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D E C I S I O N
of 16 June 2003

Case Number: T 0187/99 - 3.3.8

Application Number: 85300357.2

Publication Number: 0149565

IPC: G01N 33/53

Language of the proceedings: EN

Title of invention:
Assay method

Patentee:
Ortho-Clinical Diagnostics

Opponent:
Bio-Rad Pasteur S.A.
Bayer Corporation
Dade Behring Marburg GmbH

Headword:
Assay method/ORTHO

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request: inventive step (no)"
"Auxiliary request: inventive step (no)"

Decisions cited:
T 0606/89, T 0202/95

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0187/99 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 16 June 2003

Appellant: (Proprietor of the patent)
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Representative:
-

Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 7 December 1998 revoking European patent No. 0149565 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: F. L. Davison-Brunel
Members: T. J. H. Mennessier
C. Rennie-Smith

Summary of Facts and Submissions

I. The patentee (appellant) lodged an appeal against the decision of the opposition division dated 7 December 1998, whereby the European patent No. 0 149 565 was revoked.

II. The patent had been opposed by three parties on the grounds as set forth in Articles 100(a) and (b) EPC that the invention was not new, did not involve an inventive step and was not sufficiently disclosed. Basis of the revocation was the granted claims which were considered by the opposition division not to involve an inventive step.

III. Respondent I (opponent 1) and respondent II (opponent 2) filed observations in reply to the statement of grounds of appeal.

IV. A communication under Article 11(2) of the Rules of Procedure of the Boards of Appeal presenting some preliminary and non-binding views of the board was sent to the parties together with the summons to oral proceedings.

V. In reply to the board's communication, the appellant filed an auxiliary request together with its letter dated 13 May 2003, the granted claims being its main request.

VI. Respondents I and II filed observations relating to the board's communication and the appellant's auxiliary request. Moreover, respondent I submitted that the auxiliary request should not be admitted into the proceedings.

VII. Oral proceedings took place on 16 June 2003. As announced in its letter of 13 March 2003, respondent III (opponent 3) did not attend said oral proceedings.

VIII. Claim 1 of the main request for consideration by the board (granted claim 1) read:

"1. A method of performing an assay of an analyte in a liquid medium, comprising the use of:-
i) individual assay vessels or an array of assay vessels in fixed relationship to one another,
ii) a labelled reagent for the assay which is soluble in the liquid medium, and
iii) another reagent for the assay bound to magnetically attractable particles which are suspendable but insoluble in the liquid medium
comprising the steps of
a) incubating in the assay vessels a sample containing the analyte with the other reagents for the assay whereby the labelled reagent becomes partitioned between the liquid phase and the magnetically attractable particles in proportions which depend on the concentration of the analyte in the sample,
b) separating the liquid phase from the magnetically attractable particles, and removing the liquid phase from the assay vessels,
characterised by the labelled reagent being a component of a fluorescent or luminescent system and following

removal of the liquid phase from the magnetically attractable particles resuspending the magnetically attractable particles in another liquid medium and observing a signal generated by the labelled reagent thereon."

Claims 2 to 8 were dependent on claim 1, with claim 6 being dependent on claims 1 to 5.

IX. Claim 1 of the auxiliary request read:

"1. A method of performing an assay of an analyte in a liquid medium, comprising the use of:-
i) individual assay vessels or an array of assay vessels in fixed relationship to one another,
ii) a labelled reagent for the assay which is soluble in the liquid medium, and
iii) another reagent for the assay bound to magnetically attractable particles which are suspendable but insoluble in the liquid medium
comprising the steps of
a) incubating in the assay vessels a sample containing the analyte with the other reagents for the assay whereby the labelled reagent becomes partitioned between the liquid phase and the magnetically attractable particles in proportions which depend on the concentration of the analyte in the sample,
b) separating the liquid phase from the magnetically attractable particles, and removing the liquid phase from the assay vessels,
characterised by:
the labelled reagent being a component of a fluorescent or luminescent system,

the magnetically attractable particles being suspendable in the liquid phase without shaking for a period at least as long as the incubation time of the assay and
following removal of the liquid phase from the magnetically attractable particles resuspending the magnetically attractable particles in another liquid medium and observing a signal generated by the labelled reagent thereon." (emphasis added by the board)

Dependent claims 2 to 5 corresponded in wording to claims 2 to 5 as granted and dependent claims 6 and 7 corresponded to claims 7 and 8 as granted.

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

(24) US-A-4 256 834;

(28) EP-A1-0 030 087;

(30) I. Viinikka et al., Clin. Chim. Acta, Vol. 114, 1981, Pages 1 to 9;

(31) M. Pourfarzaneh et al., Ligand Quarterly, Vol. 5, No. 1, 1982, Pages 41 to 47;

(36) EP-A1-0 082 636;

(37) W. Klingler et al., Steroids, Vol. 42, No. 2, August 1983, Pages 123 to 136;

(51) Declaration of Gordon Coulter Forrest dated 10 September 1993.

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

Main request; inventive step of the subject-matter of claim 1

Document (28) was one of the documents which could be taken as the closest prior art for the assessment of inventive step. It described immunoassays based on fluorometry or chemiluminescence using magnetically attractable particles (MAPs). These assays differed from the method of claim 1 in that it was the fluorescence or luminescence emitted by the labelled reagent **still present in the supernatant** after separation of the MAPs which was measured. In view of that prior art, the objective technical problem could be defined as the provision of an assay method which avoided a manual process. The solution provided was to read the fluorescence or luminescence emitted by the labelled reagent fixed on the MAPs. At the priority date, the person skilled in the art knew the magnetizable particles to be dark-coloured (see document (31), page 46, right-hand column), opaque and able to absorb and scatter light when suspended in a liquid phase. He/she would have thus concluded that they had a high probability of interfering with the reading. And, besides, the sensitivity of the machines used for the reading was considered poor. Thus, it would have been expected that the presence of MAPs would have interfered so much with the reading as to make the carrying out of a useful assay impossible. Therefore, the possibility of using magnetizable particles in immunoassays would have been disregarded

as unrealistic. Evidence of this point could be found in such documents as document (30), page 3, which showed that the common practice was as described in document (28), ie to separate the MAPs and to measure fluorescence on the remaining supernatant.

The respondents had cited various documents, such as document (36), which described immunoassays based on the use of transparent, non-magnetically attractable particles. Because of the very nature of these particles, the skilled person would not have thought of combining the teachings of these documents with that of document (28). In the same manner, document (24) related to a homogeneous assay in which charcoal was used to **quench** the fluorescent signal emitted by unbound material in order to be able to measure the signal bound to clear particles. If anything, it taught away from using opaque particles as carrier for the label producing the signal to be measured. There was, thus, no reasonable expectation of success of solving the afore-mentioned problem by combining the teachings of this document with that of document (28).

Document (37) was identified by the respondents as the closest prior art. As with the previously mentioned documents, this document disclosed an immunoassay which made use of non-magnetizable particles. There was, thus, no reason why the skilled person trying to solve the afore-mentioned problem would have taken its teachings into consideration.

Auxiliary request; inventive step of the subject-matter of claim 1

In all of the documents of the prior art, the reason for using MAPs was to separate the components of the immunoassays from the biological sample. The person skilled in the art would, thus, have been inclined to look for magnetizable particles which were relatively dense and sedimented swiftly. Therefore, there was no incentive in the state of the art to choose shaking for a period of time at least as long as the incubation time. For this reason, the subject-matter of claim 1 was inventive.

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

Main request; inventive step of the subject-matter of claim 1

Document (37), which described all the technical features of the invention as defined in claim 1 with the exception that particles without magnetizable material were used instead of MAPs, represented the closest prior art. In view of the fact that the method of that document *sui generis* required a centrifugation step to separate the bound label (attached to the particles) from the unbound label (present in the liquid phase), such a step being associated with disadvantages acknowledged in the prior art (see document (31)), the objective technical problem to be solved was the provision of an immunoassay which avoided that separation step. As MAPs were known at the priority date and as their use was regarded in the

prior art (and particularly, in document (31)) as advantageously replacing centrifugation steps in immunoassays, claim 1 did not involve an inventive step.

Auxiliary request; inventive step of the subject-matter of claim 1

The additional feature contained in claim 1 of the auxiliary request was only an obvious optimisation of the claimed method, as both the disadvantage associated with continuous mixing and, thus, the advantage of using MAPs having a density or a size appropriate for them to remain in suspension were known in the prior art, more particularly from document (31).

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

All the materials and reagents, including the MAPs, useful to carry out the method as defined in claim 1 of the main request were available to the person skilled in the art at the priority date. There was no particular technical problem addressed by the invention, it was rather for the skilled person a question of choosing the more appropriate technical means. Nevertheless, if the problem-solution approach was to be used, document (37) might be regarded as the closest prior art. The use of MAPs being suspendable in the liquid phase without shaking for a period at least as long as the incubation time of the assay was to be regarded by the person skilled in the art as an obvious requirement.

XIV. Respondent III did not express any opinion in these appeal proceedings.

XV. The appellant requested that the decision under appeal be set aside and the patent be maintained as granted (main request) or alternatively on the basis of the set of claims filed with its letter of 13 May 2003 (auxiliary request).

XVI. Respondents I and II requested that the appeal be dismissed.

Reasons for the Decision

Main request (claims as granted)

Article 54 EPC, Article 83 EPC: novelty, sufficiency of disclosure

1. At oral proceedings, respondents I and II no longer objected to the claimed subject-matter on the grounds of novelty and sufficiency of disclosure. Respondent III failed to provide any arguments in writing regarding these two points. In the board's judgment, there are no documents on file which destroy the novelty of the subject-matter of claim 1, and the claimed process is reproducible on the basis of the information provided in the description of the patent in suit. Novelty and sufficiency of disclosure are acknowledged.

Article 56 EPC; inventive step of claim 1

The state of the art

2. Document (37) describes a solid phase immunoassay of unconjugated estriol in serum of pregnant women monitored by **chemiluminescence**. The immunoassay is performed in three steps. Firstly, the serum is mixed with a tracer solution (a luminogenic estriol derivative) and a suspension of anti-estriol antibodies attached to solid particles of the "Amerlex" type (which are particles without a magnetizable component). Secondly, after incubation, the particles are centrifuged and washed. Finally, they are resuspended and the light emission of the bound tracer attached to the particles is measured by oxidation with H_2O_2 using microperoxidase as catalyst.

3. Document (28) describes a solid phase immunoassay of an analyte (see pages 1 to 6 and claim 1). The immunoassay is performed in five steps. Firstly, the solution containing the analyte to be measured is mixed with an excess of magnetically attractable particles bearing a receptor capable of selectively binding the analyte (such as an antibody). Secondly, after incubation, the particles are magnetically separated. Thirdly, in one of the described embodiments (see from line 24 of page 4 to line 3 on page 5), the particles thus recovered are resuspended in a liquid comprising a labelled substance which may be a fluorometric or chemiluminescent one (see page 5, lines 11 and 12) which binds to those receptors on the particles which are not already bound to the analyte. Fourthly, after incubation, the reaction mixture is centrifuged and

resuspended. Finally, the amount of label which has become attached to the excess particle-bound receptor is measured. However, in the absence of any detailed illustration in the documents of an immunoassay employing those five steps identified above, its description (from line 11 on page 2 to line 1 on page 5) amounts to a suggestion rather than an actual disclosure.

4. Document (31) is a review about the production and use of magnetizable particles in immunoassays, this latter term being intended by the authors to include nonisotopic assays, such as assays employing fluorophores (see page 41). Magnetizable solid phase supports are described on pages 43 to 45. On page 45, right-hand column, the authors mention that "All commonly employed separation techniques ... require centrifugation...". They then go on to discuss the hazards and delays associated with centrifugation. Finally, on page 46, right-hand column, it is stated that: "The central objective of employing magnetizable particles in the separation step ... is to eliminate the need for centrifugation.". At the end of the article (see page 47, right-hand column), the following opinion is expressed: " ..., assays based on the use of antisera coupled with microparticles may require several wash and centrifugation steps. These problems can be avoided by the use of magnetizable particles ...".

The closest prior art

5. In accordance with the case law (see, for example, decision T 606/89 of 18 September 1990, reasons, paragraph 2), the closest prior art for the purpose of objectively assessing inventive step is generally that which corresponds to a similar use requiring the minimum of structural and functional modifications.

6. In the present case, the method described in document (37) and the method suggested in document (28) have the same use: measuring the quantity of an analyte in a biological sample via a fluorescent or chemiluminescent immunoassay system. They rely on the same two concepts of (i) "extracting" the analyte from the biological sample by binding it to a solid phase and (ii) evaluating its concentration "in a differential manner", ie by quantifying the amount of a labelled reagent which binds to the solid support in the presence of the analyte by measuring the amount of fluorescence due to said reagent on said support, as exemplified in document (37), and unambiguously suggested in document (28) (see from line 24 of page 4 to line 1 of page 5). How much of the analyte was bound to the particles is then deduced from these measurements.

7. What in their principles differentiates the two methods is the number of separate steps which they involve: three in the case of the method of document (37) and five in the case of the method of document (28) (see points 2 and 3, supra). Furthermore, in document (37), the particles are not magnetizable and centrifugation is used to separate them from the medium, whereas in

document (28), magnetizable particles are used which are "magnetically separated" from the medium. The signal emitted by the label attached to the particles is then measured in the presence of clear particles (document (37)) or suggested to be measured in the presence of opaque (magnetizable) particles (document (28)). In comparison to this prior art, the claimed method comprises the same number of steps as the method of document (37) but magnetic separation is used as the particles are of the magnetizable type. The amount of label is, thus, measured in the presence of opaque particles as suggested in document (28).

8. In the board's judgment, although documents (28) and (37) are in principle very close, document (37), because it provides a detailed disclosure, takes precedence over document (28), which suggests the immunoassay rather than it discloses it. In this respect, the appellant argues that the skilled person would not have considered document (37) as the closest prior art because the method disclosed does not make use of magnetizable particles. The board does not find this argument convincing. Indeed, document (37) teaches an immunoassay of the same kind as those referred to in the patent in suit. In accordance with the case law, (see decision T 202/95 of 21 July 1998), the skilled person knows everything in his sphere of competence and, therefore, he/she would be aware of the teachings contained in document (37). In the next paragraphs, the problem-solution approach is developed starting from document (37).

9. As mentioned above (see point 2, supra), document (37) discloses a solid phase immunoassay method whereby the analyte (estriol) to be measured is mixed with non-magnetizable particles ("Amerlex" particles) carrying a receptor for said analyte (anti-estriol antibodies) in the presence of a labelled reagent (a luminogenic estriol derivative) which is also capable of binding to said particles. After incubation, the particles are separated from the liquid medium by centrifugation and the amount of labelled reagent fixed on the particles is quantified by measuring the chemiluminescence caused by said reagent.

10. Starting from this prior art, the technical problem to be solved may be formulated as the provision of a simpler immunoassay for measuring the amount of a given analyte in a sample.

11. Although it is not suggested in document (37) that the method described therein could be improved upon, the formulation of this problem is nonetheless obvious as the prior art at the priority date is replete with alternative solid phase immunoassays (see document (31), pages 41 and 42, for a summary of the different assays which were available). In the board's judgment, looking for "a better method" was a concern shared by all those skilled in the art. And, thus, it took no inventive step to think of developing yet another such method.

12. The provided solution is to eliminate the centrifugation step by using magnetizable particles which enable the magnetic separation of the analyte and labelled reagent fixed on the receptor from the biological sample, followed by the measurement of the

fluorescent or luminescent signal emitted by the labelled reagent attached to the particles.

13. At the priority date of the patent in suit, the drawbacks associated with the use of centrifugation of solid phase supports, more particularly in the form of particles, in non-isotopic immunoassays had already been pointed out in document (31) (see pages 45 and 46). Furthermore, this document also describes the magnetic separation of magnetically attractable particles as **an advantageous alternative** to centrifugation (see point 4, *supra*). In the board's judgment, the combination of the teachings of this document with those of document (37) made it obvious to replace non-magnetizable particles by magnetizable particles, ie replace centrifugation by separation, when attempting to solve the afore-mentioned problem.

14. At oral proceedings, the appellant cited a number of documents including declarations, such as documents (31) and (51), as evidence that at the priority date magnetizable particles were considered opaque. In the appellant's opinion, this implied that the skilled person would have considered them as highly likely to interfere with the reading of the signal emitted by the labelled reagent fixed upon them and, therefore, he/she would not have used them in the claimed manner.

15. Whereas the board may well agree that the presence of opaque particles in close vicinity to the signal to be read could *prima facie* be thought likely to interfere with the reading, it cannot agree that this would have prevented the person skilled in the art from carrying out the method as claimed in order to solve the problem

on the basis of the combined teachings of documents (37) and (31). Indeed, this is the very alternative which is clearly envisaged in document (31): "... assays based on the use of antisera coupled to microparticles may require several wash and centrifugation steps. These problems can be avoided by the use of magnetizable particles ..". Besides, it is a point accepted by all parties that magnetizable particles were available at the priority date. Thus, it was only a matter of "try and see" whether the suggestion in document (31) could be put into practice.

16. For these reasons, it is concluded that the requirements of Article 56 EPC are not fulfilled by the main request.

Auxiliary request

Admissibility of the request into the proceedings

17. The admissibility of the auxiliary request was no longer objected to at oral proceedings. The board considers that this request was submitted in response to the communication under Article 11(2) of the Rules of Procedure of the Boards of Appeal and, thus, accepts it into the proceedings.

Amendments (Article 123 EPC)

18. Claim 1 corresponds to granted claim 6, claims 2 to 5, 6 and 7 being identical in wording to granted claims 2 to 5, 7 and 8, respectively. As granted claim 6 was dependent on granted claims 1 to 5, and as granted claims 7 and 8 were dependent on granted claim 6, the

amendments are considered to have resulted neither in the addition of subject-matter which extends beyond the content of the application as filed nor in an extension of the protection conferred. Therefore, the requirements of Article 123 EPC are met by the auxiliary request.

Inventive step (Article 56 EPC)

19. Claim 1 of the auxiliary request differs from claim 1 of the main request in that the magnetically attractable particles are said to be suspendable in the liquid phase without shaking for a period at least as long as the incubation time of the assay.

20. This added feature does not change the definition of the objective technical problem starting from document (37) as stated at point 10 (see supra).

21. The provided solution is now to eliminate the centrifugation step by using magnetizable particles which enable the magnetic separation of the analyte and labelled reagent fixed on the receptor from the biological sample, followed by the measurement of the fluorescent or luminescent signal emitted by the labelled reagent attached to the particles, said magnetizable particles being such that they fail to sediment for as long as the reaction between the particle bound receptor, the analyte and the labelled reagent takes place.

22. In the paragraph entitled "Avoidance of continuous mixing", document (31) identifies as a disadvantage the need to shake continuously the assay tubes when

employing antisera linked to a solid support. It suggests that this disadvantage may be alleviated by using magnetizable particles with a bulk density similar to that of the incubate (see page 46, right-hand column).

23. Therefore, the board considers that the person skilled in the art would have regarded it as obvious not only to replace, in the method of document (37), the "Amerlex" particles by magnetically attractable particles but also to choose those particles in such a way that they have a density approximating to that of the liquid phase, thereby avoiding, as prompted by document (31), both the need for centrifugation and continuous mixing.

24. Finally, as to the appellant's argument that the person skilled in the art would have regarded as unrealistic the reading of the fluorescence or luminescence emitted by a compound in a suspension of opaque particles, one can reply as follows. It is admitted in the patent specification (see page 5, lines 11 to 13) that, for the claimed method to be valid, it is required that "the MAPs should not attenuate the signal generated by the labelled reagent thereon by more than about 90%", which means that the appellant is satisfied if at least 10% of the light emitted by a fluorophore or luminophore is measured, whether that compound is attached to a particle or is in solution in the suspension of MAPs (the two embodiments being covered by claim 1). The person skilled in the art, aware from document (D24) (see column 28, lines 25 to 38) that 45% of the light emitted by fluorescein-labelled antibodies attached to particles in a suspension further

containing opaque particles (charcoal) may be measured, would not have been deterred from taking such a reading.

25. For these reasons, the subject-matter of claim 1 does not involve an inventive step, contrary to the requirements of Article 56 EPC, and, thus, the auxiliary request is also not allowable.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

A. Wolinski F. Davison-Brunel