Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 16 December 2022

Case Number: T 1933/19 - 3.3.04

Application Number: 14706516.3

Publication Number: 2956167

IPC: A61K39/12

Language of the proceedings: EN

Title of invention:
Methods for the release of virus-like particles

Patent Proprietor:
Intervet International B.V.

Opponent:
Boehringer Ingelheim Vetmedica GmbH

Headword:
Virus-like particles/INTERVET

Relevant legal provisions:
EPC Art. 100(b)

Keyword:
Sufficiency of disclosure - main (sole) request (no)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1933/19 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 16 December 2022

Appellant: (Patent Proprietor)	Intervet International B.V. Wim de Körverstraat 35 5831 AN Boxmeer (NL)
Representative:	Intervet International B.V. Hertford Road Hoddesdon, Hertfordshire EN11 9BU (GB)
Respondent: (Opponent)	Boehringer Ingelheim Vetmedica GmbH Binger Strasse 173 55216 Ingelheim am Rhein (DE)
Representative:	D Young & Co LLP 120 Holborn London EC1N 2DY (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 8 May 2019 revoking European patent No. 2956167 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairwoman	M. Pregetter
Members:	D. Luis Alves
	L. Bühler

Summary of Facts and Submissions

I. European patent No. 2 956 167, entitled "*Methods for the release of virus-like particles*", was granted on European patent application No. 14 706 516.3, filed as an international application published as WO 2014/125053.

II. The patent was opposed on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), under Article 100(a) EPC, and on the grounds under Article 100(b) and (c) EPC.

III. The opposition division decided to revoke the patent. As regards the main request (patent as granted), the opposition division held that the claimed invention was not sufficiently disclosed in the patent (Articles 100(b) and 83 EPC).

IV. The patent proprietor (appellant) filed an appeal against this decision. The opponent is respondent to this appeal.

V. With the statement setting out the grounds of appeal, the appellant filed claims according to auxiliary requests 1 to 10 and refiled document D25.

VI. With the reply, the respondent filed arguments and documents D26 to D28, a copy of the decision under appeal and an Annex 2 with arguments.

VII. The board appointed oral proceedings and, in a communication pursuant to Article 15(1) RPBA, informed the parties of its preliminary opinion that, *inter*

alia, the invention defined in claim 1 of the main request was not disclosed in the patent in a sufficiently clear and complete manner.

VIII. The oral proceedings before the board were held by videoconference with the agreement of the parties. During the oral proceedings the appellant withdrew its auxiliary requests 1 to 10. At the end of the oral proceedings, the chair announced the board's decision.

IX. Claim 1 of the main (sole) request (claims as granted) reads as follows:

"1. Method for the release of baculovirus-expressed Virus-like Particles (VLP's) of non-enveloped viruses from insect cells, characterized in that the method is free of lysis or cell disruption steps, and comprises the step of mixing the insect cells with a salt, wherein the salt in water solution resulting from the mixing comprises at least 300 mOsmol/l of a salt of which the cation is selected from the group consisting of the alkali metals and the earth alkali metals, and of which the anion is selected from the group consisting of Cl^- , Br^- and I^-."

X. The following documents are referred to in this decision:

D7: Viscidi, R.P. *et al.*, Clinical and Vaccine Immunology 18(10), 2011, pages 1737-1743.

D8: Pejawar-Gaddy, S. *et al.*, Cancer Immunol Immunother 59(11), 2010, pages 1685-1696.

D9: WO 2012/033911 A2

D10: WO 03/068993 A1

D11: Olejnik, A. *et al.*, Journal of Biotechnology 102, 2003, pages 291-300.

D21: Wu, J. *et al.*, Biotechnology Techniques 6(4), 1992, pages 335-340.

D22: Declaration of Dr. Banz and Dr. Gaßel, pages 1-15, dated December 2018, filed on 21 December 2018.

D25: "Vi-CELL XR Cell Viability Analyzer - Reference Manual", Beckman Coulter, October 2011.

XI. The appellant's arguments relevant to this decision may be summarised as follows.

Admittance of document D25 into the appeal proceedings

The document was a manual for a cell viability analyser and therefore reflected common general knowledge.

It had been filed in opposition proceedings in reply to the filing of document D22.

The principles applied by the opposition division when deciding not to admit the document were not the correct ones, neither were they applied in a reasonable way (see decision under appeal, page 8, last two lines). Thus, the document should be admitted into the appeal proceedings.

Main (sole) request - Claim 1

The claim should be interpreted with a mind willing to understand. Thus, the claim was directed to a method which could comprise further steps, in addition to the salt treatment recited in the claim, provided that those steps were not lysis or cell disruption steps. Interpreting the salt treatment as a lysis step would mean excluding from the method precisely the only step positively defined in the claim.

Cell lysis steps as known from the prior art included freeze-thawing, sonication, and mixing with a lysis buffer (see patent application, page 1, line 28 ff.; and documents D7, page 1738, left-hand column, first full paragraph; D8, page 3, first paragraph; D9, page 25, lines 18 to 19; D10, page 54, example 8; and D11, page 293, section 2.3).

The claimed method excluded such lysis or cell disruption steps. However, it did not exclude some level of lysis. It was therefore not of relevance whether document D22 showed some level of lysis resulting from the salt treatment. This would merely confirm what was acknowledged in the patent (see application, page 2, third paragraph).

It was nevertheless disputed that document D22 showed cell lysis. The measurements of lactate dehydrogenase (LDH) activity provided in this document could not show lysis because LDH activity correlated with cell viability instead.

Document D21 did not disclose that LDH activity was a measurement of cell lysis. This document concerned the correlation between LDH activity and loss of cell viability (see title and concluding sentence on

page 340). However, loss of cell viability was not equivalent to cell lysis. The document did not state that LDH was an intracellular protein, it merely referred to its release from cells. Therefore, it also could not be validly argued that because LDH is an intracellular protein its detection outside the cells showed cell lysis.

The patent made plausible that the majority of cells were not lysed with the salt treatment - see figure 4, showing that most cells remained intact. From figures 1 and 2, which showed the results of gel electrophoresis, it could be seen that the claimed method resulted in pure VLPs whereas the control lane in figure 1 showed multiple bands.

The respondent had provided no verifiable facts or serious doubts. In particular, the respondent had not shown that only with undue burden could the skilled person omit lysis steps and still arrive at the high concentration of pure VLPs achieved with the claimed method. For this reason, it was not relevant whether the methods used in the patent were standard for the measurement of lysis.

XII. The respondent's arguments relevant to this decision may be summarised as follows.

Admittance of document D25 into the appeal proceedings

A handbook for a particular item of equipment did not belong to the common general knowledge.

Moreover, it was of relevance to the issue of admittance whether the opposition division applied its discretion correctly. *Prima facie* this was the case:

the document could not show that, when carrying out the experiments, the skilled person had used the equipment incorrectly. Therefore the document should not be admitted.

Main (sole) request - Claim 1

The salt treatment was a method step of claim 1. The requirement that the method was free of lysis steps applied to all method steps and thus to this step too.

The claim did not define the excluded lysis steps to be those as put forward by the appellant. Therefore the excluded steps were not limited to those described in documents D7 to D11.

The methods used in the patent were not standard for measuring cell lysis. They were not suitable for that purpose and were not intended to show absence of lysis. As regards figures 1 and 2, the samples were centrifuged at 3000 rpm prior to the gel electrophoresis, so that lysed cells, partially lysed cells as well as proteins were removed before loading onto the gels. As such, these figures could not show absence of lysis. Further reasons why these figures could not show absence of lysis included: the absence of a control, i.e. a sample not subjected to salt treatment; the fact that the gel lanes in figure 1 appeared to have been sliced together from different experiments and therefore did not necessarily share the same experimental conditions; the absence of a band for VLP, i.e. the product, in the lane "WFI" in figure 2. As regards figure 4, the image was not suitable for showing whether lysis was absent. Although, on the one hand, there was no cell staining, showing that the

cells were intact, on the other hand, the figure did show some disrupted cells.

By contrast, document D22 provided evidence of cell lysis in a method carried out according to claim 1. It reported measurements of cell viability, LDH activity and cell counts after salt treatment of insect cells.

Document D21 disclosed that LDH activity was a measurement of cell lysis (see Introduction, first sentence and page 339, penultimate sentence, referring to "percent lysis"). The document additionally reported a correlation between LDH activity and cell viability. This did not, however, make its disclosure of the previously known correlation of LDH activity with cell lysis any less relevant. Furthermore, loss of cell viability was not entirely distinct from cell lysis, as shown in figures 3 and 4 of this document. Moreover, it was common sense that the detection of an intracellular enzyme, such as LDH, outside of the cell, indicated that the cell membrane had been physically disrupted.

XIII. The appellant requested that the decision under appeal be set aside and the patent be maintained as granted.

The respondent requested that the appeal be dismissed.

Reasons for the Decision

Admittance of document D25 into the appeal proceedings

1. The opposition division did not admit this document into the opposition proceedings. The board took the document into account, in accordance with the appellant's request.

2. The document was cited by the appellant to dispute the relevance of the experimental results, presented in document D22, relating to cell counting using a Vi-Cell analyser. Since this particular set of experimental results was not relevant to the outcome of the case at hand, document D25 will not be addressed further in this decision and there is no need to give reasons for its admittance into the appeal proceedings.

Main (sole) request - Claim 1

3. Claim 1 is directed to a method of preparing Virus-like Particles (VLPs) of non-enveloped virus by releasing them from insect cells. The claim recites a single method step of mixing the cells with a salt, wherein the salt, as well as the osmolarity of the resulting solution, are further defined as follows:
"*wherein the salt in water solution resulting from the mixing comprises at least 300mOsmol/l of a salt of which the cation is selected from the group consisting of the alkali metals and the earth alkali metals, and of which the anion is selected from the group consisting of* Cl^- , Br^- *and* I^-."

In the prior art as acknowledged in the patent, release of the VLPs is achieved by lysis or disruption of the insect cells (see patent, paragraph [0008], first sentence). According to the patent, the method including the salt treatment step as defined in claim 1 allows pure VLPs to be obtained because cell disruption

and lysis, with release of the whole cell content, are avoided (see paragraphs [0008] and [0009]).

Claim construction

4. Claim 1 excludes lysis or cell disruption steps. The question arises as to what is meant by a "lysis or cell disruption step". In the board's interpretation, this means any step that causes lysis or cell disruption, without any limitation as to the extent of the lysis or disruption. Therefore, the board construes claim 1 as meaning that the step of mixing with salt is also subject to the condition of causing neither lysis nor cell disruption.

5. The appellant argued that, with a mind willing to understand, the claim cannot be read as excluding precisely the only step which is positively defined in the claim, i.e. the step of mixing the insect cells with a salt. However, in the board's understanding, this argument does not contradict the claim construction as set out in the preceding point, which does not rely on excluding the salt treatment step but rather on including a salt treatment step which however is limited not only in terms of the nature and concentration of the salt but also in that it does not result in lysis or cell disruption.

6. According to the appellant, the claim is to be construed to mean that the steps excluded from the claimed method are those known as lysis steps from documents D7 to D11 as well as from the prior art acknowledged in the patent application, and include, *inter alia*, freeze-thawing, sonication and mixing with a lysis buffer. However, the claim wording does not

define the nature of the lysis steps as freeze-thawing or other as exemplified by the appellant. Therefore, this argument is not pertinent to the claim at hand.

7. The board is also not persuaded by the appellant's argument that the claim excludes lysis steps but without excluding a certain level of lysis. Such an interpretation is not supported by the claim wording, which fails to define a level of lysis or cell disruption below which a method step is not to be classified as a "lysis or cell disruption step". It is not relevant in this respect that the description of the application acknowledges that a certain level of lysis occurs with the salt treatment, because such a statement in the description does not change the features of the claim and thus its interpretation.

Disclosure of the invention (Articles 100(b) and 83 EPC)

8. According to the case law of the Boards of Appeal, it must be possible for a skilled person to reproduce a claimed step using the application as filed without exercising any inventive activity (see Case Law of the Boards of Appeal of the EPO, 10th edition, 2022, II.C.5.1).

Experimental results in document D22

9. Document D22 is an experimental report submitted to show the occurrence of cell lysis in a method according to claim 1. It describes the culture of insect cells infected with baculovirus for the expression of VLPs. The insect cells were subsequently treated with various salt solutions. Measurements of lactate dehydrogenase (LDH) activity in treated samples are reported in table 8. A control is also shown, which corresponds to

samples treated with cell medium. As can be seen from this table, the samples treated with cell medium did not show any LDH activity, whereas those treated with salt solution were positive for LDH activity (see the first row versus the second to fourth rows).

10. It has not been disputed that the salt treatment carried out in these experiments was in accordance with claim 1 and the examples in the patent.

11. What was disputed was whether the measurement of LDH activity correlates with cell lysis. Document D21 was cited in this context. It investigates the correlation between LDH activity and cell viability. It also discloses that LDH activity was known to correlate with cell lysis, see the first sentence of the Introduction and the formula for calculating the percent lysis, on page 336, as well as the penultimate sentence on page 339. Therefore, the board does not share the appellant's view that the document demonstrates that LDH correlates with cell viability instead of cell lysis.

12. Finally, the appellant argued that the respondent had not shown that the examples in the patent were not reproducible because, instead of repeating the examples, it had used different analytical techniques. However, in the present case, the relevant question in the context of sufficiency of disclosure is not whether the skilled person could repeat the experiments reported in the patent, rather, the relevant question is whether the patent teaches how to carry out the salt treatment of insect cells without resulting in cell lysis. Hence, the board does not find the appellant's argument persuasive.

13. The board concludes that the patent does not teach in a clear and complete manner how to extract VLPs from the insect cells by using the salt treatment defined in claim 1 while avoiding cell lysis, as required in the claim.

14. In view of the foregoing, the proprietor's arguments relying on the evidence in the patent are not crucial for the decision, but will be addressed for the sake of completeness. The appellant referred to figures 1, 2 and 4 to show that pure VLPs are obtained by the claimed method and that most cells remain intact.

14.1 Figure 4 shows a microscopic image of insect cells treated with salt in accordance with the claimed method. It cannot be determined from this image how many of the cells are disrupted or lysed. Moreover, the appellant acknowledged that the image shows some lysed cells. Hence,the board considers that figure 4 does not allow the conclusion to be drawn that the salt treatment does not result in cell lysis.

14.2 Figure 1 is an image of gel electrophoresis analysis of salt-treated samples (second to fifth lanes) and of a sample described as "culture harvest" (first lane), respectively. It shows that VLPs could be isolated under the conditions of the experiment described in example 2. However, in the board's view it cannot show absence of lysis, essentially because, as pointed out by the respondent, the samples which were loaded onto the gels depicted in this figure were first centrifuged. It cannot be ascertained what was removed by this treatment. A comparison with the first lane is not informative in this respect because there is no description in the patent of any treatment carried out

on the "culture harvest" sample. Figure 2 suffers from the same deficiencies.

14.3 In conclusion, the board concurs with the opposition division that the patent does not show absence of lysis as a result of the salt treatment step.

15. For the reasons given in point 13., the claimed invention is not sufficiently disclosed in the patent and therefore the ground for opposition under Article 100(b) EPC prejudices the maintenance of the patent as granted.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated