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D E C I S I O N
of 18 May 2004

Case Number: T 0522/99 - 3.3.8

Application Number: 90914243.2

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IPC: C12N 15/35

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Title of invention:
Human parvovirus B19 proteins and virus-like particles, their production and their use in diagnostic assays and vaccines

Patentee:
Rijksuniversiteit te Leiden

Opponent:
GULL LABORATORIES

Headword:
-

Relevant legal provisions:
EPC Art. 54(3)(4), 56, 84, 123(2)

Keyword:
"Product-by-process claims - main request - novelty (no)"
"Product-by-process claims - first, second and third auxiliary requests - clarity (no), added subject-matter (yes)"
"Fourth auxiliary request - novelty (yes); inventive step (yes)"

Decisions cited:
T 0412/93

Catchword:
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Case Number: T 0522/99 **-** 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 18 May 2004

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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 8 March 1999 concerning maintenance of European patent No. 0491824 in amended form.

Composition of the Board:

Chairman: S. C. Perryman
Members: P. Julia
T. J. H. Mennessier

Summary of Facts and Submissions

I. European patent No. 0 491 824 with the title "Human parvovirus B19 proteins and virus-like particles, their production and their use in diagnostic assays and vaccines" was granted with 44 claims.

II. The patent was opposed under Articles 100(a) and 100(b) EPC on the grounds that the invention was not new, it did not involve an inventive step and it was not sufficiently disclosed. The opposition division considered that the main request filed on 16 October 1998 did not fulfil the requirements of Article 56 EPC. The patent was maintained on the basis of an auxiliary request filed at the oral proceedings on 28 October 1998 before the opposition division.

III. With the statement of Grounds of Appeal, the appellant (proprietor) filed a main request and three auxiliary requests. The main and the third auxiliary request corresponded, respectively, to the main request of the decision under appeal and to the auxiliary request accepted by the opposition division.

IV. The board sent a communication to the parties indicating its preliminary, non-binding opinion.

V. In reply to the board's communication the appellant requested oral proceedings.

VI. The parties were summoned to oral proceedings on 18 May 2004.

VII. The appellant filed further observations and indicated that claims 35 to 44 of the main request as well as the corresponding claims in the first and second auxiliary requests were no longer pursued.

VIII. The respondent, which did not submit any comments on the statement of Grounds of Appeal nor on the board's communication, informed the board of its intention not to attend the oral proceedings.

IX. Oral proceedings took place on 18 May 2004 in the absence of the respondent. During the oral proceedings the appellant withdrew all auxiliary requests and filed amended first, second, third and fourth auxiliary requests.

X. The **main request** was based on claims 1 to 34 only of the set of claims filed on 16 October 1998. The subject matter of claims 1 to 11 was concerned with the VP1 protein of human parvovirus B19. Claims 1 and 6 read:

"1. Recombinant baculovirus expression vector, equipped with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19 in Spodoptera frugiperda cells."

"6. Recombinant non-fused VP1 protein of the human parvovirus B19, free of VP2 protein, formed in Spodoptera frugiperda cells according to claim 3."

Claims 2 and 3 concerned, respectively, recombinant baculovirus comprising the vector of claim 1 and Spodoptera frugiperda (Sf) cells comprising the vector or the baculovirus of claims 1 or 2. Claims 4 and 5

concerned methods of producing the VP1 protein by culturing the Sf-cells of claim 3. Claims 7 to 9 concerned the use of the VP1 protein of claim 6 or the Sf-cells of claim 3 in assays for detecting antibodies directed against the VP1 protein. Claims 10 and 11, respectively, related to a vaccine preparation comprising the VP1 protein of claim 6 and the use of this protein for inducing an immune response.

Claims 12 to 23 were concerned with the VP2 protein of human parvovirus B19 and recombinant virus-like particles thereof. Claims 12 and 18 read:

"12. Recombinant baculovirus expression vector, equipped with the genetic information that is necessary for expression of VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells."

"18. Recombinant non-fused VP2 protein of the human parvovirus B19, free of VP1 protein, formed in Spodoptera frugiperda cells according to claim 14."

Claims 13 and 14 concerned, respectively, recombinant baculovirus comprising the vector of claim 12 and Sf-cells comprising the vector or the baculovirus of claims 12 or 13. Claim 15 concerned recombinant virus-like particles consisting of VP2 protein. Claims 16 and 17 concerned methods of producing the VP2 protein or virus-like particles consisting of said VP2 protein by culturing the Sf-cells of claim 14. Claims 19 to 21 concerned the use of the Sf-cells of claim 14, the VP2 protein of claim 18 or the virus-like particles of claim 15 in assays for detecting antibodies directed against the VP2 protein. Claim 22

related to a vaccine preparation comprising the VP2 protein of claim 18 and/or the virus-like particles of claim 15. Claim 23 related to the use of said VP2 protein and/or virus-like particles for inducing an immune response.

Claims 24 to 28 concerned VP1 and VP2 proteins and virus-like particles consisting of VP1 and VP2 proteins, whereas claims 29 to 34 related to these recombinant virus-like particles. Claims 24 and 29 read:

"24. Recombinant baculovirus expression vector, equipped with the genetic information which is necessary for expression of VP1 and VP2 protein of the human parvovirus B19 in Spodoptera frugiperda cells."

"29. Recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells according to claim 26."

Claims 25 and 26 related, respectively, to recombinant baculovirus comprising the vector of claim 24 and to Sf-cells comprising the vector or the baculovirus of claims 24 or 25. Claims 27 and 28 related to methods of producing VP1 and VP2 protein and/or virus-like particles consisting of VP1 and VP2 protein culturing the Sf-cells of claim 26. Claims 30 to 32 related to the use of the Sf-cells of claim 26 or the virus-like particles of claim 29 in assays for detecting antibodies directed against the B19 virus. Claims 33 and 34 related, respectively, to a vaccine preparation comprising the virus-like particles of claim 29 and to

the use of said virus-like particles for inducing an immune response.

XI. The amended **first auxiliary request** differed from the main request on claims 6, 10, 11, 18, 22, 23 and 29. Claims 6, 18 and 29 read:

"6. Recombinant non-fused VP1 protein of the human parvovirus B19, free of VP2 protein, formed in Spodoptera frugiperda cells according to claim 3, whereafter the cells are dried in air and fixed in 100% acetone."

"18. Recombinant non-fused VP2 protein of the human parvovirus B19, free of VP1 protein, formed in Spodoptera frugiperda cells according to claim 14, whereafter the cells are sonicated, and a supernatant collected is subjected to a linear sucrose gradient."

"29. Recombinant virus-like particles consisting of VP1 and VP2 protein of the human parvovirus B19, formed in Spodoptera frugiperda cells, which cells comprise a recombinant baculovirus expression vector of claim 1 and claim 12 or a recombinant baculovirus of claim 2 and claim 13."

Claims 10 and 22 differed from claims 10 and 22 of the main request by replacement of the term "comprising" by "consisting", whereas claims 11 and 23 were amended to read "use of only" instead of "use of".

XII. The **second auxiliary request** was as the amended first auxiliary request except for the deletion of claims 24 and 25. Claim 24 of the second auxiliary request

corresponded to claim 26 of the amended first auxiliary request and read:

"24. Spodoptera frugiperda cells comprising a recombinant baculovirus expression vector according to claim 1 and claim 12, or a recombinant baculovirus according to claim 2 and claim 13."

XIII. The **third auxiliary request** comprised claims 1 to 9 of the auxiliary request accepted by the opposition division and additional claims 10 to 15. Claims 10 and 13 read:

"10. Spodoptera frugiperda cells comprising a recombinant baculovirus comprising a recombinant baculovirus expression vector equipped with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19, and a recombinant baculovirus comprising a recombinant baculovirus expression vector equipped with the genetic information which is necessary for expression of VP2 protein of the human parvovirus B19."

"13. Recombinant non-fused VP1 protein of the human parvovirus B19, free of VP2 protein, formed in Spodoptera frugiperda cells comprising a recombinant baculovirus comprising an expression vector equipped with the genetic information which is necessary for expression of VP1 protein of the human parvovirus B19 in Spodoptera frugiperda cells, whereafter the cells are spotted on glass plates, air dried, and fixed in 100% acetone for 20 minutes at -20°C."

Claims 11 and 12 related to a method of producing VP1 and VP2 protein and/or virus-like particles consisting of VP1 and VP2 protein culturing the cells of claim 10 and to a method for producing a vaccine using the method of claim 11, respectively. Claims 14 and 15 concerned the use of the cells of claim 10 in an assay for detecting antibodies directed against the B19 virus.

XIV. The **fourth auxiliary request** was as the third auxiliary request except for the deletion of claim 13.

XV. The following documents are referred to in the present decision:

D1: V.A. Luckow and M.D. Summers, Bio/Technology, Vol. 6(1), January 1988, pages 47 to 55;

D2: WO90/05538 (publication date: 31 May 1990);

D12: W.P. Sisk and M.L. Berman, Bio/Technology, Vol. 5(10), October 1987, pages 1077 to 1080;

D18: S. Kajigaya et al., Proc. Natl. Acad. Sci. USA, Vol. 88, June 1991, pages 4646 to 4650;

D31b: WO88/02026 (publication date: 24 March 1988);

D34: C.S. Brown et al., Virus Res., Vol. 15, 1990, pages 197 to 212;

D41: S. Kerr et al., J. Med. Virol, Vol. 57, 1999, pages 179 to 185.

XVI. Appellant's arguments in writing and during the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Main request

Articles 54(3),(4) EPC

Recombinant non-fused VP1 protein free of VP2 protein and recombinant non-fused VP2 protein free of VP1 protein formed in Spodoptera frugiperda (claims 6 and 18)

Document D2 referred to the limited host range of the human B19 parvovirus and to the lethal effects of B19 proteins on transformed cells which hampered the production of stable transformants and the development of assays specific for this virus. Document D2 disclosed the production of non-infectious B19 capsids in higher eukaryotic cells - Chinese hamster ovary (CHO) - using a single DNA sequence encoding the large (VP1) and small (VP2) capsid proteins. There was no reference to the isolation of any of these proteins and the document itself was concerned only with the production of non-infectious B19 capsids. The Western blot of example 3 showed the proteins forming non-infectious capsids produced in CHO cells. The gel bands in the immunoblot of Figure 4 comprised only denaturated proteins, i.e. proteins in a linear conformation without secondary and tertiary structure and without conformational epitopes. The denaturating agent sodium dodecyl sulphate (SDS) in the polyacrylamide gel electrophoresis made it impossible to recover these proteins in their original undenaturated conformation (renaturated capsid proteins). The denaturated proteins were different from

the proteins produced in Sf-cells which were in an undenaturated conformation with conformational epitopes. As shown in post-published document D41, these undenaturated antigens were essential for an accurate detection of parvovirus B19. They had an improved antigenicity which resulted in better immunoassay methods. Whereas assays using denaturated proteins detected the presence of human parvovirus B19 in about 50% of the population, undenaturated antigens resulted in positive detection for more than 70% of the population. None of these effects were disclosed in document D2 which, thus, did not anticipate these undenaturated recombinant VP1 and VP2 proteins.

Recombinant virus-like particles consisting of VP1 and VP2 protein formed in Spodoptera frugiperda (claim 29)

Document D2 disclosed the production of non-infectious parvoviral B19 capsids in higher CHO eukaryotic cells (cell line 3-11-5) using a single DNA sequence encoding the VP1 and VP2 capsid proteins. The CHO cells were deficient in dihydrofolate reductase (DHFR) and they were co-transfected with a human DHFR minigen driven by the SV40 early promoter enhancer unit. These capsids were referred to as empty capsids and they were shown to be different from viral particles of human bone marrow culture by gradient centrifugation (example 5 and Figure 6). Post-published document D18 stated that baculovirus capsids, in contrast to 3-11-5 capsids, lacked the SV40 enhancer-promoter element from the SV40 virus. It also stated that the VP1/VP2 ratio in empty capsids was higher after co-infection of insect cells than in empty capsids produced in 3-11-5 cells. Moreover, the electron microscopy in example 6 of

document D2 showed empty capsids in the nuclei of 3-11-5 cells only, whereas baculovirus capsids were in the cytosol of Sf-cells. The different cellular distribution was due to structural differences of both capsids. Thus, baculovirus capsids were not anticipated by document D2.

Amended first and second auxiliary requests
Articles 84 EPC and 123(2) EPC
Recombinant non-fused VP1 protein free of VP2 protein and recombinant non-fused VP2 protein free of VP1 protein formed in Spodoptera frugiperda (claims 6 and 18)

Claims 6 and 8 had a basis, respectively, in examples 1.11 and 4 of the application as filed. Example 1.11 referred to air drying of Sf-cells infected with baculovirus AcB19VP1L and fixed in 100% acetone. Example 4 referred to sonication of Sf-cells infected with baculovirus AcB19VP2L and to a supernatant subjected to a linear sucrose gradient. The claimed VP1 and VP2 proteins were defined as product-by-process comprising detailed features of the process for their production. These features required these proteins to have an undenaturated conformation that distinguished them from VP1 and VP2 proteins - with a linear conformation - of the immunoblot bands of document D2.

Articles 54(3),(4) EPC
Recombinant virus-like particles consisting of VP1 and VP2 protein formed in Spodoptera frugiperda (claim 29 in amended first auxiliary request and claim 27 in second auxiliary request)

Document D2 disclosed the use of a single DNA sequence encoding VP1 and VP2 proteins and there was no reference to the use of two baculovirus expression vectors. The presence of two vectors in Sf-cells allowed one to modify the VP1/VP2 ratio, which, as stated in post-published document D18, was higher after co-infection of insect cells than in capsids produced in 3-11-5 cells. Thus, empty capsids formed in Sf-cells were different from the ones disclosed in document D2.

Third auxiliary request

Articles 84 EPC and 123(2) EPC

Recombinant non-fused VP1 protein free of VP2 protein formed in Spodoptera frugiperda (claim 13)

The specific conditions used in the immunofluorescence assay of example 1.11 for preparing the VP1 protein were all recited in claim 13. These conditions produced a VP1 protein with an undenaturated conformation as shown in post-published document D41. The VP1 protein was claimed as a product-by-process comprising all relevant conditions of its method of preparation. These conditions were clearly defined and they were not suggested in document D2 nor was the relevance of the undenaturated conformation.

Fourth auxiliary request

Articles 84 EPC and 123(2) EPC

Example 3 of the application as filed was a basis for those claims concerning double infection of Sf-cells.

Article 54 EPC

No prior art disclosed the use of two recombinant baculovirus expression vectors for the production of parvovirus B19 capsid proteins in Sf-cells. Document D2 disclosed the production of parvovirus B19 empty capsids in higher eukaryotic CHO host cells using a single expression vector with a DNA sequence coding for both VP1 and VP2 proteins. There was no suggestion in document D2 to use insect cells as host cells.

Article 56 EPC

The use of two expression vectors for producing parvovirus B19 capsid proteins and empty capsids in Sf-cells was not suggested in the prior art. The Sf-insect expression system and the advantageous selection of the double infection with two baculovirus expression vectors were not obvious from the prior art. In the light of the limited host range of parvovirus B19 and the reported toxicity of B19 capsids and proteins, the skilled person had no reasonable expectation of success.

XVII. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained as main request on the basis of claims 1 to 34 only of the set of claims filed on 20 October 1998, and as auxiliary requests on the basis of one of the set of claims filed as Amended First Request, Second Request, Third Request or Fourth Request, all submitted at oral proceedings on 18 May 2004.

XVIII. No requests were made by the respondent.

Reasons for the Decision

1. In the present case, a point of law raised by all requests on file is the appropriate assessment of claims for products defined in terms of a method of production, i.e. product-by-process claims. It is established case law of the Boards of Appeal that such claims are admissible only if the products themselves fulfil the requirements for patentability, i.e. *inter alia* that they are novel and inventive (cf. "Case Law of the Boards of Appeal of the European Patent Office", 4th edition 2001, II.B.6, 172). In particular, novelty can be established only if evidence is provided that the method confers particular characteristics or distinct differences in the properties of the claimed product and the skilled person is made aware of these differences so that it can always recognize the claimed product and discard any product not having those distinct characteristics (cf. "Case Law" *supra*, I.C.3.2.7, page 72 and *inter alia* T 412/93 of 21 November 1994, point 33 of the Reasons for the Decision).

Main request

Articles 54(3),(4) EPC

Recombinant non-fused VP1 protein free of VP2 protein and recombinant non-fused VP2 protein free of VP1 protein formed in Spodoptera frugiperda (claims 6 and 18)

2. Document D2 discloses the production of non-infectious, empty capsids of human B19 parvovirus. The full-length B19 genomic clone pYT103c is digested, sub-cloned and

thereafter non-structural regions are deleted. The Chinese hamster ovarian (CHO) cell line 3-11-5 deficient in dihydrofolate reductase (DHFR) is co-transfected with DNA from two plasmid constructs, one containing a DHFR minigene and the other containing the two B19 capsid genes (encoding the large VP1 and the small VP2 protein) under the strong single B19 promoter. Co-amplification of the integrated B19 genes and the DHFR sequence as well as establishment of a 3-11-5 cell line expressing the B19 structural capsid proteins is achieved by treating the cells with increasing concentrations of methotrexate. The structural B19 capsid proteins self-assemble to form an empty, but intact, and thus non-infectious, B19 parvovirus capsid (cf. example I, pages 9 and 10). These proteins are identified by Western blot using convalescent phase antiserum containing high titre anti-B19 capsid protein IgG (cf. example III, page 11). Figure 4 shows two immunogel-bands corresponding to molecular weights of 83 kd (VP1) and 58 kd (VP2). Document D2 refers to the method of the invention as producing parvovirus structural proteins, diagnostic assays, vaccines and methods of treating diseases (cf. page 5). Even if document D2 mainly relates to non-infectious empty capsids, it explicitly states that "*the capsid proteins are isolated in substantially pure form using protocols known in the art*" (cf. line bridging pages 6 to 7).

3. It has been argued that document D2 only discloses denaturated VP1 protein in the immunogel-band of Figure 4, whereas the reference in claim 6 to the method of production of VP1 protein in Spodoptera frugiperda (Sf) cells requires the claimed VP1 protein

to have an undenaturated, non-linear conformation with all its conformational epitopes. Post-published document D41 (cited as expert opinion) has further been cited as showing that the VP1 protein produced within the Sf-cells maintains an undenaturated conformation that allows the detection of conformational epitopes and improved diagnostic assays (cf. Section XVI *supra*).

4. However, even if the claimed VP1 protein is formed in Sf-cells, claim 6 does not require said VP1 protein to be within Sf-cells. The isolation of the VP1 protein from Sf-cells is not excluded from the scope of claim 6. In fact, such a purification is explicitly contemplated in the description of the patent in suit (cf. *inter alia* page 2, lines 53 to 56 and page 3, lines 33 to 38). Example 1.10 refers to the lysis of Sf-cells, SDS-PAGE electrophoresis and nitrocellulose blotting as well as incubation with IgG-positive human sera, which reacted with the recombinant VP1 band on the gel (cf. page 6, lines 25 to 37). There is no difference between the immunoblot assay of example 1.10 and the Western blot in Figure 4 of document D2. The board fails to see any limitation of the claimed VP1 protein - either explicit or implicit (in the light of the description) - to a particular conformation.

5. There is no reference in the patent in suit to either the undenaturated or the denaturated conformation of the VP1 protein. The patent discloses the use of a VP1 protein having a denaturated (example 1.10) or an undenaturated conformation (example 1.11) for testing the antigenicity of human sera with, respectively, a Western blot assay (example 1.10) or immunofluorescence assay (example 1.11). Screening with immunofluorescence

assay of randomly selected blood donors results in 76% of the donors being positive, which is said to correspond well with the data as described for human parvovirus B19 for this age-group (cf. page 7, lines 36 to 39). There is no suggestion whatsoever of any advantage to be derived from the presence of VP1 conformational epitopes.

6. Thus, claim 6 is considered to be anticipated by document D2. Similarly, document D2 anticipates claim 18 too, which is directed to recombinant non-fused VP2 protein free of VP1 protein and formed in Sf-cells.

Recombinant virus-like particles consisting of VP1 and VP2 protein formed in Spodoptera frugiperda (claim 29)

7. Document D2 shows that recombinant non-infectious parvovirus B19 empty capsids from the CHO 3-11-5 cell line differ from intact viral particles from human bone marrow culture by isopycnic sedimentation on a sucrose or cesium chloride gradient (cf. example V, pages 11 and 12, Figure 6). These 3-11-5 capsids and their cellular distribution are characterized by electron microscopy too (cf. example VI, page 12 and Figure 7).

8. Post-published document D18 (cited as expert opinion) indicates that the co-transfection of the CHO 3-11-5 cell line with a DHFR minigene comprising a human DHFR minigene driven by the SV40 early promoter enhancer unit may result in the presence of SV40 impurities inside the recombinant empty capsids (cf. page 4650, left-hand column, lines 8 to 13). It further refers to the ratio of VP1 and VP2 in empty capsids of insect

cells as being higher than the ratio of empty capsids produced in 3-11-5 cells (cf. page 4650, left-hand column, lines 13 to 15).

9. Document D2 itself does not refer to the presence of any SV40 contamination, impurity or heterogeneity in the disclosed empty capsids. The fact that for health and safety reasons a possible minor SV40 contamination in the production of human vaccines is to be avoided makes the use of insect cells - without co-transfection with SV40 elements - significantly advantageous. However, document D18 does not show that such a contamination is actually present in empty capsids of 3-11-5 cells let alone the extent and possible importance of such a contamination. There is no technical evidence on file showing this contamination, and a practical reason for using a more convenient method cannot make up for such a demonstration. Thus, on the basis of the patent in suit, there is no distinction between the claimed empty B19 capsids and the ones disclosed in document D2.

10. There is no reference in the patent in suit to the relevance of the VP1/VP2 ratio and claim 29 is not limited to any particular VP1/VP2 ratio. Post-published document D18 (cited as expert opinion) states that the proportion of VP1 may be increased by altering the multiplicity of infection of the respective baculovirus species (cf. page 4650, left-hand column). Thus, the VP1/VP2 ratio is not a fixed value and it cannot be relied on for differentiating the empty B19 capsids of document D2 from the claimed ones.

11. Whereas by using electron microscopy example VI of document D2 identifies the presence of empty B19 capsids only in the nuclei of 3-11-5 cells (cf. page 12 and Figure 7), example IV using an immunofluorescence assay found these capsids in both nuclei and cytosol (cf. page 7, lines 18 to 20 and page 11, lines 16 to 24, Figure 5). The immunofluorescence assay shown in Figure 3 (submitted during the oral proceedings before the board) shows the presence of empty capsids in both the cytosol and, albeit minor, in the nuclei of Sf-cells too. There is, however, no evidence on file showing that the cellular distribution of empty B19 capsids produced in Sf-cells or in 3-11-5 cells is actually linked to structural differences between these capsids and not to other possible differences in the specific methods and conditions used (temperature, time of incubation, fixation, etc.). Moreover, there is no demonstration that these alleged structural differences can always be recognized by the skilled person, i.e. that they are stable and reliable, as required for establishing novelty.

12. In the light of the evidence present on file, the board considers that the empty B19 capsids produced in the CHO 3-11-5 cell line of document D2 do not differ from and thus, anticipate, the claimed empty B19 capsids produced in Sf-cells (cf. point 1 *supra*).

Amended first auxiliary request

Articles 84 EPC and 123(2) EPC

Recombinant non-fused VP1 protein free of VP2 protein formed in Spodoptera frugiperda (claim 6)

13. Claim 6 includes specific features of the method used for producing the claimed VP1 protein in Sf-cells, in particular "*wherafter the cells are dried in air and fixed in 100% acetone*" (cf. Section XI *supra*), and thereby allegedly requiring the said VP1 protein to have an undenaturated conformation that distinguishes it from the denaturated, linear VP1 protein of document D2. Example 1.11 on page 13 of the application as filed has been given as a basis for this claim.

14. The board understands that recombinant VP1 protein is found in an undenaturated conformation within Sf-cells as well as in a denaturated conformation in the immunogel-bands of document D2. However, a protein "conformation" is not an absolutely rigid state but a dynamic one defined by the three-dimensional arrangements of the side groups on the protein molecule (secondary and tertiary structure). This spatial arrangement is - up to a certain point - dynamic and flexible, particularly for those groups protruding from the surface of the protein, which are the ones underlying the main conformational epitopes of the protein. This spatial arrangement changes or shifts somewhat into different positions or conformations under different conditions and thus, modifies the nature and extent of those conformational epitopes. Denaturation is understood as the transition from an ordered state to a disordered one, a random coil, wherein the chain of amino acids forming the protein

(primary structure) assumes different arrangements randomly distributed. If only some of the ordered structure is lost, the protein is partially denaturated only. Thus, depending on the specific conditions used, the spatial arrangement of the VP1 protein changes or shifts between different intermediate conformations - including completely undenaturated, partially or fully denaturated conformations - with different conformational epitopes as well.

15. Example 1.11 refers to very specific steps and conditions, *inter alia* the infected Sf-cells, after been dried in the air, are "*fixated in 100% acetone for 20 min at -20°C*" (cf. page 13, lines 22 to 23 in the application as filed). These conditions are said to be critical for maintaining the undenaturated conformation of the VP1 protein and for performing the diagnostic assays of post-published document D41 (cited as expert opinion) (cf. Section XVI *supra*). However, the actual effect on the conformation of the VP1 protein resulting from the omission of several conditions used in example 1.11 (fixation on whatever support, for any possible time period and temperature, etc.) is not known. It is also unknown whether the claimed VP1 protein will retain the undenaturated conformation at all or else will have a - partially or completely - denaturated conformation under each and every other possible condition embraced by the claim. This information is found neither in the patent in suit nor in the application as filed, so there is no basis for the omission in the claim of these specific conditions of the immunofluorescence assay of example 1.11. Thus, the features of the method of production referred to in claim 6 do not confer any particular identifiable

characteristics on the claimed VP1 protein so that it could always and unambiguously be identified by the skilled person (cf. point 1 *supra*).

16. Moreover, whereas the corresponding claim in the application as filed is clearly directed to a specific product (recombinant VP1 protein), the scope of claim 6 is ambiguous insofar as the VP1 protein is found within an undefined mixture of debris of an insect cell depending on cell concentration, culture medium, time of culture, etc., which is dried in air and fixed in acetone to any possible support. It is not clear whether the claim embraces the recombinant VP1 protein itself alone or else in combination with said undefined composition with or without the support.

17. It is also worth mentioning that there is nothing in the application as filed to suggest the importance of air drying and fixating the VP1 protein on glass plates for carrying out the immunofluorescence assay of example 1.11. Immunofluorescence assays, including air drying and fixation of the protein, are well-known in the prior art (cf. *inter alia* document D2 on page 11, example IV). It is only by knowing the results of post-published document D41 that the skilled person is made aware of the importance of the conformation of the VP1 protein in such an immunofluorescence assay. This information, however, cannot be derived from the application as filed.

18. It follows from all the foregoing that claim 6 does not fulfil the requirements of Articles 84 and 123(2) EPC.

Recombinant non-fused VP2 protein free of VP1 protein formed in Spodoptera frugiperda (claim 18)

19. Claim 18 includes specific features of the method used for producing the claimed VP2 protein in Sf-cells, in particular "*wherafter the cells are sonicated, and a supernatant collected is subjected to a linear sucrose gradient*" (cf. Section XI *supra*). Example 4 on page 17 of the application as filed has been given as a basis for this claim.

20. Whereas the corresponding claim in the application as filed is clearly directed to recombinant VP2 protein, it is now not clear whether claim 18 embraces the VP2 protein itself alone or else in presence of sonicated cells, as a collected cellular supernatant or as a band of a linear sucrose gradient. Moreover, several specific conditions explicitly set out in example 4 of the application as filed are omitted in claim 18 and thus, neither the gradient band nor the cellular supernatant or the sonicated cells are clearly defined (cf. point 15 *supra*).

21. Thus, claim 18 is considered not to fulfil the requirements of Articles 84 and 123(2) EPC.

Article 54(3),(4) EPC
Recombinant virus-like particles consisting of VP1 and VP2 protein formed in Spodoptera frugiperda (claim 29)

22. Claim 29 requires the claimed virus-like B19 particles to be produced by co-transformation of Sf-cells with two recombinant baculovirus expression vectors (cf. Section XI *supra*). This requirement relates to the

method of production and it does not confer any specific technical feature to the resulting virus-like B19 particles themselves (cf. point 1 *supra*). The fact that using two independent baculovirus vectors makes possible to alter the multiplicity of infection of each vector so as to achieve different VP1/VP2 ratios in the resulting virus-like B19 particles, is irrelevant since claim 29 is not limited to any particular VP1/VP2 ratio at all (cf point 10 *supra*).

23. Thus, the objections raised in points 7 to 12 *supra* for claim 29 of the main request apply to claim 29 of this amended first auxiliary request, which is also anticipated by the B19 capsids of document D2.

Second auxiliary request

Articles 84 EPC, 123(2) and 54(3),(4) EPC (claims 6, 18 and 27)

24. Claims 6, 18 and 27 of the second auxiliary request correspond exactly to claims 6, 18 and 29 of the amended first auxiliary request (cf. Section XII *supra*) so the objections raised under Articles 84 and 123(2) EPC for claims 6 and 18 (cf. points 13 to 20 *supra*) as well as under Article 54(3)(4) EPC for claim 29 (cf. point 22 *supra*) of the amended first auxiliary request apply to claims 6, 18 and 27 of the second auxiliary request too, which must thus be refused.

Third auxiliary request

Articles 84 EPC and 123(2) EPC

Recombinant non-fused VP1 protein free of VP2 protein formed in Spodoptera frugiperda (claim 13)

25. Claim 13 includes further features of the method used for producing the claimed VP1 protein in Sf-cells, in particular "*whereafter the cells are spotted on glass plates, air dried, and fixed in 100% acetone for 20 minutes at -20°C*" (cf. Section XIII *supra*). As for claim 6 of the amended first auxiliary request (cf. point 13 *supra*), example 1.11 on page 13 of the application as filed has been given as a formal basis for this amendment, which allegedly requires the claimed VP1 protein to have an undenaturated conformation.

26. However, apart from the particular (glass) support and the conditions used for cell drying and fixation, no other specific steps or conditions referred to in example 1.11 are mentioned in the claim, such as *inter alia* the multiplicity of infection of Sf-cells, days and medium of culture, mixture with uninfected cells, concentration of cells/well (500 cells/well in the application, whereas a concentration as high as 5000 cells/well is reported on page 203 of post-published document D34, cited as expert opinion). The effect of these omissions on the actual conformation of the claimed VP1 protein is unknown and there is no basis in the application as filed for a claim in which these features are omitted (cf. point 15 *supra*). Moreover, the scope of the claim is ambiguous insofar as the claimed VP1 protein is found within a completely

undefined mixture of cellular insect debris, dried in air and fixed to a glass support (cf. point 16 *supra*).

27. Thus, claim 13 does not overcome the objections raised under Articles 84 and 123(2) EPC for claim 6 of the amended first auxiliary request (cf. points 13 to 17 *supra*), and the third auxiliary request too must be refused.

Fourth auxiliary request

Articles 84 EPC and 123(2)(3) EPC

28. The request comprises all the claims 1 to 9 of the request on the basis of which the patent in suit was maintained by the opposition division, and which are not a subject for consideration in this appeal since the proprietor is the sole appellant, together with claims 10 to 14 relating to Sf-cells comprising two recombinant baculovirus with the genetic information necessary for expression of the VP1 or the VP2 protein, respectively (cf. Section XIV *supra*), which do need consideration by the board. Example 3 of the application as filed has been indicated as a formal basis for these additional claims. The subject matter of these claims represents a limitation in respect of the granted claims, which embraces insect Sf-cells with either a single recombinant baculovirus for expression of both VP1 and VP2 protein or else two independent baculovirus for the expression of VP1 or VP2 protein each.

29. Claims 10 to 14 fulfil the requirements of Articles 84 and 123(2)(3) EPC.

Article 54 EPC

30. There is no document on file disclosing the production of human parvovirus B19 proteins VP1 and VP2 in Sf-cells let alone in Sf-cells comprising two recombinant baculovirus with a baculovirus expression vector each. Thus, the requirements of Article 54 EPC are fulfilled.

Article 56 EPC

31. Document D12, the closest prior art for claims directed to the VP1 protein, discloses the production of VP1 protein from human parvovirus B19 using E. coli as host cells. The document explicitly refers to VP1 and VP2 proteins and the interest of having a convenient source of viral antigens for diagnostic purposes (cf. page 1077, left-hand column, first and second paragraphs). There is, however, no suggestion to co-transfect E. coli host cells with two independent expression vectors, one for each of the two viral antigens, let alone any hint whatsoever as to the possible relevance thereof.

32. Document D31b, the closest prior art for claims directed to empty B19 capsid proteins, discloses the production of VP1 and VP2 proteins which self-assemble to empty B19 capsids using expression vectors comprising a single DNA fragment containing the VP1/VP2 structural gene with either the endogenous parvovirus promoter or an heterologous promoter (mouse metallothione promoter). The splicing of the single VP1/VP2 structural gene in mouse and canine host cells results in both VP1 and VP2 proteins which

self-assemble to produce the empty B19 capsids. There is, however, no reference to a possible co-transfection of the host cells with two independent expression vectors, one for each of the two viral antigens, let alone a hint whatsoever as to the possible relevance thereof.

33. Thus, even when account is taken of the references found in document D31b to the use of different cell lines as possible host cells (cf. page 19, lines 4 to 9 of document D31b) or to alternative convenient sources of viral antigens in document D12 (cf. page 1077, left-hand column, last paragraph), which, in principle, could lead the skilled person to known baculovirus expression systems and insect Sf-cells (cf. *inter alia* document D1), none of these documents renders the co-transfection of Sf-cells with two baculovirus expression vectors obvious. Moreover, in view of the fact that both VP1 and VP2 proteins are present in parvovirus B19 capsids in a particular VP1/VP2 ratio, the use of two expression vectors which might result in a different VP1/VP2 ratio - depending on the multiplicity of infection, efficiency of each expression vector, protein stability, etc - cannot support a reasonable expectation of success. The advantageous production of stable empty B19 capsids with altered VP1/VP2 ratio (cf. page 4650, paragraph bridging left- and right-hand columns in document D18) could not be foreseen and could only be arrived at with the benefit of hindsight.

34. Thus, the subject matter of claims 10 to 14 fulfils the requirements of Article 56 EPC.

Article 83 EPC

35. In the absence of further submissions in respect of Article 83 EPC, the board does not see any reason to deviate from the conclusions drawn in the decision under appeal. Therefore, the requirements of Article 83 EPC are considered to be fulfilled.

Conclusion

36. Thus, the board considers that the fourth auxiliary request as a whole meets all the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The matter is remitted to the first instance with the order to maintain the patent on the basis of the claims of the fourth auxiliary request submitted at the oral proceedings on 18 May 2004 and a description yet to be adapted.

The Registrar: The Chairman:

A. Wolinski S. Perryman