistic starting point.

V.2 A higher activity is at best a bonus effect and cannot confer inventiveness

42. The OD further opined that the alleged higher enzymatic activity of *Limulus* Factor C cannot be disregarded as a mere bonus effect. More specifically, the OD argued under item 21 of their PO that, "*when selecting a suitable cDNA sequence for producing horseshoe crab Factor C, the skilled person was not limited by a lack of alternatives, as the prior art disclosed Factor C sequences of T. tridentatus and C. rotundicauda. Since it was not inevitable for the skilled person to obtain the cDNA encoding L. polyphemus Factor C, they were not in a one-way street situation.*"

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We disagree with this assessment, which does not take account of the relevant facts of the case or the case law in relation to bonus effects or enhanced effects, as explained hereinbelow.

43. According to the case law of the boards of appeal, enhanced effects could not be adduced as evidence of inventive step if they emerged from obvious tests (**T 296/87**, OJ 1990, 195; **T 432/98**; **T 926/00**; **T 393/01**). In **T 308/99** the claimed use was based on a thoroughly obvious property of known substances. As in the present case, the slightly enhanced effects associated with the claimed use in comparison with substances used in prior art emerged from obvious tests. In **T 104/92** the board held that work involving mere routine experiments, such as merely conventional **trial-and-error** experimentation without employing skills beyond common general knowledge, lacked inventive step. (CLBA11 I.D.9.21.7; underline emphasis added)

44. At any rate, the OD's preliminary finding that it was not "*inevitable*" to obtain a cDNA encoding *Limulus* Factor C is not the correct legal standard. The relevant case law does not require that it would be "inevitable" to obtain the cDNA encoding *Limulus* Factor C, but rather only that embarking on such sequencing exercise would have been a **realistic option** from the prior art. Given the common general knowledge, the explicit references to *Limulus* in the closest prior art, and its prominent role as a standard endotoxin test reagent, the skilled person *would have* (not merely *could have*, as concluded by the OD) sought to determine its sequence. The clear motivation to solve the problem of *obtaining the sequence* (but also of obtaining *alternative sequences*) are proof of that, even if other alternatives existed. The fact that, based on, e.g., **D1's** disclosure of heterologous expression of Factor C, the choice of *Limulus* Factor C was a logical next step, combined with the routine nature of the task (as concluded by the OD, see item 22 of the PO), makes the technical effect a bonus effect and the solution obvious under Art. 56 EPC.

45. As we have explained above and in our previous submissions, the skilled person merely had to carry out the direct teaching of, e.g., **D1** regarding *Limulus* Factor C, which was explicitly disclosed as one of the specific "modes for Carrying out the Invention" of **D1**. In view of the OD's finding that the skilled person could have obtained the LFC sequence (item 22 of the PO), neither the implementation nor the testing of the approach suggested in **D1** involves any particular technical difficulties. Hence, **D1** puts the skilled person in a straightforward try-and-see situation (CLBA11 I.D.7.4). For instance, in **T 253/92** the subject-matter of claim 1 related to a process for the manufacture of a permanent-magnet alloy. In the board's view, a skilled person would have regarded it as obvious to try out a variety of alloys known from the prior art to be of similar composition to those of the better examples and to measure their magnetic properties. The same considerations apply to the presently claimed subject matter.

46. It is established case law that it is the normal task of a skilled person working in a certain field not to remain inactive but to seek alternatives, to be constantly occupied with the elimination of deficiencies, with the overcoming of drawbacks and with the achievement of improvements of

known devices and/or products (CLBA11, I.D.8.11). The background section in paragraph [002] of the opposed patent acknowledges that the four horseshoe crab species belong to only two genera, the genus *Tachypleus* (with the species *T. tridentatus*, *T. rotundicauda* (also known as *Carcinoscorpius rotundicauda*) and *T. gigas*) and the genus *Limulus* (with only one species, *L. polyphemus*). Given that Factor C sequences from the Genus *Tachypleus* are disclosed in **D1**, **D5** and **D6**, the skilled person would have had a high incentive to also obtain a recombinant Factor C of the only other genus, i.e., *Limulus*. Paragraph [0150]-[0151] of **D1** explicitly prompt the skilled person towards recombinant expression of Factor C, including from Limulus, and so the skilled person would naturally have been interested to obtain the Limulus Factor C and test its properties. In particular, **D1** also highlights that "*Factor C expressed in mammalian cells can have low susceptibility to activity inhibition by ions"* (paragraph [271]), and that this property may be expected for a recombinantly produced Factor C from any source including Limulus, see paragraphs [0166] and [0232] of **D1**, referring to "[t]*he Factor C of the present invention*" (see also paragraph 160 of our Reply).

47. We additionally point to the common general knowledge relating to the *Limulus* amoebocyte lysate (LAL) test at the priority date, which would inform the skilled person's reading of Limulus Factor C as a preferred recombinant protein amongst the limited choices disclosed in **D1** (or **D5** or **D6**). Significantly, the *Limulus* amoebocyte lysate (LAL) test was ***the*** compendial test for detecting endotoxin, with only one other species (*Tachypleus tridentatus*) also listed in the European and US pharmacopeias (*cf.*, chapter 2.6.14**,** first paragraph of **D34** or page 1, left column of **D35**). LAL (and to a lesser extent, TAL) thus represented the standard for endotoxin test for decades.

48. We have also referred to **D4** and **D10** as evidence of the common general knowledge in our previous submissions in this regard. For the OD's convenience, key passages from these documents are reproduced herein. For example, **D4** states that

> *"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)." (top paragraph on page 198; emphasis added)*

49. And, most noteworthy, **D10** explicitly calls out that

> *"The industry is heavily reliant upon the supply of LAL/TAL for endotoxin testing. However, scientists have now developed a recombinant version of Factor C (rFC)*[4] *that is comparable to the natural equivalent." (page 2, 1st par. under the figure caption)*

50. Hence, **D10** proves that, as of the priority date, recombinant Factor C was accepted in the art as being comparable to the natural equivalent, and the authors even concluded that "*it seems likely that rFC will become the go-to solution for BET* [bacterial endotoxin testing]". Thus, **D10** also shows that there was a desire for the development of a recombinant Factor C from Limulus instead of LAL in the technical field as of the priority date.

51. Our view is further supported by EPO case law. For instance, we refer to decision **T 53/22**, where the Board, when faced with a quite similar situation, explained that the presence of more than one possible way to implement the teaching of a prior art document does not rule out the presence of a bonus effect/one-way street situation. In the Case Law Book (CLBA11, I.D.10.8.2), **T 53/22** is referred to as highlighting that *"any additional effect stemming from the obvious combination in the case in hand (D2 in combination with common general knowledge) was a supplementary or bonus effect that could not establish an inventive step. The skilled person had* ***very few realistic options****,* ***each of which would have occurred to them as a matter of course when they considered how to realise D2's teaching*** *to realise a wastegate in a real turbocharger*" (emphasis added). More particularly, in reasons 3.5.1. of **T53/22** the Board held:

> *"3.5.1 The Board has already indicated above why it considers any further effect, and thus also the (further) reduction of misalignment, to be only secondary or supplementary to the primary effect of realizing D2's teaching. Because, as also explained above, there are only* ***very few realistic options*** *available to the skilled person when carrying out D2's teaching to press the plug into its seat by means of the actuator, and these are all known and thus obvious to them, the presence of a further effect (whether surprising or not) cannot change the fact that* ***each of these very few options is obvious****. The situation may be* ***different if there is a "multiplicity", as in "a large number", of options*** *as in the case law cited, e.g. T0192/82. There, and in other cited decisions, it was held that if a selection of one amongst many options could be associated with a (derivable and plausible) "surprising effect" then that selection was non-obvious by virtue of that surprising effect."* (emphasis added)

52. In sum, the mere straightforward *realization* of an explicit teaching in the prior art is not sufficient to render the claimed subject matter inventive, in particular if there are very few realistic options

[4] As we have pointed out before, the recombinant version referred to is recombinant TFC, but for the same reasons there was a motivation to produce a recombinant version of *Limulus* factor C instead of LAL.

available. Notably, the Board in **T 53/22** clarified that "very few realistic options" do not qualify as a "multiplicity" of options in the sense of **T 192/82**. The present case is very similar in that the skilled person would merely have to realize the direct teaching of, e.g., **D1** to use a cDNA encoding *Limulus* Factor C, and there were only very few other options available, in particular when taking into account the prominent role of *Limulus* amoebocyte lysate as a standard endotoxin test reagent.

53. We therefore stand by our position that the subject matter of claim 1 of the MR was obvious, regardless of whether or not the Proprietor's assertion of a higher activity is accepted. The same holds true for claim 2.

V.3 The alleged technical effects should not be taken into account

54. The OD preliminarily held under item 22 of their PO that "*if the objective technical problem had merely been to provide an alternative Factor C sequence from a known species of horseshoe crab, the skilled person could have obtained the L. polyphemus Factor C cDNA using routine methods, potentially leading to a lack of inventive step*" (emphasis added). While the OD concluded that the skilled person *could* have obtained the *L. polyphemus* Factor C cDNA, we emphasize that a skilled person also *would* have done so.

55. However, the OD preliminarily defines the objective technical problem as "providing an improved recombinant Factor C" based on an alleged "*increased* activity of *L. polyphemus* Factor C compared to *T. tridentatus* or *C. rotundicauda* (**D33**, fig. 4 and 7)" (*cf.*, item 21 of the OD's PO). We strongly contest the credibility and relevance of this alleged technical effect.

56. The OD's position is also not a sensible one when taking into account the correct starting point, which is the disclosure relating to *Limulus* Factor C within **D1** (or **D5,** or **D6)**, as we have explained above. If properly formulated, a comparison to another species of horseshoe crab simply does not figure in the formulation of the objective technical problem (**T 1445/21**) because the actual technical difference is merely the provision of a cDNA encoding the full-length sequence of the *Limulus* Factor C. Thus, the technical effect must be linked to the use of a cDNA in the claimed method but not in terms of enzymatic activity of the endotoxin agent produced. Proprietor's experiments are, however, not suitable to establish this link.

57. Thus, it is not appropriate to formulate the objective technical problem in terms of an *improvement*. In this regard we note once more that the Proprietor bears the burden of proof to demonstrate by appropriate evidence and to the required standard of proof that the claimed subject matter represents an improvement over the prior art, as explained in section IV.3.1 of our Reply. The

Proprietor's assertions in par. 38 of their latest submission are completely beside the point, as the statement in **T 2320/16** does not relate to a case where the objective technical problem is an alleged improvement (in contrast to the present case). Rather, as we had explained previously, according to **T 449/23** Reason 2.5.6, the burden of proof does **not** lay with the opponent to **demonstrate that the alleged technical effects were not present** (compare item 93 of our reply to Proprietor's observations).

58. The OD has so far only considered the question whether the concentration measurement of Factor C in the experiments provided in the opposed patent and in **D33** suffers from using an inappropriate antibody, but this is only *one element* of our argument.

59. In fact, the experimental evidence presented in the patent (Example 8) and **D33** is not appropriate to convincingly demonstrate higher enzymatic activity for *Limulus* Factor C compared to *T. tridentatus* (or *C. rotundicauda)* Factor C, nor that this alleged higher activity is linked to the distinguishing feature.

60. Key issues that we have previously discussed in section VI.3.2. of our Reply include:

- **Lack of comparable conditions**
- **Lack of quantitative data and controls**
- **Artificial normalization**

61. In the following we will summarize our earlier criticism relating to these defects and also provide some further comments on the Proprietor's latest submission.

Lack of comparable conditions

62. The Proprietor's comparison relies on Western blot bands using an antibody (2C12) specific for *Tachypleus* Factor C, raising serious doubts about its ability to accurately quantify *Limulus* Factor C (*cf.*, items 159-161of our Notice of Opposition). Similarly, as discussed in section IV.3.4 of our Reply, the data shown in **D33** provide at most a qualitative, relative comparison of the amount of LFC and TFC, but are unsuited for an accurate quantitative comparison (the same applies to the data in **D47**).

63. The Proprietor submitted **D45** to show that densitometry is known to allow for the measurement of protein amounts accurately. While this statement in its generality may be true enough, it depends on the specifics of the case whether an accurate quantification may be derivable from a given gel image. In the present case, the signal-to-noise ratio seems particularly problematic, it is not apparent that a background subtraction has been performed and, most importantly, the passage in **D45** explicitly mentions that the relative amount of protein in a sample can be

determined "*by comparing these measurements with known standards*", but an appropriate standard had **not** been included on the gels of the Proprietor.

64. These defects are compounded by the inappropriate use of different concentrations of Factor C in the experiments, i.e., a lack of adjusting Factor C concentration prior to adding equal *volumes* thereof. All of the experiments used firstly, a specific cascade reaction comprising Factor C of *Limulus*, *Tachypleus* or *Carcinoscorpius* in combination with *Tachypleus* Factor B as well as *Tachypleus* proclotting enzyme, and secondly, each individual experiment had highly undefined and thus, incomparable reaction conditions. Most notably, different preparations of recombinant Factor C in cell culture supernatant (unpurified) were used and with *different ratios* of the components (see IV.3.2. of our Reply).

65. In this regard we also note again that the Proprietor failed to demonstrate that Factor C is the only rate-determining enzyme under the given experimental conditions, let alone under *any* other relevant conditions. For instance, as shown in Yamashita et al., 2023 (submitted herewith as **D48**), 5 mM Ca^{2+} was able to enhance the overall reaction of the cascade reaction comprising wt FC, FB and PCE due to an enhanced activation of FB (see Fig. 2A, paragraph bridging pages 50 and 51 as well as page 52, 1st paragraph, last sentence). However, the same effect was not observed with an FB mutant with higher catalytic efficiency (K_{kat}/K_m) (see page 52, right column, last paragraph). The authors also explain in the 1st par. on page 52 that FB has the lowest catalytic efficiency within the three-enzyme cascade. More specifically, they explain that

> *"the k_{cat}/K_m value of WT chelicerase[5] B against the specific peptide substrate (k_{cat}/K_m = 5300 M^{-1} s^{-1}) is orders of magnitude lower than those of WT α-chelicerase C (k_{cat}/K_m = 370,000 M^{-1} s^{-1}) and WT CE (k_{cat}/K_m = 3,600,000 M^{-1} s^{-1})"* (emphasis added)

66. This example shows that changes in the FB activity (as the slowest enzyme in the cascade) can significantly influence the overall rate of the cascade reaction, and therefore, changes in the FB/FC ratio can indeed have an impact on the apparent performance of FC if determined in terms of output of the cascade reaction.

67. Moreover, the much higher catalytic efficiency of CE (i.e., activated PCE) as compared to FC and FB means that activation of PCE plays a major role in amplifying the signal in the LAL test and that amplification of the signal is thus placed after the activation of Factor B. This understanding is confirmed by Masakazu, 2020 (submitted herewith as **D49**; see page 36752, last par. and, 1st par. of the discussion on page 36755). The significant signal amplification by the last enzyme in the cascade, i.e., CE, further supports that minor variations in the activation of FB (e.g., due to a

[5] The authors of **D48** use the terms α-chelicerase C, chelicerase B and CE to refer to the activated forms of FC, FB and PCE, see page 48, 1st par.

different FC/FB ratio or an inaccurately determined concentration of FC) can additionally impact on the measured activity of the three-enzyme cascade, such that the (already minor) alleged difference between TFC and LFC of about 1.6/2.2-fold cannot be considered to reflect the actual difference between TFC and LFC. To sum it up, any difference in the output of the cascade reaction cannot be attributed solely and unequivocally to a difference in LFC activity.

68. The Proprietor alleged that "*one would expect to see no differences between the samples as in this case the rate would, anyhow, always be limited by the other enzymes in the cascade*" (item 45 of their latest submission). This is not in line with the evidence presented above, and also beside the point. The actual problem is that if there is not just one enzyme which is *always* rate-determining, as just explained, a change in the ratios of the components, as in the present case, can have **unpredictable** consequences on the overall reaction rate. This is why it is important to show linearity of the reaction rate depending on the amount of FC added when this amount is not held constant. No such assays with different amounts of factor C added were included. The reaction conditions for the different cascade reactions are insufficiently defined and simply not comparable, let alone reproducible.

Lack of quantitative data and controls

69. The patent lacks crucial experimental details such as actual concentrations of Factor C, relative ratios of components in the cascade system and a proper standard curve for normalization, making the results unreproducible and unverifiable (see again section IV.3.2 of our Reply).

70. In addition to the required difference of using a cDNA encoding a different amino acid sequence, the patent's example at paragraphs [190] and [191] contains **several undefined variables of the reaction solution**, the most relevant being the difference in the amount/concentration of TFC and LFC used. Due to different relative amounts of LFC and TFC (as explicitly stated in paragraph [189] of the opposed patent) in the obtained cell culture supernatants (from independent cell culture), the relative ratio of Factor C in combination with Factor B and PCE necessarily differed between LFC and TFC reaction solutions. Also, the volume of Factor C supernatant employed in the assay system in paragraph [190] remains entirely unspecified. Thus, it cannot be judged what the final concentration of each factor (and thus also their relative concentrations and ratio to FB) was and what the proportion of supernatant added within the 100 µl reaction mixture was.

Artificial normalization

71. In an attempt to make up for the above defects, the Proprietor presented *post-hoc*-normalized data in **D33** and **D47**, but we have demonstrated by a comparison of Fig. 6 of **D1** (applicant Seikagaku) with Fig. 4 of **D33 that a post-hoc normalization is not suitable to determine**

differences in specific enzymatic activity. For more details we refer to section IV.3.2 of our Reply.

72. This issue is all the more relevant as the ratio of the components of the reaction cascade may influence the overall reaction rate, as discussed above.

V.4 A more ambitious problem is not credibly solved across the whole scope claimed

73. As we have explained before in section IV.3.2 of our Reply, the Proprietor also failed to demonstrate that any effect allegedly shown is attributable to the specific LFC cDNA sequences claimed. Nor has it been credibly demonstrated that any such effect is achievable over the entire scope of the claim (IV.3.3 of our Reply).

74. The activity of Factor C is highly dependent on the expression system (e.g., host cell, expression vector, method of introduction of the vector into the cells) and reaction conditions (e.g., presence of salts). Following the established principle that each party bears the burden of proof for the facts it alleges, the onus to demonstrate that a more ambitious problem (i.e., an improvement) is solved across the whole scope claimed does not lie with the Opponent but with the Proprietor, as summarized and neatly explained in **T 449/23**.

75. In Example (8), in **D33** and in **D47**, the LFC used for the activity measurements was produced by transfection of the cDNA into mammalian cells via the ExpiCHO™ Expression System (Thermo Fisher Scientific) as described in the patent's example. Secondly, the patent at paragraph [188] discloses a specific expression plasmid, pCI-neo (Promega), for transfection into mammalian cells, such as CHO-DG44 cells. **D33** reports recombinant LFC (CHO) and recombinant LFC (HEK293). By contrast, claim 1 does not require the use of a specific expression system or a step of transfecting the cDNA into host cell. The method of claim 1 is not particularly limited as regards the way the cDNA is introduced into a host cell.

- We previously referred to **Fig. 7 of D1**, which illustrates that depending on the expression host the recombinant Factor C produced may behave differently (page 32 of our notice of opposition, item 163-164).

- Although only the supernatant from a single experiment with transfected CHO cells was analyzed in the patent (*cf.* 167 of our notice of opposition), a specific type of host cell is not required by the claimed method. In view of Fig. 7 of **D1 (i.e. the degree of inhibition varied in accordance with the type of injection and the type of host for expressing Factor C)**, it is simply not credible that whatever the host, the Factor C protein produced will exhibit higher activity towards the detection

of endotoxin (mAbs/min) as compared to *Tachypleus* Factor C which has been artificially expressed with any other system of one's choosing.

- In items 165-167 of our notice of opposition, we have presented evidence (Figure 4 of **D28**) that even the method by which the Factor C coding sequence is introduced into a given host cell can influence its activity (i.e., virus infected vs. transfected Sf9 cells).

76. As pointed out in item 168 of our notice of opposition and as may be taken from Figure 7 of **D1**, Factor C also **behaves quite differently depending on which salts are present** in the reaction solution for the activity measurement. This aspect is particularly relevant to the culture solution or culture supernatant comprising recombinant Factor C, since 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.

77. The broad claim scope, which does not limit the expression system or the reaction conditions, means that any effect demonstrated for highly homologous cDNAs encoding Factor C under specific conditions (e.g., expressed in a very specific expression system using a particular vector, transfection method and ExpiCHO or HEK293 cells and compared only in a particular cascade reaction) cannot be credibly extrapolated to the entire claim scope.

78. Since the MR is not limited to a specific sequence, an improvement has to be demonstrated even for sequences that are very close to the Tachypleus sequence, given that the TFC protein sequence is 92% identical to the claimed LFC sequences (*cf.*, par. 49 of our Notice of Opposition) and the claim requires an identity of only 95% thereto, which is very close.

79. In fact, the data in the opposed patent and in **D33** are also limited to a specific cascade reaction comprising Factor C of *Limulus*, *Tachypleus* or *Carcinoscorpius* and *Tachypleus* Factor B as well as *Tachypleus* proclotting enzyme, so generalizing any alleged effect to *any* reaction system comprising (only) LFC is not justified. See also, e.g., items 163-169 of our Notice of Opposition or section IV.3.3 of our reply to the Proprietor's observations.

80. We again refer to the comments made by the then applicant during prosecution of the opposed patent. In **D26**, applicant pointed to pre-published Fig. 2 of Tokunaga (cited as **D22** in our notice of opposition) showing a lower maximum rate of changes in absorbance of native Factor C purified from hemocyte lysate of *Limulus Polyphemus* ("NFLC") upon activation by alpha-chymotrypsin than native Factor C purified from hemocyte lysate of *Tachypleus* ("NTLC"). (Activity of NTFC (about 1.0 ΔA_{405nm}/5min) is higher than that of NLFC (about 0.6 ΔA_{405nm}/10 min, which is converted

to about 0.3 ΔA_{405nm}/5 min)). There is no evidence at all that purified recombinant LFC will exhibit higher activity than purified recombinant TFC in a single-enzyme reaction upon activation with alpha-chymotrypsin.

81. In fact, there is not even evidence that comparison of LFC alone vs. TFC alone would show an improvement when activated under *any* other conditions, such as by LPS.

82. There are thus many variables that may influence the activity of the Factor C. In line with the discussion, important parameters include (but are not limited to):

- FC sequence
- Expression system
- Expression vector
- Expression host
- Method of transfection
- Presence of different salts
- Method of activation of FC (by LPS, chymotrypsin, …)
- Single enzyme reaction vs. enzyme cascade reaction
- Ratio of FC, FB and proclotting enzyme
- Detection reaction

83. Since the alleged technical effect of "increased enzymatic activity" is not credibly demonstrated across the whole claim scope or attributable to the distinguishing feature, it cannot be used to formulate an ambitious objective technical problem. Thus, the problem should be reformulated less ambitiously as the *provision of an alternative recombinant Factor C sequence*.

84. The OD concluded that "*the skilled person could have obtained* the *Limulus Factor C cDNA using routine methods"* (PO, item 22). We maintain that the skilled person *would* have done so because it would have been an obvious next step for the skilled person.

VI. The Patent is Insufficiently Disclosed (Art. 100(b) and Art. 83 EPC)

85. The OD believes that the MR is sufficiently disclosed and therefore complies with Art. 83 EPC. According to item 13 of their PO, "*the reference to a culture solution or supernatant in claim 1 inherently implies the cultivation of whole cells, thus excluding the use of cell-free expression*

systems. Any potential discrepancy between the claimed subject-matter and a disclosure in the patent may, at most, result in a lack of clarity but is not a reason to deny sufficiency of disclosure."

86. However, the opposed patent itself explicitly teaches in paragraph [0037] that "[t]*he recombinant first protein can be produced by, for example, utilizing a heterologous expression system or a cell-free protein synthesis system".* This statement clearly indicates that cell-free expression systems are intended to be covered by the broad term "artificially expressing a protein" in claim 1, in line with **G 1/24**. As explained under item 200 of our Notice of Opposition with reference to **D30**, cell-free expression systems typically use extracts (i.e., cellular extracts but not whole cells) or lysate solutions. But in such a cell-free system, the expressed protein *cannot* be collected from a "culture solution or culture supernatant" because no cells are cultured. This makes the claim, within its entire scope as properly interpreted, unworkable.

87. The OD's assessment that the claim "inherently implies the cultivation of whole cells" contradicts the explicit teaching in the opposed patent. In fact, the claim covers cell-free protein synthesis systems regarding the process step of artificially expressing a protein, but the method of collecting the protein in the form of "*culture solution or supernatant*" is incompatible with these systems, so the invention is not enabled over this part of its scope. This is a fundamental insufficiency, not merely a clarity issue.

88. The OD further opined that **D31** does not raise serious doubts, substantiated by verifiable facts, that polypeptides falling within the scope of the claims could be expressed as soluble proteins in COS-1 cells and further that the selection of appropriate cell lines is considered to fall within the abilities of the skilled person.

89. We reiterate that the opposed patent states in paragraph [0040] that the host cell "*is not particularly limited as long as it can functionally express the protein from the first cDNA*" and provides examples of mammalian cells including "primates". COS-1 cells are a specific example of primate cells. We have explained under item 202 of our Notice of Opposition that **D31** reports the experimental finding that when Factor C is produced using COS-1 cells, the expressed protein is "associated with the cellular debris" and thus, it is *not secreted* into the culture medium.

90. If the protein is not secreted, it cannot be collected as a "culture solution" or "culture supernatant", as discussed in greater detail in the context of Art. 123(2) EPC. The OD's argument that **D31** does not prove that polypeptides *could not* be expressed as "soluble protein" in COS-1 cells fails to address the critical point. The claim feature of collecting the recombinant protein as a solution fraction ("culture solution or supernatant") introduces as a requirement that the protein is **secreted** by the cells. A lack of secretion of rFC generated from COS-1 cells which is evidenced in **D31** (page 360, right col. 2nd par) is a direct factual contradiction between a claimed feature and a specific embodiment falling within the broad scope of the claim.

91. Hence, we as the opponent have raised serious doubts substantiated by verifiable fact that the invention is insufficiently disclosed.

92. In our view, the OD's PO attempts to construe claim 1 quite narrowly to avoid a finding of lack of sufficiency. However, the patent's own disclosure explicitly includes cell-free expression systems and broadly defined host cells (including primate cells like COS-1) within the method of artificially expressing a protein.

93. Therefore, we respectfully disagree with the OD's PO and maintain that the claimed subject matter does not meet the requirements of Article 83 EPC.

VII. Auxiliary Requests

94. For the avoidance of doubt, we do maintain all objections raised under Added Matter and Inventive Step against the present ARs 1-11 (see section II.2 and IV.6. of our Reply). We also submit that the reasons set out in section II above also prejudice maintenance of the opposed patent in the form of any of ARs 12-29 even if these are admitted into the proceedings. Should they be admitted, we reserve the right to further comment thereon (especially under Art. 123(2) EPC, Art. 56 EPC).

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Enclosures
Documents **D48-D51**

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