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Datasheet for the decision of 19 October 2021

Case Number: T 2035/18 - 3.3.01

Application Number: 11746586.4

Publication Number: 2609428

IPC: G01N33/53

Language of the proceedings: EN

Title of invention:
POTENCY TEST FOR VACCINE FORMULATIONS

Patent Proprietor:
Intervet International B.V.

Opponents:
Sagittarius Intellectual Property LLP
Zoetis Services LLC

Headword:
Antigen content in mixture of PCV-2 and M. hyo antigens/ INTERVET

Relevant legal provisions:
EPC Art. 100(b), 100(a), 56
RPBA Art. 12(4)

Keyword:
Grounds for opposition - sufficiency of disclosure, inventive step (yes)
Late-filed evidence - admitted (no)

Decisions cited:
T 0435/91, G 0008/91

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Beschwerdekammern

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Case Number: T 2035/18 - 3.3.01

D E C I S I O N
of Technical Board of Appeal 3.3.01
of 19 October 2021

Appellant 1: (Opponent 1)	Sagittarius Intellectual Property LLP 19-21 Chapel Street Marlow Buckinghamshire SL7 3HN (GB)
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Respondent: (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)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 15 June 2018 rejecting the opposition filed against European patent No. 2609428 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairwoman	T. Sommerfeld
Members:	M. Pregetter
	R. Romandini

Summary of Facts and Submissions

I. European patent No. 2609428 is based on European patent application No. 11746586.4, which was filed as an international application published as WO2012/025612.

The patent as granted contains 11 claims. Claims 1, 9 and 10 as granted read as follows.

"1. A method for the determination of an antigen content of a first antigen in a mixture of at least a composition comprising the first antigen and a composition comprising (i) a second antigen and (ii) antibodies that are capable of binding with the first antigen, the method comprising the steps of,
A dissociating antigen-antibody complexes in the mixture, formed between the first antigen and the antibodies, and
B determining the antigen content of the first antigen by means of an immunoassay,
characterized in that the first antigen is a porcine circovirus type 2 (PCV-2) antigen and the second antigen is a Mycoplasma hyopneumoniae antigen."

"9. A method for the determination of an antigen content of a PCV-2 antigen in a mixture of at least a composition comprising the PCV-2 antigen and a composition comprising a M. hyo antigen, the method comprising the steps of,
A mixing the two compositions, and
B determining the antigen content of the PCV-2 antigen by means of an immunoassay,
characterized in that the M. hyo antigen is obtained

from a culture that does not comprise swine serum."

"10. A method for the determination of an antigen content of a first antigen in a mixture of at least a composition comprising the first antigen and a composition comprising a second antigen, the method comprising the steps of,
A separating the second antigen from antibodies that are capable of binding with the first antigen in a composition comprising the second antigen and the antibodies,
B mixing the second antigen with a composition comprising the first antigen, and
C determining the antigen content of the first antigen in the mixture by means of an immunoassay,
characterized in that the first antigen is a PCV-2 antigen and the second antigen is a Mycoplasma hyopneumoniae antigen."

II. The following documents, cited during the opposition and appeal proceedings, are referred to below.

(1) US 2009/0317423

(2) Declaration by Dr Rhona Banks, 7 November 2016, 4 pages

(4) US 4,459,359

(5) Patton et al., J. Immunol. Methods, 2005, 304, 189-195

(7) Coombes et al., J. Immunol. Methods, 2009, 350, 142-149

(11) Opriessnig et al., J. Vet. Diagn. Invest., 2007,

19, 591-615

(12) Dodig S., http://www.biochemia-medica-com/content/interferences-quantitative-immunochemical-methods, 2009, 12 pages

(13) Allan G.M. and Ellis J.A., J. Vet. Diagn. Invest., 2000, 12, 3-14

(14) Freundt E.A., in: Methods in Mycoplasmology, vol. 1, chapter C7, 127-135

(15) Kricka L. J., Clin. Chem., 2000, 46(8), 1037- 1038

(16) Kroll M.H. and Elin R.J., Clin. Chem., 1994, 40(11), 1996-2005

(17) Selby C., Ann. Clin. Biochem., 1999, 36, 704-721

(18) US 4,703,001

(21) McNeilly F. et al., J. Vet. Diagn. Invest., 2002, 14, 106-112

(22) EMEA: "Note for guidance: Requirements for combined veterinary vaccines", CVMP/IWP/52/97-FINAL, effective as of 1 January 2001, 6 pages

(38) Experimental data, submitted on 23 October 2018, 2 pages

III. The patent was opposed under Article 100(a), (b) and (c) EPC on the grounds that the claimed subject-matter lacked an inventive step, was not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, and extended beyond

the content of the application as filed.

The opposition division rejected the oppositions.

IV. Both opponents appealed this decision.

Appellant 1 (opponent 1) submitted document (38) with the grounds of appeal.

V. The respondent (patent proprietor) submitted auxiliary requests 1 to 8 with its reply to the grounds of appeal.

VI. By letter dated 16 August 2021, appellant 2 (opponent 2) submitted further arguments.

VII. Oral proceedings before the board took place on 19 October 2021.

VIII. The appellants' arguments, in so far as they are relevant to the present decision, may be summarised as follows.

Admission of document (38)

Document (38) had been submitted in a timely manner with the statement setting out the grounds of appeal. It could not have been filed earlier, as it was only from the opposition division's decision that the opponents learned that their arguments had been considered to be "speculative". Furthermore, and more particularly in view of auxiliary request 2, which defined incubation with acids, it was a highly pertinent document.

Sufficiency of disclosure

Claim 1 of the patent in suit required close to 100% recovery of the antigen in its method for determination; otherwise this claim could not provide a contribution to the art. It was, however, clear from the patent in suit that the use of well-known dissociation agents in a method according to claim 1 resulted in failure, see Tables 2 and 5, which disclosed non-working embodiments. Furthermore, the dissociation level in these examples was conflated with the recovery level, as dissociation could be accompanied by damage to the antigen, and no way to distinguish between the two was described. Only one very specific set of conditions that allowed for the determination of the PCV-2 antigen content by a method according to claim 1 was disclosed. However, as this did not enable the method to be carried out over the whole scope of the claim without undue burden, this single working embodiment was not sufficient according to established case law, in particular in view of T 435/91 and in view of the functional nature of the feature of dissociating antigen-antibody complexes. The description did not provide any guidance on further working embodiments or conditions. Furthermore, there were no examples relating to claims 9 or 10. In short, as the failures were not exceptional and there were no indications that could lead to success, finding the appropriate conditions to carry out the claimed methods represented an undue burden. Consequently, the claimed methods could not be carried out over their whole scope.

Inventive step

Document (1) represented the closest prior art. It disclosed a combination vaccine comprising PCV-2 and M. hyo antigens. The person skilled in the art who was following the teaching of document (1) would have to perform potency tests, which were a regulatory requirement, in the mixture comprising the PCV-2 and M. hyo antigens. The technical problem was thus to provide a method for reasonably accurately measuring PCV-2 antigen in a mixture of PCV-2 and M. hyo antigens. This technical problem had not been solved over the whole scope of claim 1, for the same reasons that led to a lack of sufficiency of disclosure. However, the solution to the technical problem was obvious in any case. Problems with the potency tests would have been apparent immediately. Having discovered them, the person skilled in the art would naturally have attempted to solve these problems. For potency testing of combination vaccines the person skilled in the art would have used an immunoassay, as, for example, disclosed in document (7). The person skilled in the art, faced with problems in the immunoassay, would have immediately identified the cause of the problem and the solution to it from their common general knowledge. The following knowledge was part of their "mental furniture": first, that M. hyo was grown in swine serum, which was textbook knowledge, as disclosed in document (14) (page 129, first paragraph and Media A26 and FF); secondly, that infections with PCV-2 were endemic in pig populations, meaning that all swine sera contained PCV-2 antibodies (document (13), page 6 and document (11), page 592); and thirdly, that the primary cause of problems in immunoassays lay in interferences concerning the analyte. Here, document (12) presented four options for such

interference, of which the second option, interference with an antibody, was the most compelling in relation to the present case. Similar information could be found in document (16). From common general knowledge, the person skilled in the art would have immediately concluded that PCV-2 antibodies from swine serum of the M. hyo culture interfered in the immunoassay, as also stated by Dr Banks in document (2). The solution to this problem of interference was provided by document (4), which the person skilled in the art would have consulted, given its title. When implementing the solution disclosed in document (4), the person skilled in the art would have immediately arrived at the subject-matter of claim 1. Concerning the subject-matter of claims 9 and 10, it was obvious to avoid interferences either by culturing M. hyo in a medium which did not comprise swine serum or by entirely removing interfering antibodies from the sample, as suggested by document (17) (page 712, item A in Table 3).

Appellant 2 further argued that the subject-matter of the claims was not inventive over the disclosure of document (21) in combination with document (18) and in view of document (18) in combination with document (1). While M. hyo antigen was present in the mixture to be tested according to the patent in suit, the method did not relate to the determination of the M. hyo antigen.

Starting from document (21), which related to the quantification of PCV-2 antigen and disclosed the presence of PCV-2 antigens in a very high percentage of swine sera, the difference was that PCV-2 antigen was tested after dissociating antigen-antibody complexes formed by PCV-2 antigen and antibodies binding to PCV-2. The effect of this difference was improved

accuracy of the test of document (21). The technical problem was thus the provision of a PCV-2 antigen test with improved accuracy. The subject-matter was obvious in view of document (18) and common general knowledge, especially since the interference from antibodies in the test sample was well known, for example from documents (15) or (16). Concerning the subject-matter of independent claims 9 and 10, the difference being the avoidance of swine serum (in the case of claim 9) or the separation step A (in claim 10), the technical problem could only be seen as the provision of an alternative method of determining PCV-2 antigen. With the knowledge that antibodies against PCV-2 were present in swine serum and that antibodies in a test sample might react with the analyte, it would have been obvious to avoid swine serum in the culture of any antigen, including M. hyo, or to remove these antibodies, as suggested by document (18) (column 1, paragraph 3); in other words to use entirely conventional methods.

Document (18) was directed to the same purpose as the patent in suit, namely the accurate detection of a complexed analyte, for which it suggested pre-treatment by dissociating antigen-antibody complexes. The difference between the claimed subject-matter and the disclosure of document (18) was that a specific antigen was to be assayed with the method of document (18). The problem was thus the provision of a further application for the test of document (18). The person skilled in the art would have realised that the method of document (18), i.e. removing the cause of the interference by dissociating the analyte from antibodies against it prior to performing the immunoassay, was perfectly applicable to the antigen combination of document (1), and consequently would

have applied the method to the mixture of document (1). In respect of the subject-matter of claim 9, the skilled person would have avoided swine serum, as its use was expensive and contrary to animal welfare. The same line of argument as that provided for claim 1 applied to claim 10 as well, since document (18) already suggested removing interfering antibodies (column 1, lines 41 to 45).

IX. The respondent's arguments, in so far as they are relevant to the present decision, may be summarised as follows.

Admission of document (38)

Document (38) could and should have been filed in first-instance proceedings. It was not a response to the decision under appeal, since the opposition division kept to its preliminary opinion, provided in the annex to its summons. There was thus no reason to file the document so late. Furthermore, the content of document (38) had many flaws.

Sufficiency of disclosure

Most importantly, the claimed invention was not about determining an antigen by an immunoassay, but was at a higher conceptual level. The contribution to the art had to be seen in the realisation that there was a problem with determining PCV-2 antigen in a mixture comprising PCV-2 and M. hyo antigens, in finding the cause of the problem and in providing ways of solving it. Thus, the patent in suit as a whole disclosed a general concept and the principles for carrying it out. For details about certain process steps, the prior art and common general knowledge as cited by the appellants

in the discussion of inventive step provided guidance. In particular, reference was made to documents (2), (4), (5) and (18). No serious doubts substantiated by verifiable facts had been raised by the appellants. As required by case law, the patent in suit described at least one way to carry out the invention. In fact, several ways were described, and guidance on ways that did not give acceptable results was provided. In addition, the claims did not require a total dissociation and there was no level of accuracy defined. For practical purposes it was enough to determine that there was enough antigen present in the mixture to represent a protective amount.

Inventive step

The closest prior art was document (1). It contained several embodiments in which separate vaccines comprising either PCV-2 or M. hyo antigen were mixed shortly before administration. The patent in suit related to means for enabling a ready-to-use combination vaccine of PCV-2 and M. hyo antigens by providing a method for determining the content of PCV-2 antigen. The difference was thus that document (1) did not determine the PCV-2 content in the mixture. The inventive contribution could be seen in realising the problem for the determination of the PCV-2 antigen content in the mixture, in identifying its cause and in solving it. By the problem-solution approach, the technical problem was the provision of a reliable test for the determination of PCV-2 antigen using a more convenient combination vaccine. This problem was solved by the subject-matter defined in the claims. There was no pointer to these solutions: combining the prior art documents as the appellants had done was the product of hindsight. There were many possible interferences when

the final product, including all its components, was tested. It was not straightforward to point to one particular cause. Once it had been found that the immunoassays were compromised, the following steps had to be performed: identifying the cause as antibodies, identifying the source of the interfering antibodies as the M. hyo preparation, establishing that the reason for the presence of these antibodies lay in the culturing method of the M. hyo, and then providing a solution to the compromising of results.

Document (21) did not represent the closest prior art. It concerned the epidemiology of PCV-2 virus infections in pigs and used ELISA and PCR methods for this. It did not relate to M. hyo, vaccines, or potency testing.

Document (18) did not represent the closest prior art either. It related to testing in serum samples comprising both antigen analytes and antibodies against such antigens. The aim was to detect the antigen, not to quantify it. Document (18) relied on aggressive pH-dependent chaotropes to denature proteins so that antibodies disintegrated and so were removed from the antigen. PCV-2, M. hyo, antigen quantification, vaccine manufacture and potency testing were not mentioned.

X. The final requests of the parties were as follows.

The appellants requested that the decision under appeal be set aside and the patent be revoked. Furthermore, they requested that auxiliary requests 1 to 8 not be admitted.

The respondent requested that the appeals be dismissed. Alternatively, it requested that the patent be maintained in amended form, based on any of auxiliary

requests 1 to 8, all of which had been filed with the reply to the statements setting out the grounds of appeal. Furthermore, it requested that document (38) and appellant 2's submissions of 16 August 2021 not be admitted.

Reasons for the Decision

1. The appeals are admissible.

1.1 *Amendments*

The ground for opposition pursuant to Article 100(c) EPC was not substantiated during the appeal. In view of the character of the *inter partes* appeal proceedings and the principle of equal treatment of the parties, it cannot be expected that the board "provide on its own, an elaborate and full reasoning, substituting itself for that opponent which remains passive" in relation to this ground for opposition. As clarified in the case law (see decision G 08/91 of the Enlarged Board of Appeal, OJ 1993, 346, point 10.1 of the Reasons), "it is not the function of the Boards of Appeal to carry out a general review of decisions at first instance, regardless of whether such a review has been sought by the parties" (see G 08/91, point 10.2).

1.2 Consequently, in view of the lack of any substantiation by the appellant(s), the board has no reason to depart from or to review the assessment made by the opposition division in the question of added matter. As a result, the ground for opposition pursuant to Article 100(c) EPC does not prejudice the maintenance of the patent.

2. *Admission of document (38)*

Document (38) was submitted by appellant 1 with its statement setting out the grounds of appeal. It contains experimental data relating to the determination of PCV-2 antigen.

In the annex to the summons to oral proceedings before the opposition division, the opposition division gave a positive preliminary opinion on sufficiency of disclosure. The decision under appeal finds that there is sufficiency of disclosure, and bases this on reasons following the same line as that in the annex. The finding of the decision under appeal on sufficiency of disclosure cannot thus be seen as a surprise that would allow the filing of new experimental data in response. Consequently, the experimental data could and should have been filed earlier.

Thus, the board decided not to admit document (38) into the appeal proceedings, pursuant to Article 12(4) RPBA 2007 (being applicable pursuant to Article 25(1) and (2) RPBA 2020).

3. *Sufficiency of disclosure*

3.1 All the independent claims define a "method for the determination of an antigen content". An interpretation of the term "antigen content" to equate it with any arbitrary proportion of the amount of antigen in the composition is incompatible with the way a person skilled in the art would ordinarily understand it, especially in the context of immunisation and vaccines. However, this term does not imply a restriction to 100% accuracy or recovery, either. The term "determination of an antigen content" thus means that a concrete idea

of the amount of antigen in the composition is obtained when the method is carried out.

3.2 The method according to claim 1 comprises a step of "dissociating antigen-antibody complexes". In the context of claim 1 it is clear that this dissociation must be carried out in a manner that allows the antigen content to be determined by an immunoassay, implying that the dissociated antigen retains the required reactivity.

3.3 From the documents cited and the arguments provided by the parties, it can be taken that the dissociation of an antigen-antibody complex as such and the determination of an antigen content by an immunoassay as such are routine steps.

In particular, documents (4), (5), (7), (13), (18) and (21) were cited.

Document (4) relates to a process for determining the presence of an antigen or antibody in a sample wherein this antigen or antibody exists in the form of an immune complex which is dissociated by using a dissociating buffer, such as, inter alia, buffers based on urea or solutions of low or high pH (abstract; column 4, lines 5 to 10). The process is useful in the detection of virtually all antigens or antibodies sequestered within immune complexes (column 7, lines 19 to 20).

Document (5) is entitled "An acid dissociation bridging ELISA for detection of antibodies directed against therapeutic proteins in the presence of antigen". The dissociation step takes place in acidic medium (abstract).

Document (7) relates to ELISA for lot release of combination vaccines. Antigen content of diphtheria toxoid antigen is determined by ELISA.

Document (13), in the context of a review article on the epidemiology of PCV-2 infections, mentions ELISA for detecting PCV-2 infections in pigs (page 6, left-hand column, first paragraph).

Document (18) relates to immunoassays in the form of ELISA, and discusses dissociation to avoid impaired performance in a serum sample including a serum antibody having a specificity for the particular analyte (column 1, lines 34 to 45; column 2, line 68 to column 3, line 5). The serum antibody is dissociated and denatured at low pH with a chaotrope from an acid stable antigen (claim 1).

Document (21), in the context of diagnosis of postweaning multisystemic wasting syndrome in pigs, uses ELISA for PCV-2 detection.

3.4 The present case concerns a particular antigen-antibody complex, namely a complex comprising PCV-2 antigen.

The inventors have found that the determination of the PCV-2 antigen content requires the avoidance of interferences (patent in suit, paragraphs [0007] and [0008]). Means for allowing the determination of the PCV-2 antigen content by avoiding these interferences are the technical contribution of the patent in suit. For this purpose, claim 1 proposes a step of dissociating antigen-antibody complexes in the mixture. The particularities of the appropriate dissociation are left to the expertise of the person skilled in the art.

In support, certain examples and data are provided by the patent in suit. These data show that dissociation by acids, in particular if a certain protocol is followed, leads to the determination of the PCV-2 antigen content with an acceptable accuracy (Examples 4 to 7). On the other hand, dissociation using other common agents gives unacceptable results (Example 3 and Table 2). This is observed in paragraph [0064] of the patent in suit. In short, the patent in suit discloses ways to determine the PCV-2 antigen content and ways that do not lead to the determination of this content. These ways, seen collectively, provide guidance to the person skilled in the art when putting the claimed subject-matter into practice.

Taking into consideration the guidance provided in the description of the patent in suit and common general knowledge, the tests to be carried out by the person skilled in the art for putting claim 1 into practice, although they may be numerous, do not necessitate carrying out a research programme. There is thus no undue burden.

3.5 Consequently, in view of the fact that at least one way of carrying out the invention is disclosed and that dissociation of antigen-antibody complexes in the context of immunoassays is part of common general knowledge, the person skilled in the art is enabled to perform routine tests to find further practical solutions falling within the scope of claim 1.

3.6 *Further arguments*

3.6.1 It was argued that "dissociating" represents a functional definition.

There is no doubt that the method step of dissociating according to item A of the claim under consideration is functionally disclosed. However, the term "dissociating" as such has a well-established meaning in the art. This is supported by the use of the term "dissociating" and "dissociation" in the prior art relating to the same or similar situations, i.e. the breaking up of an antigen-antibody complex, see point 3.3. Consequently, the board fails to see any ambiguity or lack of disclosure in this functionally defined feature. Moreover, it has not been argued that the person skilled in the art cannot determine whether dissociation as such has taken place.

3.6.2 The appellants argued that Table 2 does not make it possible to differentiate between dissociation and recovery. However, the crucial point of the data in Table 2 is whether a method using particular means for dissociation allows the PCV-2 antigen content to be determined. With this in mind, the percentage of recovery of PCV-2 antigen is the pertinent point. There is no need to distinguish between effects related to dissociation and recovery.

3.6.3 Furthermore, the appellants submitted that the situation under consideration is comparable to the situation in T 435/91 (OJ EPO 1995, 188).

The board agrees that the situation is comparable in so far as a person skilled in the art is capable of determining whether dissociation has taken place and in that at least one way to carry out the invention is disclosed. However, as stated above, the board considers, contrary to the finding in T 435/91, that the subject-matter of granted claim 1 as a whole can be carried out by the person skilled in the art. Unlike

the situation in T 435/91, the functional definition does not concern a component of a composition of matter but a process step. As explained above, there is no doubt that the person skilled in the art is capable of dissociating antigen-antibody complexes. The only, potentially burdensome, issue is to determine dissociation conditions that lead to a dissociated antigen responsive to quantification by an immunoassay. However, contrary to the situation in T 435/91, where sufficiency of the functional feature was discussed in the context of the technical contribution to the art made by the disclosure of the invention, the technical details of the dissociation do not constitute the contribution to the art in the present case. Here, these details form part of common general knowledge. A finding of lack of sufficiency of disclosure, whether relating to functional features or for other reasons, can only be taken on the basis of facts of the individual case, as acknowledged in T 435/91 (page 9, first paragraph). In the present case, the board considers the subject-matter to be sufficiently disclosed, for the reasons given in point 3.5 above.

3.7 No arguments concerning the subject-matter of claims 9 and 10 were submitted during the appeal, other than that the patent in suit does not contain any examples of these claimed methods. Examples are not required to establish sufficiency of disclosure since, as in the case of claim 1, the procedural steps defined in these claims are part of common general knowledge.

3.8 The ground for opposition pursuant to Article 100(b) EPC does not prejudice the maintenance of the patent.

4. *Inventive step*

4.1 The patent in suit relates to methods for the determination of an antigen content of a first antigen in a mixture comprising two or more antigens. This determination of antigen content is to be made in the context of potency tests for an antigen in a combination vaccine (paragraph [0001]). The antigen of which the content is to be determined in the presence of a second antigen is PCV-2 antigen. The second antigen is a Mycoplasma hyopneumoniae antigen (paragraph [0025]).

Claims 1, 9 and 10 define three alternative methods for the determination of the content of PCV-2 antigen in mixtures containing PCV-2 antigen and M. hyo antigen.

4.2 While all parties agreed that document (1) represents the closest prior art, appellant 2 further considered documents (18) and (21) as starting points for the problem-solution approach.

4.3 *Document (1) as closest prior art*

Document (1) discloses immunogenic compositions comprising PCV-2 antigen and M. hyo antigen (claim 1). The aim is to provide a combination vaccine comprising PCV-2 and M. hyo antigens in sufficient amounts to confer a protective immune response (paragraph [0010]). The antigen contents of PCV-2 and M. hyo antigens are determined prior to mixing the antigens, as can be inferred from Table 2.

4.3.1 The difference between the subject-matter of claims 1, 9 and 10 of the patent in suit and the disclosure of document (1) is thus that the patent in suit seeks to

determine the antigen content in the mixture comprising the PCV-2 and M. hyo antigens.

4.3.2 The technical problem is the provision of a method for the determination of the content of PCV-2 antigen in the mixture.

The board considers that this problem is solved by the methods defined in claims 1, 9 and 10 of the patent in suit. Reference is made to the reasoning given for sufficiency of disclosure.

4.3.3 Starting from document (1), the skilled person would have needed to determine the contents of the antigens, including the content of PCV-2 antigen, in the combination vaccine itself. Such an antigen content determination is a regulatory requirement, see document (22), point 2.4.2. Furthermore, the board considers it obvious that the person skilled in the art would have resorted to immunoassays. Immunoassays are well established in the field and, being in vitro tests, are relatively easy to perform and to standardise. In addition, the person skilled in the art, following routine procedures when carrying out immunoassays, would have been confronted with obviously incorrect results from these assays. The subsequent behaviour and considerations by the person skilled in the art need to be discussed in more detail.

Confronted with a method that leads to incorrect results, the person skilled in the art would have been faced with finding the reason(s) for these. In immunoassays, two major components are usually involved: the sample to be tested (comprising the analyte) and the reagents used in the tests (comprising the antibody or antibodies used for detection).

Incorrect or erroneous results may be due to either of the two major components or to incidents during the assay (issues relating to handling, degradation, etc.) which do not arise in the control. As the appellants and the respondent limited themselves to discussion of the sample, so will the board.

Interferences within the sample that render the antigen unavailable (or available only to a limited extent) have been described in the art. This is acknowledged in paragraph [0007] of the patent in suit. Several documents relating to such interferences were submitted.

Document (12), a review article, discusses some of the possible interferences in quantitative immunochemical methods. In its abstract, it lists cross-reactivity with endogenous and exogenous non antibody-structured substances, cross-reactivity with endogenous and exogenous antibody-structured substances, the hook effect and the matrix effect. While it seems that the hook effect can be ruled out quite easily, the other effects require detailed research. The listing of these effects, as in the abstract of document (12), might be seen to imply that these interferences are quite "simple". However, a study of the details disclosed in document (12) immediately shows that it is not only four specific options that have to be checked for but also a variety of underlying possibilities and mechanisms, see, for example, the discussion of the various mechanisms underlying the option "cross-reactivity with endogenous and exogenous non antibody-structured substances" in Figure 1. For the second option, cross-reactivity with endogenous and exogenous antibody-structured substances, detailed considerations as to antibodies to be expected in the sample are

necessary, as provided in the corresponding section of document (12). Similar considerations as to the other options apply to the last option, the matrix effect, which, in the absence of a detailed discussion of compounds to be expected in the mixture under consideration, can only be speculated on. In the context of vaccines, it seems that such compounds would either be known or would originate from the two antigen preparations. In short, there are many possible sources of interferences, contrary to the conclusions of document (2).

Documents (15) and (16) also identify numerous possible compounds that may cause interferences.

All these possible compounds have to be checked for if they are to be identified as causes of the interferences.

The closest prior art itself provides no information concerning constituents other than the two antigens, let alone concerning possibly interfering constituents. The appellants focused their arguments as to the cause of the interferences on M. hyo and its cultivation in swine serum.

However, the production process(es) of the M. hyo antigen, as used for example in the preparations of document (1), which are described in paragraph [0047] of that document as being the whole M. hyo bacterin (in inactivated, live modified or attenuated form), a chimeric virus, or polypeptides comprising at least an immunogenic amino acid sequence of M. hyo, and in the examples, have not been discussed by the appellants. They merely submitted that M. hyo as such is always cultivated in swine serum. However, it appears to be an

oversimplification to reduce a M. hyo antigen preparation to "M. hyo antigen in swine serum". The fact that PCV-2 infections are widespread in pigs (see documents (11) and (13)) does not equate to a direct disclosure that all M. hyo antigen preparations necessarily contain PCV-2 antibodies. Swine serum may well be the usual culture medium for M. hyo (see document (14)). Also, PCV-2 antibodies may well be present in most M. hyo antigen preparations. However, no document discussing the actual production process for the or a M. hyo antigen preparation involving swine serum, and thus pointing to the presence of compounds usually or often present in swine serum, is on file. It is of course possible that the person skilled in the art, after determining that the interfering agent in the mixture containing the PCV-2 and M. hyo antigens was a PCV-2 antibody, would look for the source of this antibody. However, such a follow-up of the underlying reasoning for the presence of the PCV-2 antibody could only be undertaken once the presence of the PCV-2 antibodies had been identified as the problematic factor, and is thus irrelevant to the present decision.

The appellants have not convincingly shown that a project enabling identification of the cause of the interferences would have been within the competence and routine work of the person skilled in the art. Consequently, the identification of (PCV-2) antibodies as the cause of the interferences in the immunoassays under consideration goes beyond what can be expected of the person skilled in the art.

4.3.4 In short, while the board has come to the conclusion that the person skilled in the art would have realised that methods for the determination of the PCV-2 antigen content in a mixture also comprising M. hyo antigen

were not straightforward, it would not have been obvious to the person skilled in the art that the problem was specifically linked to the presence of interfering antibodies against PCV-2.

As a consequence of this finding, the further process steps defined in the independent claims would not have been obvious to the person skilled in the art either, even in view of documents (4) (for dissociation) and (17) (for removing interfering antibodies) or considerations such as animal welfare. In other words, a person skilled in the art who was unaware that incorrect results of the immunoassays were linked to the presence of antibodies against PCV-2 in the sample would have no reason to resort to a method step of dissociating antigen-antibody complexes, to the use of a M. hyo antigen in the mixture obtained from a culture that does not comprise swine serum, or to a method step of separating the M. hyo antigen from antibodies that are capable of binding with the PCV-2 antigen.

Since the solution to the technical problem is not obvious to the person skilled in the art, the subject-matter of claims 1, 9 and 10 of the patent as granted involves an inventive step over document (1) (Article 56 EPC).

4.4 *Further starting points*

Document (21) evaluates the use of antigen-capture ELISA for the detection of PCV-2 in tissue samples from diseased and non-diseased pigs (abstract).

Document (18) relates to an immunoassay for assaying an acid-stable antigen potentially bound by serum antibody in a serum sample (claim 1). A pretreatment involving

chaotropic acids dissociates the serum antibody from the analyte (column 2, line 62 to column 3, line 17).

The difference between the subject-matter of independent claims 1, 9 and 10 of the patent in suit and the disclosure of documents (21) and (18) is the presence of a M. hyo antigen in the composition that undergoes immunoassay. For document (18), a further difference is the presence of PCV-2 antigen.

Starting from document (21), the problem formulated by appellant 2, i.e. the provision of a PCV-2 antigen test with improved accuracy, cannot lead to the subject-matter of the claims of the patent in suit. Document (21) discusses immunoassays in the context of analysing tissue samples of pigs in the context of diagnosing a certain syndrome in the pigs. An improvement in accuracy, independently of whether method steps leading to the improvement are obvious or not, could not have led the person skilled in the art to carry out the method on a mixture of PCV-2 antigen and M. hyo antigen, as required by the patent in suit.

Alternatively, starting from either document (21) or (18), the technical problem can be seen as the provision of a further application for the immunoassays in these two documents.

The solution to this technical problem is the use of a method according to document (18) for determining the PCV-2 antigen content in a mixture comprising PCV-2 antigen and M. hyo antigen.

In the absence of any document disclosing the interaction of PCV-2 antigen with a (PCV-2) antibody in a mixture comprising PCV-2 antigen and M. hyo antigen,

the skilled person would not have any incentive to select such a mixture when looking for a further application for the immunoassays according to documents (21) and (18), and thus would not arrive at the method of claim 1 of the patent in suit.

The same reasoning applies mutatis mutandis to the subject-matter of independent claims 9 and 10, which is not obvious either.

The subject-matter of claims 1, 9 and 10 of the patent as granted involves an inventive step in view of either document (21) or (18) as closest prior art (Article 56 EPC).

4.5 The ground for opposition pursuant to Article 100(a) and Article 56 EPC does not prejudice the maintenance of the patent.

5. Since no further grounds for opposition were submitted, the maintenance of the European patent is not prejudiced, as found by the decision under appeal rejecting the oppositions.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairwoman:

M. Schalow T. Sommerfeld

Decision electronically authenticated