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D E C I S I O N
of 18 January 1995

Case Number: T 0063/94 - 3.3.4

Application Number: 81200768.0

Publication Number: 0045103

IPC: G01N 33/54

Language of the proceedings: EN

Title of invention:
Method for the determination of antigens with the aid of two or more monoclonal antibodies

Patentee:
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Opponent:
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Pasteur Sanofi Diagnostics
Boehringer Mannheim GmbH Patentabteilung
HYBRITECH INC.

Headword:
Determination of antigens/AKZO NOBEL N.V.

Relevant legal provisions:
EPC Art. 56, 114(2)

Keyword:
"Inventive step (no) - obvious replacement"

Decisions cited:
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Catchword:
-

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Case Number: T 0063/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 18 January 1995

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office dated 11 November 1993 concerning maintenance of European patent No. 0 045 103 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: L. Galligani
S. C. Perryman

Summary of Facts and Submissions

I. European patent application No. 81 200 768.0, claiming priority from NL 80 04308 of 28 July 1980, was granted as European patent No. 0 045 103 on 12 December 1984 with seven claims.

II. Notice of Opposition against the European patent was filed by five opponents who requested the revocation of the patent on the grounds of Article 100(a) and (b) EPC. During the opposition proceedings the following documents were inter alia relied upon:

(1) EP-A-0 042 755 [cited under Article 54(3) EPC];
(2) US-A-3 791 932;
(3) Uotila et al, Mol.Immunol., June 1980, Vol. 17, pages 791 to 794;
(4) Köhler G. and Milstein C., Nature, 1975, Vol. 256, pages 495 to 497;
(5) R.Ekins, "Radioassay methods" in "Radiopharmaceuticals II: Proceedings 2nd International Symposium on Radiopharmaceuticals, March 19 to 22, 1979, Seattle, Washington" published in October 1979, Book Coordinator: J.A. Sorenson, Salt Lake City, Utah, USA.

Document (5) had been submitted by its author pursuant to Article 115 EPC together with other observations.

The Opposition Division in a first decision dated 7 November 1988 revoked the patent on the ground that it lacked novelty under Article 54(3) EPC having regard to document (1), and indicated that otherwise it was satisfied that there was an inventive step, without, however, giving reasons for this view.

An appeal was lodged against this decision by the Patentee.

By its decision T 3/89 dated 14 February 1990 (not published in the OJ of the EPO), the Technical Board of Apppeal 3.3.2 found that Claims 1 to 7 filed at oral proceedings before it on 14 February 1990 had a basis in the original application and did not extend the scope of the granted claims, thus satisfying the requirements of Article 123(2) and (3) EPC. The Board, stating expressly that it was dealing only with the question of novelty, held the claims to be novel under Article 54(3) EPC over document (1), and remitted the case to the Opposition Division for further prosecution.

III. The opposition proceedings were resumed and inter alia the following further documents were allowed into the proceedings under Article 114(1) EPC:

(6) R.S. Accolla et al, Proc.Natl.Acad.Sci.USA, Vol. 77, No. 1, January 1980, pages 563 to 566;
(7) E.D. Sevier, Clinical Microbiology Newsletter, Vol. 2. No. 3, February 1980, pages 1 to 2.

On 11 November 1993 the Opposition Division issued an interlocutory decision within the meaning of Article 106(3) EPC whereby it decided that the patent met the requirements of the EPC, in particular that the subject-matter of the claims on file, i.e. the claims filed on 14 February 1990, involved an inventive step.

Independent method claims 1 and 5 read as follows:

"1. Method for determining antigens by means of an immunological reaction, whereby the antigen must enter into a bond with at least two antibody molecules at least one of which is labelled, characterized in that

hereby two or more different sorts of monoclonal antibodies are used directed against the same antigen; with the exception of the use of two monoclonal antibodies one of which is bound to a solid surface and rendered insoluble and the other of which is labelled, in sandwich assays."

"5. Method for determining antigens by means of an immunochemical reaction, whereby the antigen must enter into a bond with at least two antibody molecules at least one of which is labelled with red blood cells (haemagglutination), polystyrene latex spheres (latex agglutination), finely-suspended dyestuff particles or metal sol particles, characterized in that hereby two or more different sorts of monoclonal antibodies are used directed against the same antigen."

Independent product Claims 6 and 7 concerned test kits for antigen determination.

The Opposition Division considered that, in spite of the fact that in general the replacement of polyclonal antibodies by monoclonal antibodies could be seen as obvious, the use of two or more different monoclonal antibodies against the same antigen according to the claimed method was non-obvious having regard to document (2), which represented the closest prior art, also when document (6) was taken into account. The Opposition Division accepted that Table 1 of the patent specification demonstrated a surprising increase of sensitivity linked to the use of particular pairs of different sorts of monoclonal antibody, in comparison with the use of only one monoclonal antibody. This was treated as an indication supporting the existence of an inventive step.

IV. The Appellants (Opponents III and IV) lodged an appeal against this decision and submitted new evidence, including document:

(8) GB-A-2 029 571

in respect of the issue of inventive step with the statement of grounds of appeal.

The Respondent (Patentee) filed observations in response to the said statement and requested that the appeals be declared as inadmissible because they were merely aimed at delaying the procedure as demonstrated in particular by the fact that the opposing parties relied on evidence never put forward before. In its submission this late evidence should not be admitted into the proceedings.

V. In a communication pursuant to Article 11(2) of the rules of procedure of the boards of appeal, the Board informed the parties that under Article 114(2) EPC late-filed evidence could be disregarded and indicated that inventive step of the claimed subject-matter was the only point at issue. The Board pointed out that the decision under appeal was the first fully reasoned decision on the patent in suit giving reasons for its conclusion on inventive step.

VI. By letter dated 16 December 1994, Appellant IV sent further observations together with new documents.

VII. By letter dated 16 January 1995, the Respondent filed further submissions together with four auxiliary requests as follows: auxiliary request I (Claims 1 to 5); auxiliary request II (Claims 1 to 6); auxiliary request III (Claims 1 to 5) and auxiliary request IV (Claims 1 to 4).

VIII. Oral proceedings took place on 18 January 1995.

At oral proceedings the Respondent withdrew its request that the appeals be declared inadmissible. A further auxiliary request V (Claims 1 to 7) was filed and some amendments were proposed to the requests already on file.

In the auxiliary request I, Claim 1 differed from Claim 5 of the main request only in that the term "immunochemical" had been replaced by the term "immunological".

In the auxiliary request II, Claim 1 was identical to Claim 1 of the main request.

In the auxiliary request III, Claim 1 differed from Claim 5 of the main request as shown below (with additions in bold-type letters and deletions shown in italics in square brackets):

"1. Method for determining antigens by means of an **immunological** [*immunochemical*] reaction, **wherein** [*whereby*] the antigen must enter into a bond with at least two antibody molecules at least one of which is labelled with [*red blood cells (haemagglutination), polystyrene latex spheres (latex agglutination),*] finely-suspended dyestuff particles or metal sol particles, **and wherein** [*characterized in that hereby*] two or more different sorts of monoclonal antibodies [*are used*] directed against the same antigen **are used**."

In the auxiliary request IV, Claim 1 differed from Claim 1 of the main request as shown below (with the addition in bold-type letters, and the deletion shown in italics in square brackets):

"1. Method for determining antigens by means of an immunological **agglutination** reaction, whereby the antigen must enter into a bond with at least two antibody molecules at least one of which is labelled, characterized in that hereby two or more different sorts of monoclonal antibodies are used directed against the same antigen [*; with the exception of the use of two monoclonal antibodies one of which is bound to a solid surface and rendered insoluble and the other of which is labelled, in sandwich assays*]."

In the auxiliary request V, Claim 5 was identical to Claim 5 of the main request, except that the term "immunochemical" in line 2 was replaced by the term "immunological".

IX. The Appellants submitted essentially that the substitution of polyclonal antibodies by monoclonal antibodies in a "two-site" or "sandwich" assay method was obvious for the skilled person in view of the known advantages of monoclonal antibodies. In their submissions, the reported increase in sensitivity was not surprising for the skilled person as it derived from the occupation of two determinants on the antigen, instead of one.

X. The Respondent essentially argued that the increase in sensitivity in "two-site" or "sandwich" assays observed when proceeding according to the present invention was not to be expected for the skilled person in view of the known narrower sensitivity of monoclonal antibodies in comparison with polyclonal antibodies. In view of this knowledge, the skilled person would not have chosen as a second antibody a monoclonal antibody, but rather a polyclonal antibody.

XI. The Appellants requested that the decision under appeal be set aside and the patent be revoked.

The Respondent requested as main request that the appeals be dismissed and that the patent be maintained (on the basis of Claims 1 to 7 filed on 14 February 1990), and as auxiliary requests one to five, respectively, that the decision under appeal be set aside and the patent be maintained on the basis of one of auxiliary requests I to V respectively filed at the oral proceedings on 18 January 1995.

Reasons for the Decision

1. The appeals are admissible.

Late-filed evidence

2. Of the material sought to be relied on by the parties only at a very late stage, the Board was prepared to admit into the proceedings only document (8) published just before the priority date of the patent in suit, because this contains a helpful summary of two site assays with polyclonal antibodies. As to the other material sought to be relied on, the Board regarded this as not filed in due time, and exercised its discretion under Article 114(2) EPC to disregard it, as it appeared no more relevant than other material already on file, and in some cases it was not even clear when it was published or whether it was prior art or not.

The main request: inventive step (Article 56 EPC)

3. The only point at issue in respect of this request is the inventive step of Claims 1 to 7 filed on 14 February 1990. For this purpose it is only necessary to discuss the independent method Claims 1 and 5.

4. The Board sees in document (5) the closest prior art. This document is a review of radioassays and other related labelled reagent techniques. In particular, the article discusses solid-phase "sandwich" or "two-site" assay methods in which use is made of two separate radioisotopically-labelled antibodies that recognise two different binding sites on the same antigen molecule (see, in particular, page 223 and Figure 2). The use of some other non-isotopic labels such as enzyme or fluorescent labels, is also discussed (see page 235 onwards). In its final part (see pages 238 to 239), the article outlines two major obstacles to be overcome, these being the reduction of "background" signals and the improvement of the purity of the labelled reagents, i.e. - in general - labelled antibody. In respect to this latter point, the article states as follows: "Resolution ... is in sight in consequence of the exciting new developments pioneered by Milstein and others involving in-vitro fusion of mouse myeloma and antibody producing spleen cells. These techniques, now under intensive examination in my own laboratory hold out the promise of in-vitro monoclonal antibody production in unlimited amount, giving us for the first time the opportunity to tailor molecules possessing exactly the structural-recognition capacity which we require for use in two-site assay systems." In the conclusion, the view is expressed that the use of monoclonal antibodies will generate antibody-based

analytical methods with improved speed, sensitivity and precision (see, page 239, last paragraph of the discussion).

5. In view of the cited prior art document the problem to be solved is to be seen in overcoming one or more of the above disadvantages of the prior art "two-site" or "sandwich" assay methods.

As a solution thereto the patent in suit proposes a method for antigen determination and means therefor (test kits) wherein use is made of at least two different sorts of monoclonal antibodies directed against the same antigen, one of which is labelled. The said method can be carried out in soluble, suspension or solid phase. The solution proposed in independent method Claims 1 and 5 is based fundamentally on the same principle. The major difference between the Claim 1 and Claim 5, is that Claim 5 is further limited to one sort of monoclonal antibody being labelled with particular labels, namely with red blood cells (haemagglutination), polystyrene latex spheres (latex agglutination), finely-suspended dyestuff particles or metal sol particles. Moreover, the method according to Claim 1 excludes by way of a disclaimer "sandwich assays" in which two monoclonal antibodies are used, one of which is insolubilized by bonding to a solid surface and the other is labelled.

On the basis of the whole body of evidence in the present case, including the results presented in the patent specification (see, for example, Table 1) and put forward during the prosecution of the case (see, for example, the Table reported in the minutes of oral proceedings before the Opposition Division on 5 October 1993), the Board is satisfied that the method of Claim 1 or Claim 5 solves the underlying technical problem.

6. The development by G. Köhler and C. Milstein [see document (4)] of the methods of in vitro production of monoclonal antibodies constituted a major breakthrough in the field of immunochemistry, as it permitted the synthesis of unlimited amounts of single species of antibody directed against a single antigenic site. It is also generally accepted that the applicability of monoclonal antibodies to immunoassays was immediately apparent to the skilled person.

7. The relevant question to be asked in the present case is whether the skilled person, faced with the underlying technical problem, would have in a straightforward manner replaced the polyclonal antibodies by monoclonal antibodies in a "two-site" or "sandwich" assay with the reasonable expectation of any improvement.

8. With respect to the above question, the divergent positions of the parties were essentially as follows.

According to the Appellants, the skilled person had definite reasons to proceed to the replacement in question in view of the many known advantages of monoclonal antibodies, such as their availability in large amounts, their specificity for single antigenic determinants and the constant affinity therefor [see document (7)]. In their submissions, there was no prejudice in the art against such a replacement; on the contrary, there was an incentive to use monoclonal antibodies [see also documents (3) and (5)]. In the view of the Appellants, the person skilled in the art would have empirically tried for a combination of monoclonal antibodies which produced as good an assay as possible. In their submission, the method suggested in the patent in suit to obtain a good combination was just this (see the patent specification, page 4, lines 48 to 53).

The Respondent maintained that, although the advantages of monoclonal antibodies such as their reproducibility and their high specificity were recognised in the art, the skilled person was also aware of the fact that they had a lower affinity than polyclonal antibodies. This lower affinity was due partly to the fact that monoclonal antibodies were prepared from spleen cells whereas affinity maturation of antibodies takes place in the bone marrow, and partly to the difficulties in selecting with respect to affinity. The skilled person in order to improve "sandwich" or "two-site" assays had several options open such as, for example, the search for better polyclonal antibodies or the substitution of only the first polyclonal antibody by a monoclonal antibody. However, he or she would not have readily replaced **both** antibodies by monoclonal antibodies in such assays, being aware of the lower sensitivity of the latter.

9.1. The Board observes that Claims 1 and 5 at issue are quite generally worded and cover methods for antigen determination in which at least any two different monoclonal antibodies directed against the same antigen are used, irrespective of their specificity and/or affinity characteristics. As stated also in the specification (see page 4, lines 48 to 53), "not every arbitrary combination of two or more antibodies will satisfy reasonable requirements regarding sensitivity and specificity, and it is even possible that such a combination will not be at all effective. **Establishing the best combination depends exclusively on empiricism.**" (emphasis added). Thus, the claimed innovation lies merely in the proposal of using a combination of at least two monoclonal antibodies in "sandwich" or "two-site" assays, the burden being then left to the skilled reader to find empirically the appropriate combination

of antibody partners. As a matter of fact, the present patent specification gives virtually no contribution to lessening this burden of work.

9.2 The skilled person would have readily derived the suggestion of replacing both polyclonal antibodies by monoclonal antibodies in "sandwich" or "two-site" assays from document (5) (see, in particular, page 239). The reproducibility of monoclonal antibodies, their availability in large amounts as well as their specificity and the constant affinity for single antigenic determinants would have constituted an incentive in this direction. The skilled person would have seen in monoclonal antibodies the suitable substitutes for products (polyclonal antibodies) which had disadvantages such as the non-reproducibility and low specificity. Based thereupon, the skilled person would have expected the replacement of **not only one but both** polyclonal antibodies by monoclonal antibodies to bring about an improvement of the assays. The skilled person would, of course, have been aware of the fact that the identification of monoclonal antibodies with affinity and specificity characteristics required for the assay of an analyte could be in some cases arduous and time-consuming. However, these technical difficulties, which underlie also the present patent specification, would not have constituted for the skilled person a prejudice against the replacement because, he or she knew that the finding of a suitable antibody, whether by conventional or hybridoma-based techniques, varied considerably from one analyte to another and was merely a matter of time, perseverance, and luck, and that the benefits of finding a suitable monoclonal antibody which could be reproducibly produced justified in general the efforts required.

9.3 In the Board's view, the arguments based on the affinity characteristics of monoclonal antibodies in comparison with polyclonal antibodies which have been put forward by the Respondent (see point 8, third paragraph, above) are irrelevant in the present case. In fact, the underlying technical problem here is not specifically the improvement of the sensitivity of "sandwich" or "two-site" assays, but the overcoming of one or more of the disadvantages of these assays, i.e. - in other words - the generic improvement of any aspect of such assays. The solution proposed, as reflected by the general wording of the claims, embraces any combination of at least two different monoclonal antibodies against the same antigen, including combinations which are no better than would be expected from adding the affinities of the two separate antibodies. Such solution is fully in line with the suggestion in document (5) to replace polyclonal antibodies by monoclonal antibodies.

9.4 In any case, as the skilled person was certainly aware of the fact that sensitivity of an assay determined the lower limit of detection, it was desirable to ensure the highest possible sensitivity and this was merely a matter of finding empirically at least two appropriate antibody partners.

9.5 On behalf of the Respondent it was also argued that the skilled person would not contemplate operating within the scope of the claims because there was no certainty that a successful assay against any particular antigen could be produced. This would be a strong argument, if the claims had been directed to an assay against a particular antigen, and the description had shown that this was difficult but provided a reproducible way of overcoming these difficulties. Here, however, the claims cover assays against any antigen, and the suggested way of carrying out the claimed method is embarking on a

process of trial and error using only the information to be found in the prior art on polyclonal assays and the making of monoclonal antibodies. If this prior art knowledge is not sufficient to make an assay against a particular antigen, the Board can find nothing in the specification that would enable the skilled man to do so. The specific embodiments described identify (e.g. by deposit) neither the monoclonal antibodies used, nor the hybridomas used to make them, in a way that would enable the specific embodiments to be reproduced. The specific embodiments thus convey only the information that pairs of unidentified monoclonal antibodies do exist with which much better results can be achieved than by using a single sort of these unidentified antibodies. This information does not reduce the amount of work anybody else would have to do to come up with an assay against an antigen they wish to test for. The Board understood from the co-inventor who gave evidence, that in Example 1 the five antibodies referred to as A, B, C, D and E had in fact been selected after testing the antibodies from more than a hundred hybridomas producing monoclonal antibody against HCG, so that the amount of work in producing a suitable assay against any one antigen is very substantial. Yet in the patent, the Respondent treats this amount of work as reasonable for making something falling within his claims. On this basis it seems fair to the Board to assume that a skilled person seeking to solve the problem above posed would also have thought this amount of work reasonable, and would, on the basis of the indications existing in the prior art, inevitably have arrived at something falling within the scope of Claims 1 and 5, when adapting prior art polyclonal assays at least for some antigens.

9.6 The Board, therefore, concludes that the solution proposed in general terms in both Claim 1 and Claim 5 at issue is not different from what a person of average skill would have derived in a straightforward manner from the prior art. In this respect, it is further observed that both the manner in which the antigen-antibody reaction complex is formed (in solution, in suspension, in solid phase; cf. Claims 1 and 5) and the specific nature of the label recited (Claim 5) are features of ancillary importance which in the present case do not contribute to inventive step, being for the skilled person merely routine aspects of putting the assay method into practice [in this respect, see, for example, the review of the state of the art made in the introductory part of document (8)]. In respect of the labels, it is observed that the patent specification itself acknowledges at page 1, lines 27 to 30 that these labels have been employed to label [polyclonal] antibodies. Whereas reliance on the requirement of such labels may serve to render a claim novel, it does not introduce any new aspect into the consideration of inventive step.

10. In conclusion, the Board considers that Claims 1 and 5 at issue lack an inventive step and, therefore, the main request which contains them cannot be allowed.

The auxiliary requests: inventive step (Article 56 EPC)

11. As regards auxiliary requests I to V, as the claims involved are essentially more restricted than the claims of the main request, the Board sees no objections to them under Articles 54 and 123 EPC. However each of them contains at least one claim that lacks inventive step.

12. Thus, Claim 1 of auxiliary request I is essentially identical to Claim 5 of the main request, and thus lacks an inventive step for the reasons given above in relation to that claim.

13. Claim 1 of auxiliary request II is identical to Claim 1 of the main request, and lacks an inventive step for the reasons given above in relation to that claim.

14. Claim 1 of auxiliary request III is essentially the same as Claim 5 of the main request, subject to the further limitation that the label is limited to being finely-suspended dyestuff particles or metal sol particles. As explained in point 9.6 above, this is known for assays using polyclonal antibodies, so that the arguments for lack of inventive step remain as for Claim 5 of the main request.

15. Claim 1 of auxiliary request IV differs from Claim 1 of the main request essentially in that the manner in which the immunological determination is made is specified, this being agglutination. However, as already stated (see point 9.6, above), the Board is of the opinion that this feature cannot contribute to inventive step because for the skilled person it is merely a question of routine to decide the manner in which the antibodies are reacted with the antigen. In fact, the principles of antibody-antigen reactions are fundamentally the same in suspension, soluble or solid phase. Moreover, no particular improvement and/or advantage linked to agglutination has been put forward by the Respondent. Thus, in the Board's view, also Claim 1 of auxiliary request IV does not involve an inventive step.

16. Claim 5 of auxiliary request V is essentially identical to Claim 5 of the main request, and thus lacks an inventive step for the reasons given above in relation to that claim.

17. As each of the auxiliary requests contain a claim for which an inventive step cannot be recognized, none of these auxiliary requests is allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

A. Townend U. Kinkeldey