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**D E C I S I O N**  
**of 3 May 2005**

**Case Number:** T 1052/02 - 3.3.4

**Application Number:** 91904867.8

**Publication Number:** 0466914

**IPC:** C12Q 1/00

**Language of the proceedings:** EN

**Title of invention:**

Immuno-chromatographic assay and method of using same

**Patentee:**

PACIFIC BIOTECH INC.

**Opponent:**

UNILEVER N.V. / UNILEVER PLC

**Headword:**

Immuno-chromatographic method/PACIFIC BIOTECH

**Relevant legal provisions:**

EPC Art. 54, 56, 83, 84, 123(2)(3)

**Keyword:**

"Main request - unallowable amendment - (yes)"

"First auxiliary request: filed at the oral proceedings -  
admissible - (yes); unallowable amendment - extension of scope  
of protection - (no); clarity - sufficiency of disclosure -  
novelty - inventive step - (yes)"

**Decisions cited:**

T 0002/81, T 0522/96, T 1126/97

**Catchword:**

-



Case Number: T 1052/02 - 3.3.4

**D E C I S I O N**  
**of the Technical Board of Appeal 3.3.4**  
**of 3 May 2005**

**Appellant:**  
(Opponent)

UNILEVER N.V.  
P.O. Box 760  
NL-3000 DK Rotterdam (NL)

UNILEVER PLC  
Unilever House  
Blackfriars  
London EC4 4BQ (GB)

**Representative:**

Hagemann, Heinrich, Dr.rer.nat., Dipl.-Chem.  
Meissner, Bolte & Partner  
Postfach 86 03 29  
D-81630 München (DE)

**Respondent:**  
(Proprietor of the patent)

PACIFIC BIOTECH INC.  
9050 Camino Santa Fe  
San Diego  
California 92121 (US)

**Representative:**

Whitaker, Iain Mark  
Marks & Clerk  
St. Albans Office  
45 Gosvenor Road  
St. Albans  
Hertfordshire AL1 3AW (GB)

**Decision under appeal:**

**Interlocutory decision of the Opposition  
Division of the European Patent Office posted  
24 April 2002 concerning maintenance of the  
European patent No. 0466914 in amended form.**

**Composition of the Board:**

**Chair:** U. M. Kindeldey  
**Members:** G. L. Alt  
G. E. Weiss

## Summary of Facts and Submissions

I. The appeal was lodged by the opponent (appellant) against the decision of the opposition division to maintain European patent No. 0 466 914 on the basis of the Auxiliary Request II pursuant to Article 102(3) EPC. The patent with the title "Immunochromatographic assay and method of using same" had been granted on the basis of claims 1 to 19. It had been opposed under Article 100(a) EPC for lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), under Article 100(b) EPC and Article 100(c) EPC. The opposition division found that claim 1 of the amended main request was not allowable under Article 123(2) EPC and that the claims of Auxiliary Request I contravened the requirement of Article 123(3) EPC.

Claims 1 and 13 as granted read:

"1. An assay involving the interaction of a ligand and an antiligand, in which one of the reagents used in the assay comprises either said ligand or antiligand, and nonlabeled particle is provided in admixture with said ligand or antiligand to minimize nonspecific binding, characterized in that the assay is executed on a chromatographic support layer along which said admixture moves, wherein said ligand or said antiligand is attached to a first mobile particle capable of moving through said support layer without agglomeration upon the occurrence of said interaction, and said nonlabeled particles comprise second mobile particles ranging from 0.2 $\mu$ m to a size such that said particles can move by capillary action through the support layer and formed of the same material as the first particle,

wherein the ratio of the second particles to the first particles in the assay is 1:1 to 3:1"

"13. A method for minimizing agglomeration of ligand-labeled or antiligand-labeled particles used in an assay involving the use of labeled and nonlabeled particles, wherein said label interacts with analyte, characterized by the steps of providing said labeled particles, said nonlabeled particles, and analyte on a support layer, wherein both the labeled particles and the nonlabeled particles range from about 0.2 $\mu$ m to a size such that said particles can move by capillary action through the support layer and formed of the same material, permitting interaction of said label and said analyte, and moving said labeled particles through said support layer away from a first zone into a second zone subsequent to said interaction, without agglomeration of particles due to said interaction."

II. The patentee (respondent) replied to the statement setting out the grounds for appeal and requested to dismiss the appeal, that is to say, to maintain the patent on the basis of the claims according to Auxiliary Request II found by the Opposition Division to fulfil all the requirements of the EPC.

Claims 1 and 12 of this request (now the Main Request), read:

"1. A single analyte assay involving the interaction of a ligand and an antiligand, in which one of the reagents used in the assay comprises either said ligand or antiligand, and nonlabeled particle is provided in admixture with said ligand or antiligand to minimize

nonspecific binding, characterized in that the assay is executed on a chromatographic support layer along which said admixture moves, wherein said ligand or said antiligand is attached to a first mobile particle capable of moving through said support layer without agglomeration upon the occurrence of said interaction, and said nonlabeled particles comprise second mobile particles ranging from 0.8 $\mu$ m to a size such that said particles can move by capillary action through the support layer and formed of the same material as the first particle, wherein the volume to volume ratio of the second particles to the first particles in the assay is 1:1 to 3:1."

"12. A method for minimizing agglomeration of ligand-labeled or antiligand-labeled particles used in an assay involving the use of labeled and nonlabeled particles, wherein said label interacts with analyte, characterized by the steps of providing said labeled particles, said nonlabeled particles, and analyte on a support layer, wherein both the labeled particles and the nonlabeled particles range from 0.8 $\mu$ m to a size such that said particles can move by capillary action through the support layer and formed of the same material, permitting interaction of said label and said analyte, and moving said labeled particles through said support layer away from a first zone into a second zone subsequent to said interaction, without agglomeration of particles due to said interaction."

The request contained thirteen claims dependent on claim 1 and three claims dependent on claim 12.

- III. Oral proceedings were summoned, accompanied by a communication from the board summarizing some of the issues to be discussed.
- IV. In the course of the oral proceedings the respondent filed an auxiliary request.

Claim 1 of the First Auxiliary Request read:

"1. A method for minimizing agglomeration of ligand-labeled or antiligand-labeled particles used in an assay involving the use of labeled and nonlabeled particles, wherein said label interacts with analyte, characterized by the steps of providing said labeled particles, said nonlabeled particles, and analyte on a support layer, wherein both the labeled particles and the nonlabeled particles are 0.8 $\mu$ m size such that said particles can move by capillary action through the support layer and formed of the same material, permitting interaction of said label and said analyte, and moving said labeled particles through said support layer away from a first zone into a second zone subsequent to said interaction, without agglomeration of particles due to said interaction."

The request comprised three dependent claims.

- V. The following documents are mentioned in this decision:

D1: GB-A-2 204 398

D2: US-A-4,680,274

VI. The appellant's arguments as far as they are relevant for the present decision may be summarised as follows:

*Main request*

*Amendments - Article 123(2) EPC*

The volume to volume ratio of second particles to first particles of 1:1 to 3:1 in claim 1 was neither explicitly nor implicitly disclosed in the application documents as filed. The same was true for the size of the second mobile particles, i.e. "0.8 $\mu$ m to a size such that that said particles can move by capillary action through the support layer".

*First Auxiliary Request*

*Admissibility into the proceedings*

The First Auxiliary Request was late filed. It was not prima facie allowable. Therefore it should not be admitted into the proceedings.

*Amendments - Article 123(2) EPC*

The value of 0.8  $\mu$ m was disclosed as part of a range in the application documents as originally filed. However, this was not a basis for the individualized value.

*Extension of scope of protection - Article 123(3) EPC*

In claims 1 and 13 as granted the size of the particles was precisely defined by a range of values "0.2 $\mu$ m to a size such that said particles can move by capillary

action through the support layer". Now the size was defined as being "0.8µm size such that said particles can move by capillary action through the support layer". Due to its unusual wording this feature could be construed as if the particle size was defined merely by the ability to move through the support layer with the consequence that embodiments fell under the claim which had not been fallen under it before, thus, extending the scope of protection.

*Clarity - Article 84 EPC*

Due to the double definition of the particle size, i.e. "0.8µm" on the one hand and "size such that said particles can move by capillary action through the support layer" on the other, the claim lacked clarity since it was not apparent to the reader of the claim whether the two features were relevant in combination or individually.

*Sufficiency of disclosure - Article 83 EPC*

In the patent specification it is reported that when the assay was carried out with a plain second particle (Example I), a positive signal was not visible. This demonstrated that there was an embodiment of the invention that did not work.

*Novelty - Article 54 EPC*

Document D1 disclosed on page 6 that "an ideal size range for the particles is from about 0.05 to about 0.5 microns". This disclosure included however implicitly that the particles may be greater, i.e. also 0.8 µm.



Moreover, document D1 disclosed as an embodiment an assay where two types of mobile particles were added for detection (pages 17 and 18). This was the same situation as in the patent in suit.

*Inventive step - Article 56 EPC*

Document D1 was the closest prior art document the only difference between its teaching and that of the patent in suit being the size of the particles. Since no effect was adhered to this feature, the problem to be solved was to find an alternative assay. In the absence of any effect a particle size of 0.8  $\mu\text{m}$  was an arbitrary selection and therefore not inventive. Moreover, the teaching of document D2 was not restricted to a particle size of 0.2  $\mu\text{m}$  or smaller but envisaged larger particles as well.

- VII. The respondent's arguments as far as they are relevant for the present decision may be summarised as follows:

*Main Request*

*Amendments - Article 123(2) EPC*

The examples disclosed that the effect of reduction of agglomeration became better the higher the concentration of BSA (bovine serum albumin)-coated particles relative to antibody-coated particles was. This was a general teaching from which the skilled person would understand that he could use a whole range of ratios.

The patent in suit generally disclosed that the particles had to have a size such that they were capable to move through the support layer. The value of 0.8  $\mu\text{m}$  would have been understood by the skilled reader as the exemplification of one of all the possible sizes. Therefore, it could represent either the upper or the lower endpoint of a range.

*First Auxiliary Request*

*Admissibility into the proceedings*

The First Auxiliary Request was prima facie allowable.

*Amendments - Article 123(2) EPC*

A particle size of 0.8  $\mu\text{m}$  was explicitly disclosed in the application document as originally filed.

*Extension of scope of protection - Article 123(3) EPC*

Claim 1 as granted related to a range of particle sizes: "0.2 $\mu\text{m}$  to a size such that said particles can move by capillary action through the support layer". Claiming one of the values comprised in the range, namely 0.8  $\mu\text{m}$ , was a limitation rather than an extension.

*Clarity - Article 84 EPC*

The feature "size such that said particles can move by capillary action through the support layer" was an explanation rather than a limiting feature. This might

be an over-definition, which did however not entail a lack of clarity.

*Sufficiency of disclosure - Article 83 EPC*

Example I was a comparison between plain and BSA-coated particles and, as could be seen from a comparison of Figures 1A, 2A and 2B, both had the advantage of minimizing agglomeration. Thus, this example did not demonstrate that an embodiment of the invention did not work.

*Novelty - Article 54 EPC*

Document D1 did not disclose a method for minimizing agglomeration.

*Inventive step - Article 56 EPC*

Apart from the particle size of 0.8  $\mu\text{m}$  a further difference between document D1 and the patent in suit was that document D1 used labelled and non-labelled particles. The subject-matter of the claims could neither be derived from document D1 alone nor in combination with document D2 which taught the use of smaller particles.

VIII. Requests

The appellant (opponent) requested that the decision under appeal be set aside and that the European patent No. 0 466 914 be revoked.

The respondent (patentee) requested that the appeal be dismissed (main request) or, in the alternative, to set aside the decision under appeal and to maintain the patent on the basis of the claims 1 to 4 filed at the oral proceedings (first auxiliary request).

## **Reasons for the Decision**

Main request

Amendments - Article 123(2) EPC

1. The appellant argues that the feature in claim 1 "wherein the volume to volume ratio of the second particles to the first particles in the assay is 1:1 to 3:1" has no basis in the application as filed.
2. The passages in the application documents as originally filed dealing with the ratio of second to first particles are the following:
  - (i) page 6, lines 27-28: "Ab-latex and BSA-latex are mixed together in varying ratios depending upon the test to be performed."
  - (ii) page 6, lines 34-37: "Of course, the ratios can vary substantially, with greater amounts of protein-labeled latex resulting in greater reduction of nonspecific binding."

(iii) page 6, last line continued on page 7: "The amount of latex (or other particle) that does not have antibody on can be any amount that is effective to appreciably decrease nonspecific binding, or false positives."

(iv) Example I - Strep A Test: antibody-latex - BSA-latex ratio 1:2;

(v) Example II - Occult Blood Test: antibody-latex - BSA-latex ratio: 1:1 or 1:3;

3. Hence, as can be seen from the above-cited passages, the application documents as originally filed do not disclose explicitly the ratio of 1:1 to 3:1. Apart from the specific values mentioned in the Examples (passages (iv) and (v) above), the application documents as originally filed contain general indications on possible ratios, cited in passages (i) to (iii) above. According to the case law of the Boards of Appeal on amendments a general disclosure is not regarded as a clear and unambiguous disclosure of a specific value. Therefore, the application documents as originally filed are not interpreted as disclosing implicitly the range of ratios of 1:1 to 3:1.

4. Thus, the amendment contravenes the requirements of Article 123(2) EPC.

5. Since the claim is invalid for that reason alone, there is no need to consider the issue of the particle size.

*First Auxiliary Request*

*Admissibility into the proceedings*

6. The appellant objected to the admissibility of the First Auxiliary Request because it was only filed during the course of the oral proceedings and not clearly allowable.

The Boards of Appeal have developed criteria for deciding on the admissibility of late-filed requests. Decision T 1126/97 of 13 December 2001, for example, summarizes conditions under which late amendments are admissible:

- (i) there should be some justification for the late filing;
  - (ii) the subject-matter of the new claims should not diverge considerably from the claims already filed, in particular they should not contain subject-matter which has not previously been claimed.
  - (iii) the new claims should be clearly allowable in the sense that they do not introduce new objections under the EPC and overcome all outstanding objections.
7. As to the first condition, the present board could accept as a justification for the late filing that the respondent had no reason to believe that the opposition division's decision might be overturned by the board in view of its "neutral" communication which only

- summarized the issues. It should be stressed however that, even if the circumstances may not suggest it, there is always a danger that a request might be refused. Therefore, it is advisable to file auxiliary requests as early as possible in order to minimize the danger of them not being admitted into the proceedings.
8. The rationale behind the second condition is that it is difficult, and therefore contrary to the principle of fairness, for an opponent to deal properly with subject-matter which significantly differs from previously claimed subject-matter. In the board's view subject-matter may be regarded as "significantly different" or "diverging considerably" when it requires examination of for example, a new solution to a new technical problem, or, in other words, when it creates a "new case". However, in the present case, claim 1 of the First Auxiliary Request corresponds to claim 13 as granted with the only exception of the definition of the particle size ("0.8 $\mu$ m size such that said particles can move by capillary action through the support layer" instead of "0.2 $\mu$ m to a size such that said particles can move by capillary action through the support layer"). This is not regarded to be a considerable divergence in the above sense.
9. As to the last condition, the notion of "clear allowability" does not - as implied by the appellant's argumentation - mean that an amendment must be acceptable without any consideration. Rather it means that it should not introduce new objections under the EPC - which is not the case here - and overcome all outstanding objections - which is the case as can be seen from the reasons below.

10. Hence, the board decides to admit the First Auxiliary Request into the proceedings.

*Amendments - Article 123(2) EPC*

11. The appellant argues that the feature "wherein both the labelled particles and the non-labelled particles are 0.8 $\mu$ m size" is not disclosed in the application documents as originally filed, because "0.8 $\mu$ m" is not disclosed as an individual value, but merely as part of a range.
12. It is true that "0.8 $\mu$ m" is disclosed in the application documents as originally filed as the endpoint of a range (page 3: "0.1 $\mu$  to about 0.8 $\mu$  in diameter"). However, the explicit mentioning of "0.8 $\mu$ m" gives the skilled person the unambiguous indication that the particles can take that size. Hence, the board considers that the application documents as originally filed provide a clear and unambiguous basis for a particle size of 0.8  $\mu$ m. The view that the endpoint of a range is recognised as a distinct, disclosed value is supported by case law of the Boards of Appeal on ranges establishing that an end point of a general range can be combined with the end point of a sub-range of that general range to a new range without infringing the requirements of Article 123(2) EPC (for example T 2/81 of 1 July 1982, point 3 of the Reasons or T 522/96 of 7 May 1998, point 2.1 of the Reasons).



*Scope of protection - Article 123(3) EPC*

13. In claim 1 as granted the particle size is defined as a range of "0.8 $\mu$ m to a size such that said particles can move by capillary action through the support layer...". As pointed out below under "Clarity", the expression in claim 1 of the First Auxiliary Request "...0.8 $\mu$ m size such that said particles can move by capillary action through the support material..." is regarded to refer specifically to the value of 0.8  $\mu$ m. By restricting a range to a single value the scope of protection is limited. Thus, the requirement of Article 123(3) EPC fulfilled.

*Clarity - Article 84 EPC*

14. The board does not see a lack of clarity arising from the expression "wherein both the labelled and the non-labelled particles are 0.8 $\mu$ m size such that said particles can move by capillary action through the support material". Since "0.8 $\mu$ m" is a distinct value, the expression "such that said ...".. can only be regarded as a description of a property of particles of this size, rather than an alternative definition or a limiting feature. Hence, the clarity-requirement of Article 84 EPC is fulfilled.

*Sufficiency of disclosure - Article 83 EPC*

15. Example I describes a comparison between immunoassays carried out with carbohydrate antigen of group A Streptococcus (StrepA antigen) and either latex particles coated by anti-Strep A antigen-antibodies

alone (Figure 1A) or in admixture with BSA-coated (Figure 2B) or plain latex particles (Figure 2A).

According to the appellant, Figure 2A of Example I demonstrates that an embodiment of the invention does not work, namely when plain, i.e. uncoated latex particles are used as agglomeration stoppers because then, as could be seen from the figure and as concluded in the disclosure of the example, "the positive signal was not visible".

The board does not concur with the appellant's view. The invention as claimed is a method to minimize agglomeration. Thus, whether there is a lack of sufficiency of disclosure or not, hinges on the evidence demonstrating whether or not the skilled person is in a position on the basis of the disclosure in the patent in suit and if necessary in combination with the common general knowledge to carry out the invention, i.e. to achieve a minimization of agglomeration. However, no such evidence is provided by Example I. On the contrary: A comparison of the degree of agglomeration of the assays of Example I demonstrates that when the test is carried out with antibody-coated particles alone (Figure 1A) agglomeration takes place and is observable as a distinct band, whereas when a mixture of either plain or BSA-coated particles and labelled particles (Figures 2A and 2B) is used, the moving particles are visible as a "smear" which is indicative of a reduction of agglomeration. Thus, Figure 2A of Example I is no evidence that an embodiment of the claimed method does not work.

16. The appellant's second argument, namely that the method formulated in the claims is so unclear that a person skilled in the art does not know what to do, cannot convince the board, either. Sufficiency of disclosure is assessed on the basis of the application as a whole - including the description - and not of the claims alone. However, the only evidence to which the appellant has pointed in order to demonstrate a lack of sufficient disclosure on the part of the description, is, as has been concluded above, not relevant.

Thus, the requirements of Article 83 EPC are fulfilled.

*Novelty - Article 54 EPC*

17. Document D1 discloses on pages 17 and 18 an multi-analyte assay specific for apolipoprotein A1 and B. Two different types of particles are added to the support layer, anti-apolipoprotein A1 and B antibodies attached to a label. According to page 6 of document D1 the label may be, for example, a coloured latex particle of a maximum diameter of not greater than 0.5  $\mu\text{m}$ .

This disclosure of the addition of a mixture to the support layer as claimed is not novelty-destroying for the subject-matter of claim 1 for two reasons: Firstly, the assay disclosed in document D1 relies on smaller particles (not greater than 0.5  $\mu\text{m}$  versus 0.8  $\mu\text{m}$  in claim 1) and secondly, the particles in the mixture are **both** coated with analyte-specific binding reagent, whereas in the assay of the patent in suit only one type of particle is specifically coated, but not the other.

Therefore, the subject-matter of claim 1 is novel over the disclosure of document D1.

*Inventive step - Article 56 EPC*

18. In the context of the problem and solution approach the Boards of Appeal have repeatedly pointed out that the starting point for assessing inventive step, i.e. the closest prior art, is a document relating to subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common. Thus, the purpose underlying the present invention has to be determined.
19. The authors of the patent in suit have noticed that in binding assays using binding-reagent-coated particles to capture the analyte, especially latex-coated particles, the particles tend to spontaneously agglutinate so that the amount of particles moving along the support and being able to react is reduced. Consequently, the signal relied on for the decision of whether the assay is considered negative or positive becomes faint, or even invisible, entailing the danger of false negative or positive results. Hence, the purpose underlying the patent in suit is to provide means that reduce or eliminate this spontaneous agglutination or agglomeration.
20. This objective is met by the subject-matter of claim 1 by providing the following mixture to a chromatographic support: (i) labelled particles, wherein the label is the compound reacting with the analyte and (ii) non-

labelled particles, i.e. particles not carrying the label, both types of particles having a size of 0.8  $\mu\text{m}$ .

The examples indicate that a mixture of latex particles coated with antibody and either latex particles coated with bovine serum albumin or plain latex particles reduces agglomeration of the particles. Hence, the underlying problem is solved by the patent in suit.

21. None of the prior art documents on file serves the purpose of reduction of particle agglomeration in immunoassays:

Document D1 aims at improving the convenience of use of an immunoassay. It is for example stated on page 2, last paragraph "The present invention is concerned [...] to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.". The objective of document D2 is to avoid the influence of contaminating factors in samples which react unspecifically with the binding reagents in immunoassays. Moreover, the agglomeration of particles is not mentioned in these documents which are the only prior art documents on file, the further document on file, document D3, being the International application corresponding to the European patent application of the patent in suit.

22. In view of the disclosure of documents D1 or D2 alone or in combination, a skilled person would neither be prompted to formulate the problem recited in point 18 above nor to solve it in the suggested way.

Therefore, the subject-matter of claims 1 and its dependent claims 2 to 4 involve an inventive step and meet the requirements of Article 56 EPC.

## Order

### For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the first instance with the order to maintain the patent in the following version:
  - claims 1 to 4 filed at the oral proceedings
  - description : pages 2, 3 and 5 filed at the oral proceedings; page 4 of the patent specification;
  - drawings of the patent specification.

Registrar:

Chair:

P. Cremona

U. Kinkeldey