BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

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

Datasheet for the decision of 15 December 2005

Case Number: T 0327/04 - 3.3.04

Application Number: 98201056.3

Publication Number: 0870508

IPC: A61K 39/145

Language of the proceedings: EN

Title of invention:
Influenza vaccine

Patentee:
DUPHAR INTERNATIONAL RESEARCH B.V

Opponents:
Sanofi Pasteur
GlaxoSmithKline Biologicals s.a.

Headword:
Influenza vaccines/DUPHAR

Relevant legal provisions:
EPC Art. 54, 56, 111(1)

Keyword:
"Main request: Novelty (yes); Inventive step (yes)"
"Remittal (yes)"

Decisions cited:
T 0990/96

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0327/04 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 15 December 2005

Appellant: (Patent Proprietor)
DUPHAR INTERNATIONAL RESEARCH B.V
C.J. van Houtenlaan 36
NL-1381 CP Weesp (NL)

Representative:
Oser, Andreas
Prüfer & Partner GbR
Patentanwälte
Sohnckestrasse 12
D-81479 München (DE)

Other party (Opponent 01)
Chiron Corporation
4560 Horton Street
Emeryville, California 94608-2917 (US)

Representative:
Hallybone, Huw George
Carpmaels and Ransford
43 Bloomsbury Square
London WC1A 2RA (GB)

Respondent: (Opponent 02)
Sanofi Pasteur
2, avenue du Pont Pasteur
F-69007 Lyon (FR)

Representative:
Ayroles, Marie-Pauline
Sanofi Pasteur
Direction propriété Intellectuelle
2, avenue Pont Pasteur
F-69007 Lyon (FR)

Respondent: (Opponent 03)
GlaxoSmithKline Biologicals s.a.
89 Rue de l'Institut
B-1330 Rixensart (BE)

Representative:
Stephen, Robert John
GlaxoSmithKline
Corporate Intellectual Property (CN9 25.1)
980 Great West Road
Brentford, Middlesex
TW8 9GS (GB)

Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 26 January 2004 revoking European patent No. 0870508 pursuant to Article 102(1) EPC.

Composition of the Board:

Chair: U. Kinkeldey
Members: R. Gramaglia
G. Weiss

Summary of Facts and Submissions

I. European Patent No. 0 870 508 (application No. 98 201 056.3), with the title "Influenza Vaccine" was granted on the basis of 9 claims, of which claims 1 and 3 read as follows:

"1. Influenza surface antigen vaccine from Influenza Viruses propagated on animal cell culture obtainable by the method of claim 3 and having a host cell DNA content equal to or less than 25 pg per dose.

3. Method for the preparation of surface antigen proteins from Influenza Viruses propagated on an animal cell culture comprising the subsequent steps of:

a. treatment of the whole virus containing fluid obtained from the cell culture with a DNA digesting enzyme, and

b. adding a cationic detergent,

followed by isolation of the surface antigen proteins."

Claims 2 and 4 to 9 related to specific embodiments of the vaccine according to claim 1 or the method according to claim 3, respectively.

II. Notices of opposition were filed by opponents (01) to (03) requesting the revocation of the European patent on the grounds of Article 100 (a) and (b) EPC. The opposition division revoked the patent because it came to the conclusion that the subject-matter of claims 1

and 3 as granted (sole request) lacked an inventive step.

III. As regards the objection under Article 83 EPC, the opposition division informed the opponents in a communication dated 23 December 2002 (see page 2) that this objection had to be substantiated and that the onus of proof lay on the opponents. However, no further evidence was submitted by the opponents, so that the issue of Article 83 EPC was neither discussed at the oral proceedings before the opposition division, nor dealt with in the decision under appeal.

IV. The patentee (appellant) filed an appeal against the decision of the opposition division. The statement of grounds of appeal included amended sets of claims (1st to 30th auxiliary requests).

V. Opponent (01) withdrew its opposition by letter dated 9 November 2004.

VI. With a letter dated 12 September 2005, one of the respondents (opponent O3) submitted test reports relating to the DNA measurement, the slot-blot analysis and the reproducibility of Example 3 of the patent, in order to show insufficiency of disclosure under Article 83 EPC.

VII. The appellant asked that the above test reports be disregarded as being submitted too late.

VIII. Oral proceedings were held on 15 December 2005.

IX. The following documents are cited in the present decision:

D5 Merten O.-W. et al., Novel Strategies in Design and Production of Vaccines, Edited by Cohen S. and Shafferman A., Plenum Press, New York, pages 141-151 (1996);

D6 Brands R. et al., Proceedings of the Third International Conference on Options for the Control of Influenza, Cairns, Australia, 4-9 May 1996, pages 683-693;

D7 US-A-4,064,232;

D8 Bachmayer H., Intervirology, Vol. 5, pages 260-272 (1975);

D9 Hagen A.J. et al., Biotechnol. Appl. Biochem., Vol. 23, pages 209-215 (1996);

D10 Hagen A.J. et al., Biotechnol. Prog., Vol. 12, pages 406-412 (1996);

D11 EP-A-0583142;

D13 WO-A-95/24468;

D14 WO-A-90/10058;

D15 Huyghe B.G. et al., Human Gene Therapy, Vol. 6, pages 1403-1416 (1995);

D16 WO-A-96/27677;

D18 WO-A-97/08298;

D19 WO-A-96/21035;

D20 Mendonça R.Z. et al., Brazilian J. Med. Biol. Res., Vol. 26, pages 1305-1317 (1993);

D21 Mitra G. et al., Ann. N. Y. Acad. Sci., Vol. 782, pages 422-431 (1996);

D22 Crainic R. et al., Develop. Biol. Stand. Vol. 46, pages 275-279 (1979);

D26 "Summary basis for approval" for Myoscint® of Centocor B.V. (1996);

D37 US-A-5,173,418;

D38 Jennings R. et al., Vaccine, Vol. 2, pages 75-80 (March 1984);

D39 Del Sal G. et al., BioTechniques, Vol. 7, No. 5, pages 514-518 (1989);

D42 WO-A-92/13002;

D44 EP-A-0370163;

D54 Influvac® 96/97 Solvay Arzneimittel;

D56 Halperin W. et al., AJPH, Vol. 69, No. 12, pages 1247-1251 (December 1979);

D57 ABPI Compendium 1996-1997;

D58 VAQTA® (inactivated hepatitis A vaccine) leaflet from Merck & CO., Inc, USA (March 1996);

D60 Takizawa T. et al., Journal of General Virology, Vol. 74, pages 2347-2355 (1993);

D62 Experimental test report dated 3 June 2004.

X. The submissions by the appellant, insofar as they are relevant to the present decision, can be summarized as follows:

Novelty (Article 54 EPC)

- The value "equal or less than 25 pg/dose" (hereafter: "≤ 25 pg/dose") in present claim 1 was a valid and reliable distinguishing feature since it could be accurately and reliably measured by the slot blot hybridization method used in the patent in suit.

- A lack of novelty of the claimed subject matter had not been substantiated.

- Decision T 990/96 (OJ EPO 1998, 489) did not apply to the present case because this decision related to the question of purity of chemical compounds, while the opposed patent dealt with the purification of a biological material, for which prior attempts to reach a particular degree of purity by conventional methods had failed. Therefore, it was permissible to rely on the

achievement of a higher degree of purity to gain novelty over the prior art.

– The technical teaching of document D6 was not enabling for obtaining the vaccine having less than 50 pg DNA/dose.

– The methods for DNA removal described in documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44 did not deal with influenza surface antigen vaccines and were not applicable to the present situation. There was no certainty to obtain the required purity on influenza vaccines expressed as a ratio pg residual DNA vs. µg of hemagglutinin (HA). The preparation of different virus vaccines was designed individually and specifically to the virus of concern, as the various viruses differed significantly in terms of the overall structure, (RNA, DNA), the virus surface (enveloped, non-enveloped) and size.

Inventive step (Article 56 EPC)
Claim 3

– The closest prior art was represented by documents D5, D7 or D8 dealing with the production of flu vaccines.

– There was no implicit teaching in document D6 of using the detergent cetyl trimethyl ammonium bromide (CTAB) in the preparation of the Influvac® vaccine. A consideration of further documents D54 and D57 (Influvac® vaccine only) was not acceptable.

– In any case even if document D6 could be combined with documents D54 and/or D57, the skilled person could not derive any information as to which solubilisation procedure had been used for a down-stream processing of the vaccine made on Madin Darby Canine Kidney (MDCK) cells.

– The cationic detergent CTAB was used in prior art methods for the purpose of selective solubilisation of hemagglutinin and neuraminidase versus other viral components, not to reduce the DNA content.

– DNA digesting enzymes were disclosed in connection with virus types other than influenza virus. The main purpose was to reduce viscosity after cell lysis. However, cell lysis as described in documents D9 to D11, D15, D16 and D37 did not occur in the case of influenza virus preparation.

– The biological process involving influenza virus infection on cells in culture was clearly distinct from cell lysis. An envelopped virus like influenza virus used cellular membranes for cell assembly (budding and cell necrosis), not cell disruption as in lytic viruses.

Claim 1

– It was only by following the technical means of claim 3 that the product of claim 1 could be arrived at.

XI. The submissions by the respondents, insofar as they are relevant to the present decision, can be summarized as follows:

Novelty (Article 54 EPC)

- The slot blot hybridization method used in the patent in suit for measuring the residual DNA levels was so imprecise that the value ≤ 25 pg/dose in present claim 1 could not be used to distinguish it from the very close "less than 50 pg/dose" value disclosed in document D6.

- The only alleged distinctive feature of claim 1 (c.f. ≤ 25 pg/dose) vis-à-vis the influenza virus surface antigen vaccine disclosed by document D6 containing less than 50 pg of residual DNA was the purity degree of the vaccine. However, it was not permissible to rely on the achievement of a higher degree of purity to gain novelty over the prior art, unless prior attempts to reach a particular degree of purity by conventional methods had failed (see decision T 990/96, supra).

- But several conventional methods for DNA removal were available to the skilled person (see documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44). These protocols for DNA removal could be repeated until the DNA content fell beneath the prescribed threshold. Therefore, according to decision T 990/96 (supra), document D6 disclosed said influenza virus surface antigen vaccine in all grades of purity as far as the removal of contaminating DNA was concerned.

Accordingly the subject matter of claim 1 was not novel in view of document D6.

Inventive step (Article 56 EPC)
Claim 3

- The closest prior art was represented by document D6.

- There was an implicit teaching in document D6 of using the detergent CTAB in the preparation of both the Influvac® and the MDCK-based vaccines.

- The only difference between the process described in document D6 and that of present claim 3 lay in the addition of a nuclease (see step a) of claim 3).

- If it were denied that document D6 provided an implicit disclosure of using a cationic detergent in the preparation of the MDCK vaccines, the only differences between the process described in document D6 and that of present claim 3 lay in the addition of both a cationic detergent and a nuclease (see steps a) and b) of claim 3).

- The problem to be solved was reducing the contaminating host cell DNA to a minimal level.

- The addition of a cationic detergent was either implicit in document D6 or a mandatory step for solubilising the outer membrane proteins in order to produce a subunit vaccine, an expedient known from documents D7 and D8.

– As for the addition of a nuclease, the skilled person would turn to the prior art relating to the application of DNA digesting enzymes such as Benzonase and DNase for the DNA removal from biological materials (see documents D9 to D11, D13 to D16, D18, D19, D37 and D44).

– The use of a nuclease prior to detergent treatment was the most obvious choice to be done (see document D37, column 8, lines 53-55 and column 9, last paragraph).

Claim 1

– The subject matter of claim 1 lacked an inventive step in view of the obviousness of the method of claim 3, used for its production.

– If the vaccine according to document D6, exhibiting a level of contaminating DNA < 50 pg/dose were administrated intradermally, it would require 1/5 of the dose (see document D56, page 1249, r-h column, first paragraph) and consequently it would exhibit the required DNA level ≤25 pg/dose.

– It would have been obvious to modify the process for producing the vaccine disclosed by document D6 by incorporating therein a conventional purification step to remove DNA (see documents D9 to D11, D13 to D16, D18 to D22, D26, D37 and D44), and optionally a solubilisation step (see document

D8) and to arrive in an obvious way at the vaccine of claim 1.

XII. The appellant requested that the decision under appeal be set aside and that the patent be maintained as granted (main request) or, alternatively, that the patent be maintained in amended form on the basis of the auxiliary requests 1 to 30 filed with letter dated 4 June 2004.

The respondents requested that the appeal be dismissed or to remit the case to the department of first instance for further prosecution on the Article 83 EPC issues.

Reasons for the Decision

Main request
Novelty (Article 54 EPC)
Claim 3

1. The novelty of the subject matter of claim 3 has not been questioned by the respondents and the board agrees that none of the prior art documents discloses the combination of steps (a) and (b) above, let alone in that order, in the context of preparing virus surface antigen proteins from influenza viruses propagated on an animal cell culture.

Claim 1

Reliability of the method for measuring the residual DNA levels

2. The respondents argue that the slot blot hybridization method used in the patent in suit for measuring the residual DNA levels is so imprecise that the value ≤ 25 pg/dose in present claim 1 could not be used to distinguish it from the very close figure "< 50 pg/dose" disclosed in document D6. However, the board considers this objection to pertain to the issue of insufficiency of disclosure (Article 83 EPC), in the sense that the patent may be insufficient for failure to provide a reliable method for measuring the residual DNA levels. For the reasons given by the board in points 53 to 57 infra, the case is remitted to the department of first instance for examining the requirements of Article 83 EPC. The issues of this decision will thus be the novelty and inventive step questions.

Degree of purity suited to gain novelty over the prior art or not?

3. According to decision T 990/96 (see points 7 and 8 of the reasons), conventional methods for the purification of low molecular organic compounds are within the common general knowledge of the skilled person. Therefore, a document disclosing a low molecular chemical compound and its manufacture makes normally available this compound to the public in the sense of Article 54 EPC in all desired grades of purity, unless all prior attempts to achieve a particular degree of purity by conventional purification processes have failed.

4. Based on the above decision, the respondents argue that the degree of purity (c.f. "less than 25 host cell DNA per dose" in present claim 1) cannot confer novelty over the prior art, unless prior attempts to reach a particular degree of purity by conventional methods have failed. In the respondents' view document D6 discloses all grades of purity either in the starting material comprising the influenza virus surface antigen (i.e., the virus-containing fluid obtained by cell culture on MDCK-cells) or in the purified influenza virus surface antigen containing less than 50 pg of residual DNA (see page 689, first full paragraph) per 30 μg HA of dose (see page 685, under "Vaccines specifications").

5. The respondents further argue that the present situation does not represent an exception as set forth in decision T 990/96, since, at the priority date of the patent in suit, several conventional methods for DNA removal (involving e.g.: (i) phenol extraction, ethanol precipitation; (ii) chromatography such as anion-exchange chromatography; (iii) nuclease treatment of virus fluid followed by one or several capture steps including e.g. ultrafiltration, or (iv) the combination of the above techniques) were available to the skilled person. As examples of conventional methods for DNA removal, the respondents refer to processes disclosed in e.g., document D9, describing the purification of the VAQTA® vaccine containing less than 4 pg DNA per dose, as confirmed by document D58 (see first page, under "Description": "less than 4.10^{-4} mcg of DNA"), document D14, showing a decrease in DNA content by means of DNase treatment (see page 86, lines 25-30), document D22, describing a vaccine which contains less

than 8.2 pg cell DNA per dose (page 277, last-but-one paragraph) and document D44 (see page 4, line 31: "DNA was undetectable"). Reference is also made to document D37, teaching the use of Benzonase for the removal of nucleic acids from a biological material by degradation of long nucleic acid sequences into easy-to-remove shorter fragments (see last paragraph of column 2).

6. The appellant maintains that document D6 fails to provide an enabling disclosure for the skilled person to arrive at an influenza virus surface antigen vaccine containing less than 50 pg of residual DNA. The board accepts in favour of the respondents that the only possible distinctive feature of the vaccine of claim 1 vis-à-vis the influenza virus surface antigen preparation disclosed in this document lies in the achievement of a higher degree of purity.

7. The product covered by present claim 1 is an influenza virus surface antigen vaccine meeting certain criteria, inter alia that of exhibiting extremely low amounts of host-cell DNA expressed as a **ratio** of the residual DNA relative to the dose ("≤ 25 pg/dose"). A dose is defined in the patent in suit as 50 µg HA (see page 4, line 32). Therefore, it was neither relevant nor sufficient that at the priority date, contaminating DNA could easily be removed by conventional means from any kind of biological material in solution (virus, protein or else) so that the residual DNA content be below the threshold (25 pg) prescribed by claim 1 at issue. Rather, the decisive issue to be answered by the board is whether or not applying to the purified influenza surface antigen preparations disclosed by document D6 the methods for DNA removal pointed out by the

respondents would result in a vaccine exhibiting **a ratio** ≤ 25 pg residual DNA/50 µg HA according to present claim 1.

8. The board, furthermore, notes that the methods for DNA removal described in documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44 do not deal with influenza surface antigen vaccines. Therefore, these techniques are prima facie not applicable to the present situation since the preparation of different virus vaccines is designed individually and specifically to the virus of concern, as the various viruses differ significantly in terms of the overall structure (RNA, DNA), the virus surface (enveloped, non-enveloped) and size. This view is supported by the fact that documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44 are limited to the respective virus type, rather than describing universal methods for DNA removal from biological materials in solution (virus, protein or else). The different methods may have a significant impact on the overall result, in the sense that these differences may not only affect DNA removal, but also the removal of other components of the system, such as the desired product, namely the HA and neuraminidase surface antigen proteins, the precursors thereof, or other components such as DNA nuclease relevant for the process of the invention. It may happen that a change will remove more DNA but increase retention of influenza viruses and thus decrease the yields of e.g. HA. Thus, the decreased yield of HA would negatively affect the DNA product ratio stated in present claim 1.

9. A closer analysis of documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44 confirms the view arrived at

by the board that preparation methods dealing with other viruses cannot be readily applied to an influenza virus system.

10. In the process according to documents D9 to D11, a nuclease is used to avoid problems (low yields of the virus due to the formation of large aggregates between nucleic acids and the Hepatitis A virus (HAV)) which result from the HAV harvest by means of detergent-effected cell lysis (see e.g. document D9, page 210, 1-h column, lines 5-16 and page 214: "Conclusion"). However, cell lysis by means of e.g. the detergent Triton has to be avoided when dealing with the preparation of influenza virus vaccines, because this would disrupt the influenza virus already at this initial stage. The same conclusion applies to the method described in document D13, also involving a complete lysis of the host cells (see Examples 6 and 7).

11. Document D14 relates to a method for the production and purification of a hepatitis B virus vaccine. Hepatitis B virus is a DNA virus, whereas the influenza virus is a RNA virus. Moreover, in document D14 hepatitis B surface antigen particles are secreted into the culture medium using corresponding recombinant constitutive expression plasmids (see claim 1). This is fundamentally different from the situation of influenza virus preparations which involve the processing of whole virus containing fluids. While a decrease in DNA content by means of DNase treatment is described (see page 86, lines 25-30), it is not certain that this expedient would achieve a ratio ≤ 25 pg residual DNA/50 µg HA according to present claim 1, interpreted in the light of the description (page 4, line 32).

12. Document D15 relates to the purification of an adenovirus encoding human p53 by column chromatography (see title). The use of Benzonase is described on page 1404, end of l-h column, however, in the context of cell lysis which is a cause of contamination/yield problems in the subsequent ion-exchange chromatography (see page 1407, l-h column, third paragraph). However, cell lysis has to be avoided when dealing with the preparation of influenza virus vaccines, because this would disrupt the influenza virus already at this initial stage (see point 10 supra).

13. Document D16 relates to the purification of viral vectors for the delivery of therapeutic genes, a situation which is basically different from the preparation of surface antigen proteins from influenza viruses. Moreover DNase or Benzonase is also used in the context of cell lysates before ion exchange and affinity chromatography steps are carried out (see pages 17-18, under the heading "Lysis of Unencapsulated Nucleic Acids, Nuclease Treatment").

14. Document D19 relates to the preparation of recombinant retro-viral particles (see page 28). DNase is used to digest exogenous DNA and to improve the yield of purified recombinant retro-viral particles in the subsequent column chromatography step (see passage bridging pages 29 and 30). It is not certain that applying this expedient to the case of HA would achieve a ratio $\leq$ 25 pg residual DNA/50 µg HA according to present claim 1, interpreted in the light of the description (page 4, line 32).

15. Document D22 describes the purification of an inactivated polio virus (PV) cultured on human HeLa cells according to a protocol including phenol extraction, ethanol precipitation, filtration and a DEAE-sepharose column chromatography. The inactivated PV was shown to contain less than 8.2 pg cell DNA per dose (see page 277, last-but-one paragraph). However, there is no evidence that applying the above purification protocol valid for the non-enveloped PV to the enveloped flu virus would achieve a ratio ≤ 25 pg residual DNA/50 μg HA according to present claim 1, interpreted in the light of the description (page 4, line 32).

16. Document D37 relates to the purification of enzymes of Serratia spp. (nuclease, lipase and phospholipase) produced in E. coli (successively named Benzonase after the applicant of D37, Benzon Pharma) and teaches their use for the removal of nucleic acids by degradation of long nucleic acid sequences into easy-to-remove shorter fragments (see last paragraph of column 2). However, the nuclease is used in the context of reducing the viscosity of the cell lysate (see column 7, lines 25-27 and column 8, lines 54-56). Therefore, the conclusions arrived at under points 10 and 12 supra also apply to the purification process disclosed in this document.

17. Document D44 deals with the purification of monoclonal antibodies in a cell culture fluid, a situation different from preparing surface antigens from an animal cell culture. DNase is used to digest exogenous DNA and to improve the yield of purified recombinant proteins in the subsequent column chromatography step (see page 2, lines 27-30 and page 4, Example 3). It is

not certain that applying this technique to the case of HA, a molecule different from antibodies, would achieve a ratio ≤ 25 pg residual DNA/50 µg HA according to present claim 1, interpreted in the light of the description (page 4, line 32).

18. The respondents also maintain that the above protocols for DNA removal can be repeated ad libitum until the DNA content falls below the prescribed threshold. However, even if repetition of the protocol lowers the **absolute** residual DNA concentration, it would not alter the **ratio** of residual DNA/µg HA, which is a constant linked to the degree of "stickiness" of the residual DNA to the glycoproteins HA and neuraminidase.

19. In conclusion, there is no evidence before the board that at the priority date of the patent in suit, the skilled person applying to the purified influenza surface antigen preparations disclosed by document D6 the methods for DNA removal pointed out by the respondent (see documents D9 to D11, D13 to D16, Dl8, D19, D22, D37 and D44) would result in a vaccine exhibiting a ratio ≤ 25 pg residual DNA/50 µg HA.

20. Therefore, the board considers that the degree of purity in present claim 1 (c.f. "less than 25 host cell DNA per dose") is indeed suited to gain novelty over the influenza virus surface antigen described in document D6, be the latter document enabling or not.

Inventive step (Article 56 EPC)

Claim 3

Closest prior art

21. The parties were not in agreement as to which document represented the closest prior art. The appellant viewed documents D5, D7 or D8, dealing with the production of flu vaccines, as the closest prior art, whereas the respondents considered more appropriate to depart from document D6.

22. Document D5 relates to the propagation of influenza virus on animal cells and its isolation. Both documents D7 and D8 disclose the selective solubilisation by means of cationic detergents of the antigens (hemagglutinin and neuraminidase) of influenza virus propagated on embryonated eggs.

23. Document D6 pertains to a MDCK cell culture-based process for producing the influenza virus vaccine, which process has been adapted from the known Influvac® egg-based process (see page 684, first sentence under the heading "Production method of a MDCK- and egg-derived vaccine"). It is stated on page 685, first full paragraph of this document that the new process was "largely based" upon this existing process involving ion-exchange chromatography, ultrafiltration, ultracentrifugation, formaldehyde inactivation and solubilisation. The issue of contaminating host cell DNA is specifically emphasized under the heading "Vaccine specifications" (see page 685), stating that "The MDCK-derived vaccine contained host-cell DNA at levels well below the WHO-recommended limit of 100 pg/dose".

24. Two critical features of the process of present claim 3 are inter alia (i) its capacity to yield a vaccine with a contaminating host cell DNA level ≤25 pg/dose (see

claim 1), (ii) the propagation of the virus on animal cells. The board observes that only document D6 simultaneously deals with features (i) and (ii) above, whereas document D5 is concerned with feature (ii) only and documents D7 and D8 do not address any of these features. Therefore, applying the principle according to which the closest prior art is that dealing with a similar problem and requiring the minimum of structural and functional modifications to arrive at the invention, the board concludes that document D6 represents the closest prior art.

Problem to be solved

25. The objective problem to be solved departing from the disclosure of document D6 is seen in the provision of an improved method for the preparation of influenza surface antigen proteins in order to obtain a vaccine which has reduced host-cell DNA content. The concern regarding cell-substrate DNA was the possible integration of the abnormal DNA into host DNA (see e.g., document D21, page 422, second paragraph).

26. The solution to this problem is provided by the method of present claim 3, comprising the subsequent steps of

a) treatment of the whole virus containing fluid obtained from the cell culture with a DNA digesting enzyme, and

b) adding a cationic detergent,

in that order.

27. The results in the Table on page 4 of the patent in suit and the additional test report D62 provided by the appellant show that the above problem has indeed been solved. Document D62 even shows that if steps (a) and (b) occur simultaneously, no advantageous effect turns up (see Table 1, column 3: "1000 pg DNA/mg proteins" ≈ 50 pg DNA/50 μg proteins).

Implicit teaching or not of using CTAB in document D6

28. The respondents argue that the skilled person, knowing that the Influvac® egg-based process utilized the detergent CTAB for subunit solubilisation, would understand from document D6 that CTAB was also used to prepare the subunit vaccine from the virus grown on MDCK cells. In support of the above view, documents D54 and D57, mentioning traces of CTAB in the final Influvac® product, were cited.

29. However, in the board's judgement, even by combining the teaching of document D6 with that of documents D54 and/or D57, the skilled person could not unambiguously derive any information as to which solubilisation procedure had actually been used for the downstream processing of the MDCK cell culture-isolated influenza virus described in document D6. This is because document D54 mentions traces not only of the cationic detergent CTAB but also of the anionic detergent "Natrium Desoxycholat" and of the non-ionic detergent "Polysorbat 80", while document D57 also mentions "Natrium Desoxycholat" besides CTAB. But in the light of document D42, illustrating the suitability of cationic, anionic or non-ionic detergents for solubilising the influenza virus (see page 2, line 2

from the bottom to page 3, line 10), the skilled person could reasonably assume that **any** of CTAB, sodium deoxycholate and polysorbate 80 had been used for dissociating a virus in order to obtain the sub-units. In conclusion, there is no unambiguous teaching in document D6 read in the light of documents D54 and 57 of utilizing the detergent CTAB for subunit solubilisation. Nor is there any guidance in document D6 which might lead the skilled person looking for an improved method for the preparation of an influenza subunit vaccine, to add a nuclease (i.e., steps a), b) in claim 3), let alone in that specific order.

Inventive step of the proposed solution

30. The relevant question to be answered by the board is whether it was obvious or not to modify the process described in document D6 by adding a nuclease and a solubilisation step with a cationic detergent (with the nuclease added before the detergent) in the expectation of solving the problem referred to under point 26 supra and obtaining the advantageous effect highlighted under point 27 supra.

31. As regards the addition of a cationic detergent (see step b) in present claim 3), the respondents argue that this was an obvious mandatory step for solubilising the outer membrane proteins in order to produce a subunit vaccine (see documents D7 and D8). However, the board notes that the use of a cationic detergent was not the only way open to the skilled person wishing to solubilise the outer membrane proteins. In fact, document D42 (see page 2, line 2 from the bottom to page 3, line 10) and document D38 (see page 75, l-h

column, lines 1-4 under "Introduction") show that anionic and non-ionic detergents and other dissociating agents could also be used for solubilising the surface glycoproteins hemagglutinin and neuraminidases of the influenza virus.

32. As for the addition of the nuclease (see step a) in present claim 3), it is the respondents' view that the skilled person would turn to the prior art relating to the application of DNA digesting enzymes such as Benzonase and DNase for removing unwanted DNA from biological materials (see documents D9 to D11, D13 to D16, D18, D19, D37 and D44).

33. However, the board notes that in the processes described in documents D9 to D11, D13, D15, D16, D18 and D37, the DNA digesting enzymes are disclosed in connection with virus types other than influenza virus with the purpose to reduce viscosity arising from cell lysis with a detergent, during the preparation of Hepatitis A virus vaccines (see document D9, page 210, r-h column, line 13: "lysate" and line 28: "Nuclease digestion"; document D10, page 407, Figure 1: "Lysate" and "Nuclease treatment; document D11, Figure 1: "Triton → Nuclease" and document D13, Example 7.1 on pages 36-37), adenovirus or retro-virus vectors (see document D15, page 1404, bottom of l-h column; document D16, page 6, lines 12-13; document D18, page 16, lines 1-3 and page 17, lines 29-35) and during the preparation of biological material in general (see document D37, column 8, lines 31 and 51 and column 7, lines 27-30). However, no viscosity problems arise during the preparation of an influenza virus sub-unit

vaccine (see document D5, page 143, under "Virus Purification").

34. Relying on the legend to Fig. 1 ("Effect of influenza virus infection on the lysis of MDCK") of document D60 and on page 4, line 9 of the patent in suit ("to remove the cell debris"), the respondents argue that the influenza virus is also capable to induce cell lysis. In the board's view, however, while document D60 states that, in the case of influenza virus propagated on MDCK cell culture, virus replication is not a direct cause of cell lysis, which occurs by apoptosis (see page 2353, bottom of l-h column), there is no evidence before the board that the extent of said cells lysis is such that viscosity problems arise.

35. Document D14 relates to Hepatitis B virus (a DNA virus) and deals with the purification of secreted surface antigen (HBsAg) particles (see pages 10 and 11 of D14). The major contaminants are said to be high molecular weight protein complexes (see page 12, point (3)). Document D19 deals with the purification of recombinant retroviral vector particles (see Example 4 on page 56). Document D44 deals with an enzymatic purification process for monoclonal antibodies or proteins expressed in cell culture fluids. These are situations different from the preparation of a sub-unit vaccine based on surface antigen glycoproteins of influenza virus (a RNA virus), which involves inter alia processing fluids containing the whole virus.

36. In summary, while the use of DNA digesting enzymes for lowering the DNA content was disclosed in several documents, it was in situations which were prima facie

basically different from that of the preparation of an influenza virus sub-unit vaccine. Therefore, it could not be foreseen that applying the methods described in these documents would achieve a ratio ≤ 25 pg residual DNA/50 µg HA, a ratio which is linked to the specific degree of "stickiness" of the residual DNA to the HA and neuraminidase glycoproteins.

37. The board also notes that other means directed to DNA removal as such were known and applied as distinct alternatives to DNA digesting enzymes, e.g. DNA removal gels (see document D10, page 410, r-h column, line 4) and DNA filters (see document D11, page 14, line 20).

38. In conclusion, it was prima facie not obvious to modify the process described in document D6 by adding a nuclease and a solubilisation step with a cationic detergent in the expectation of solving the problem referred to under point 26 supra.

39. But even if the board accepted, for the sake of reasoning, that the prior art provided a hint to modify the process described in document D6 by adding a nuclease and a solubilisation step with a cationic detergent, it would still remain to be decided whether the order of addition (nuclease before detergent), a critical feature for obtaining the advantageous effect looked for (see point 27 supra), was obvious or not in view of the prior art.

40. In connection with the above question, the respondents argue that document D37 (see bottom of column 9) indicates that the nuclease may be used with a detergent at an early stage. However, this early

addition of the nuclease serves the purpose of reducing the viscosity of the cell lysate and to remove the bulk of the nucleic acid present, not to fine-tune the trace DNA (see ibidem, lines 63-65). Moreover, the board observes that when dealing with antigens and vaccines, document D37 (see column 10, lines 35-57) prescribes that the addition of nuclease should serve the purpose of removing the nucleic acid of such infectious agents, optionally with the help of a detergent to make the nucleic acid available to the nuclease. Therefore, the above passage suggests that the nuclease should be added after (or together with) the detergent has exposed the pathogen's DNA or RNA. This order of addition is contrary to the order "nuclease first, detergent after" stated in present claim 3.

41. In conclusion the subject matter of present claim 3 satisfies the requirements of Article 56 EPC.

Claim 1

42. For the same reasons highlighted under points 22 to 24 supra, document D6 represents the closest prior art underlying the vaccine of present claim 1.

43. The problem to be solved can be seen in the provision of an improved influenza surface antigen vaccine which contains ≤ 25 pg host cell DNA/dose. The results in the Table on page 4 of the patent in suit and the additional test report D62 show that the above problem has indeed been solved.

44. The board has first to dismiss the respondents' argument of a lack of inventive step of the vaccine of

claim 1 arising from the obviousness of its preparation method (claim 3), as the latter has been found by the board not to follow from the prior art in an obvious way (see point 41 supra).

45. In a further line of argument, the respondents maintain that if the vaccine according to document D6 (< 50 pg/dose) were administrated intradermally, it would require 1/5 of the dose (see document D56, page 1249, r-h column, first paragraph) and consequently it would obviously exhibit the required DNA level ≤25 pg/dose. This also applied if the vaccine were turned into a child dose (1/2 of the adult dose). However, even if such measures would lower the absolute residual DNA concentration, they would not alter the ratio residual DNA/μg HA. This is because taking 1/5 or 1/2 of a dose would also proportionally lower the μg HA taken.

46. Finally, the respondents argue that it would have been obvious to modify the process for producing the vaccine disclosed by document D6 by incorporating therein conventional purification steps to remove DNA (see documents D9 to D11, D13 to D16, D18 to D22, D26, D37 and D44) and possibly a solubilisation step (see document D8) and to arrive at the vaccine of claim 1.

47. However, in the context of the novelty (see points 8 to 17 supra), the board has already denied that combining the purification method described in document D6 with the methods for DNA removal described in documents D9 to D11, D13 to D16, D18, D19, D22, D37 and D44 would yield a vaccine exhibiting a ratio ≤ 25 pg residual DNA/50 μg HA according to present claim 1,

interpreted in the light of the description (page 4, line 32).

48. Therefore, the impact of the further methods for reducing the DNA levels disclosed in documents D20, D21 and D26 on the process described in document D6 has to be considered.

49. Document D20 discloses the preparation of a human rabies vaccine (see e.g. the abstract) propagated on animal cells (VERO cells). The procedure described in document D20 involves ultracentrifugation in a sucrose gradient (see page 1307, under "Virus concentration and purification"). It is reported on page 1312, l-h column that host residual DNA was quantified at levels of 10 pg per vaccine sample. However, no definition of the term "sample" is given, so that the skilled person is not in a position to know the real ratio in terms of residual DNA/μg antigen. There is also no evidence before the board that applying ultracentrifugation in a sucrose gradient to the preparation of an influenza subunit vaccine would achieve DNA levels below 25 pg/dose of flu surface antigen vaccine.

50. Document D21 describes an artificial model system wherein contaminants (labelled protein or DNA) were added to a glycoprotein partially purified from a mammalian cell-culture and then clearance of the contaminants from the recombinant glycoprotein was tested, using immunoaffinity chromatography as a purification method (see e.g., page 425 under "Experimental"). The skilled person would not derive from this model system far from the "real world situation" any hint that immunoaffinity chromatography

would be suitable to successfully obtain a flu vaccine product where the host cell DNA content is below 25 pg/dose.

51. Document D26 relates to a monoclonal antibody (Myoscint®), reporting levels of host cell DNA < 10 pg per dose (see page 19). It is merely stated on page 1, line 2 from the bottom that "the monoclonal antibody is produced by standard hybridoma technology" without giving any details as to how to arrive at such low levels of host cell DNA. Moreover, document D26 deals with a situation different from the preparation of a sub-unit vaccine based on surface antigen glycoproteins of influenza virus (a RNA virus), which involves inter alia processing fluids containing the whole virus.

52. In summary, there is no evidence before the board that the vaccine of claim 1 can be produced through an obvious modification of the process described in document D6. Therefore, since the route to the vaccine of claim 1 was applying the non-obvious process of claim 3, it must be concluded that the subject matter of present claim 1 does not follow from the prior art in an obvious way. This conclusion also applies to dependent claims 2 and 4 to 9, relating to specific embodiments of the vaccine according to claim 1 or the method according to claim 3, respectively.

Remittal

53. According to Article 111(1) EPC the board of appeal may either exercise any power within the competence of the department which was responsible for the decision appealed or remit the case to the department for

further prosecution. Remittal to the department of first instance is at the discretion of the board.

54. In particular, remittal is taken into consideration by the boards in cases where a first instance department issued a decision solely upon some particular questions which are decisive for the case against a party and leaves another essential issue outstanding. If, following appeal proceedings, the appeal on the particular issue is allowed, the case is normally remitted to the first instance department for consideration of the undecided issues.

55. The issue of Article 83 EPC has not been discussed at the oral proceedings before the opposition division (see paragraph III supra) and the respondents, who provided further test reports in order to show insufficiency of disclosure under Article 83 EPC (see paragraph VI supra), are asking remittal to the first instance for examination of Article 83 EPC.

56. Thus, a fundamental requirement for the grant of a patent, like sufficiency of disclosure has not yet been examined by the first instance. Consequently, the examination of the oppositions was not carried out in a way to put the board in a position to decide now, on the basis of a comprehensive examination of the first instance, whether or not the substantial requirements of the EPC are met by the present patent, which, considering the economical aspect of the procedure, would be the most preferable situation.

57. In conclusion, although being aware that this could lead to a considerable delay of the procedure, the

board considers it to be justified and appropriate to allow the present set of claims to be examined by two instances, and decides therefore, at its discretion under Article 111(1) EPC, to remit the case to the first instance for further prosecution.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance for further prosecution.

The Registrar: Chair:

P. Cremona U. M. Kinkeldey