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**D E C I S I O N**  
**of 27 January 2000**

**Case Number:** T 0681/98 - 3.3.4

**Application Number:** 88303744.2

**Publication Number:** 0291194

**IPC:** G01N 33/543

**Language of the proceedings:** EN

**Title of invention:**

Immunoassays and devices therefor

**Patentee:**

UNILEVER N.V.

**Opponent:**

VEDALAB

Carter-Wallace Inc.

GENZYME CORPORATION

Andrea von Preen

Int. Mycoplasma

Technical Chemicals & Products Inc.

ORAMON Arzneimittel GmbH

Pharma Peter

The Ultimate Pharma et Health Products GmbH

**Headword:**

Test device/UNILEVER

**Relevant legal provisions:**

EPC Art. 56

**Keyword:**

"Inventive step (yes) - amended claims"

**Decisions cited:**

T 0296/93

**Catchword:**

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Boards of Appeal

Chambres de recours

Case Number: T 0681/98 - 3.3.4

**D E C I S I O N**  
**of the Technical Board of Appeal 3.3.4**  
**of 27 January 2000**

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**Respondent:** UNILEVER NV  
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**Decision under appeal:** Decision of the Opposition Division of the  
European Patent Office posted 11 May 1998  
rejecting the opposition filed against European  
patent No. 0 291 194 pursuant to Article 102(2)  
EPC.

**Composition of the Board:**

**Chairman:** U. M. Kinkeldey  
**Members:** L. Galligani  
C. Holtz

## Summary of Facts and Submissions

- I. Six opposing parties lodged an appeal against the decision by which the oppositions against the European patent No. 0 291 194 were rejected. Of them, three (appellants IV to VI) were interveners according to Article 105 EPC. The grounds of opposition were lack of novelty and lack of inventive step (Article 100(a) EPC).

Claim 1 as granted reads as follows:

"An analytical test device comprising a hollow casing (30) constructed of moisture-impervious solid material and containing a dry porous carrier (10) which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone (14) on the porous carrier and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, the device incorporating means (32) enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed, characterised in that the label is a particulate direct label."

Dependent claims 2 to 22 concerned specific embodiments of the test device, while claim 23 related to a method using it.

II. The following documents, which were discussed before the opposition division, are still of relevance for the appeals:

(1) EP-A-0 149 168;

(2) WO-A-86/04683;

(3) EP-A-0 186 799;

(6) WO-A-86/03839.

III. Appellants I and VI (opponents 01 and 10) requested an accelerated prosecution of the case having been sued for infringement of the patent.

IV. The respondents (patent proprietors) replied to the statements of grounds of the appellants and challenged the admissibility of the interventions by appellants IV to VI (opponents 08 to 10).

V. The board issued a communication with a provisional opinion on the issue of the admissibility of the interventions of appellants IV to VI. The communication also indicated the points to be discussed at oral proceedings. In reply thereto, the respondents filed six auxiliary requests, and appellants V and VI submitted an expert opinion.

VI. Oral proceedings took place on 26 and 27 January 2000.

The respondents withdrew their request that the interventions of appellants IV to VI be considered inadmissible.

On the second day of the proceedings, the respondents filed a new main request (claims 1 to 22) and auxiliary requests I and II in replacement of all previous requests, which had been considered not allowable by the board.

Claim 1 of the **main request** read as follows:

"An analytical test device comprising a dry porous carrier (10), unlabelled specific binding reagent for an analyte which unlabelled reagent is permanently immobilised in a detection zone (14) on the porous carrier and is therefore not mobile in the moist state, and in the dry state in a zone (12) upstream from the detection zone a labelled specific binding reagent for the same analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, characterised in that the porous carrier and the labelled specific binding reagent are contained within a hollow casing (30) constructed of moisture-impervious solid material, the porous carrier communicates directly or indirectly with the exterior of the casing such that liquid test sample can be applied to the porous carrier, the casing incorporates means (32) enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed, the label is a particulate direct label, the labelled reagent is



contained in a first zone (12) of the dry porous carrier, and the unlabelled reagent is immobilised in a detection zone spatially distinct from the first zone, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the detection zone."

Dependent claims 2 to 21 concerned specific embodiments of the test device, while claim 22 related to a method using it.

VII. The appellants had no objections under Article 123 and 54 EPC against the main request on file. They essentially objected that the claimed subject-matter lacked an inventive step having regard in particular to the combination of the teaching of document (6) with that of documents (1), (2) and/or (3).

According to the appellants, the concept of a self-containing test device usable without need for professional skill was already known from document (1). This document described a capillary tube made of glass or synthetic resin (cf page 11, lines 28 to 31), which had the same function of a hollow casing, said tube containing a porous solid matrix (cf page 7, lines 15 to 27) which contained the labelled reagent for binding the analyte in a first zone where the sample was applied, and, in a separate location, a zone wherein substances for taking up the migrating labelled complex were immobilised (cf pages 9 to 13).

Documents (2) and (3) described test devices based on the same principle of placing in a first zone of a support a labelled reagent capable of binding to the

analyte, said reagent being picked up by a liquid sample and diffusing into the support through a separate zone with an immobilised combination partner after which detection took place.

In all these documents, the proposed labelling included inter alia chromogenic substances, in particular enzymes or fluorescent compounds giving rise to a colour visible either directly or by light of an exciting wavelength.

It would have been obvious for the skilled person to use in such systems particulate direct labels, like colloidal gold, especially in view of document (6) which had shown that such labels, after having brought into suspension by a liquid sample, could diffuse into a porous matrix (cf Example X). This demonstration of the mobility of particulate labels within a porous matrix rendered ineffective the respondents' argument that the skilled person would have expected the particulate labels to have an anchoring effect. Otherwise, claim 1 had necessarily to contain as an essential feature the pre-treatment of the porous carrier with a glazing material. However, claim 1 at issue did not contain the said feature, and thus its subject-matter was obvious from the combination of the teachings of the prior art, as these invited the skilled person to use particulate direct labels as shown in document (6) in the test systems according to documents (1), (2) or (3).

VIII. The respondents argued that none of the quoted prior art documents provided a teaching which could be linked with the teaching of other prior art documents so as to

render obvious the combination of features which characterised as a whole the claimed test device. In particular, none of the citations suggested to the skilled person that a reagent labelled with a particulate direct label could be incorporated in a dry porous support and that, after being resuspended by an applied liquid sample, it would migrate through the support to a different zone. Particulate materials were expected to have rather an anchoring function (cf documents (1) and (3)). Moreover, document (6) did not suggest that the test sample should be applied to a region spatially distinct from the test zone, as the test solution was applied to the test zone of the solid phase.

- IX. The appellants requested that the decision under appeal be set aside and that the patent be revoked.

The respondents requested that the decision under appeal be set aside and that the patent be maintained on the basis of claims 1 to 22 of the main request or any of the auxiliary requests I or II, all submitted in the oral proceedings.

## **Reasons for the Decision**

### *Admissibility of the interventions*

1. While the opposition/appeal proceedings were pending, appellants IV to VI filed their interventions within the prescribed time limit of three months from the date on which infringement proceedings were instituted

against them, this being the date on which the writ was served (cf. eg T 296/93, OJ EPO 1995, 627). Thus, their interventions under Article 105 EPC are admissible.

*Main request: Formal admissibility under Article 123 EPC*

2. The claims at issue differ from the claims as granted essentially in that claim 1 has been reformulated by introducing therein the features of granted claim 2 ("the labelled reagent is contained in a first zone (12)...") and by further specifying (i) that the labelled specific binding reagent for the analyte is "in the dry state in a zone (12) upstream from the detection zone", (ii) that "the porous carrier and the labelled specific binding reagent are contained within a hollow casing" and (iii) that the casing (the device in the granted claim) incorporates means of observation. The remaining claims have been renumbered and their references to the preceding claims correspondingly amended.
3. The said features are of a restrictive nature and find support both individually and in their combination in the application as filed (cf eg page 3, second paragraph and Figures 1 to 5). Thus, there are no objections under Article 123(2) and (3) EPC.

*Novelty*

4. None of the appellants had any novelty objections against the claims at issue. Nor does the board have any objections in this respect. Thus, novelty is acknowledged.

*Inventive step*

5. Document (6) is considered to represent the most appropriate starting point for the evaluation of inventive step.

This document illustrates a solid phase diffusion assay performable in the kit form also by non-technical personnel (cf page 9, lines 11 to 12 and 17 to 18), whereby a sample containing an analyte to be tested is first mixed with a labelled binding substance, then applied to a region of an insoluble support (eg a nitrocellulose membrane) bearing immobilised adsorbent molecules and allowed to diffuse therein. The diffusion pattern is visualized and measured. In order to focus on the point of application of the sample, the document proposes placing a sheet of plastic or tape with a small hole on the support. As for the labelling substances, the document refers to a number of possibilities (cf page 14, line 13 to page 15, line 23), including dye particles such as colloidal gold or silver which are said to allow direct visualisation of the results (cf passage bridging pages 14 and 15, and page 29, lines 14 to 17). The latter embodiment is exemplified in Examples IX to XIII. In Example X, in view of a pregnancy test, the following practical assay format is described: a swab containing lyophilised gold-labelled anti-human chorionic gonadotropin (HCG) monoclonal antibodies is wetted with a sample of urine suspected to contain HCG, and immediately brought into contact with a nitrocellulose membrane bearing immobilised polyclonal antibodies against HCG via the opening in the membrane cover and held in place for about 30 seconds. A red

spot which is said to be obtained at concentrations of HCG higher than 50mIU/ml, indicates a pregnancy. Lower concentrations are said to produce no visible spot. The purpose is thus to provide a test system of the type "yes/no".

6. Starting from this document, the problem to be solved can be defined as the provision of an alternative assay format for easy use by non-technical personnel.
  
7. As a solution, claim 1 proposes a test device comprising a hollow casing which contains a dry porous carrier which bears in a first zone a specific binding reagent for an analyte, said reagent being labelled with a particulate direct label, and, in a detection zone spatially distinct from the first zone, an unlabelled specific binding reagent for the same analyte in immobilised form, the two zones being arranged such that liquid sample applied to the porous carrier can pick up the labelled binding reagent and permeate via the first zone into the detection zone. The said casing incorporates means enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed. The proposed solution is thus a self-containing test device providing to the user a "yes/no" type of answer. The said device puts together elements which, admittedly, are known, either individually or in some combinations, from the prior art.
  
8. The relevant questions in relation to inventive step are what measures the skilled person, faced with the stated technical problem, would have considered adopting in the light of document (6) and other related

prior art, and whether these would have led him or her to combine the different elements so as to obtain a test device as claimed.

9. A skilled person can be expected to seek, within the normal design procedures, modifications or simplifications of known devices for the sake of obtaining a more handy or convenient product. Thus, in the present case, the skilled person, faced with the stated problem, starting from the knowledge of document (6), would have tried to optimise the assay arrangement described therein, eg in view of a pregnancy test (cf *ibid.* Example X), so as to render it more user friendly, simple and reliable. For this, the skilled person would have intervened, for example:

- at the level of the covering, as document (6) pointed to the desirability of focusing on the point of application of the sample (cf page 14, first paragraph) and indicated that covering could be accomplished by alternate means (cf Example XIII, last paragraph); and/or
- at the level of the insoluble support, which had to be suited for particular needs (cf page 15, lines 24 to 29) and for which one could envisage some form of housing or protection so as to facilitate handling and/or prevent damages; and/or
- at the level of the means used for the transfer of the sample (eg capillary tube, micropipet, microbiological loop or swab; cf page 14, lines 2 to 4 and Example X) in order to ensure a reliable single point application and minimise losses of

sample.

10. Although Example X of document (6) gave to the skilled person some indications that a lyophilised (thus, dry) binding reagent labelled with dye particles such as colloidal gold, when wetted with a sample of urine, could be transferred by contact to the nitrocellulose membrane and diffuse therein at some extent, nothing in document (6) would have suggested including the dry labelled binding reagent **directly** in a first zone of the support membrane from which it would permeate into a spatially distinct zone of the same for detection. The emphasis in the document is rather on detection at the place of application (cf Example X to XII), ie in correspondence of the opening in the covering. The question thus arises whether such a measure would have been readily suggested by a related prior art document.
11. In this respect, the appellants made reference in particular to documents (1), (2) or (3), all of which, in their view, directed the skilled person's attention to such an assay format.
12. Document (1) describes capillary tubes containing matrix material which contains a labelled reagent that binds to an analyte from a liquid sample with which the tubes are put in contact. The thus formed complex moves by capillarity upwards to a region bearing in immobilised form an uptaking substance for the same analyte, where the resulting labelled complex is visualised and measured. The recommended labels are radioactive isotopes, enzymes or fluorescent compounds, no indications being given about the possible use of a particulate direct label. A reference to particulate



material, inter alia gold, is made only when illustrating the possible candidates as solid matrix material (cf page 7, lines 15 to 34).

13. Document (2) describes a clinical test device in the form of tube, strip, pad etc. where a biological liquid containing an analyte to be determined is contacted sequentially with specific enzyme-labelled reagents, with enzyme-labelled reagents immobilised on the solid support and with chromogenic substrates capable of producing an evidencing reaction. The device can be subdivided into zones such that the liquid flowing up or down the support will react first with the non-immobilised labelled reagents, and then pass to the zone(s) with the immobilised reagents and chromogenic substrates, where the colour for quantitative and/or qualitative determination develops (cf eg Figures 6a and 6b), the arrangement of the latter zone(s) being dependent upon the type of assay (competition or sandwich). Also this document does not provide any suggestion in the direction of using a label in the form of a particulate direct label.
  
14. Document (3) is concerned with a diagnostic strip wherein a labelled agent dissolves in the applied sample and binds to the analyte to be detected. The formed complex moves to the detection zone where another binding reagent for the same analyte is immobilised. Among various known labels, enzymes requiring chromogenic substrate systems or substrate systems which produce fluorescence or chemiluminescence are said to be preferred. Also here there is no mention of the possible use of a particulate direct label. A reference to a dispersion of particles is made only in

relation to fixing components within the solid phase zone (cf page 9, last paragraph).

15. In the board's judgement, the skilled person would not have readily envisaged combining any of the test systems according to documents (1), (2) or (3) with a particulate direct label as used in the assay format according to document (6) because all said systems relied on the use of soluble labelled reagents expected to be freely mobile within solid supports, including particulate solid supports. In spite of document (6) showing the transfer of colloid particles-labelled reagents taken up by a urine sample from a swab when this is applied to an area of a nitrocellulose matrix, the skilled person would still not have expected them to be sufficiently mobile between two spatially distinct zones within solid supports like those of documents (1), (2) and (3). Although document (6) refers in Example X to some diffusion of the gold-labelled complex into the nitrocellulose membrane, this is clearly limited to the area of application as shown by the further indication eg in Examples XI to XIII that in order to enhance diffusion additional measures have to be taken such as negative or positive pressure or hydrophilic material.

For the same reasons, the skilled person would also not have readily contemplated modifying the assay format exemplified in document (6) according to the model offered by the test systems of documents (1), (2) and (3), eg by creating on the insoluble matrix a first zone containing the labelled binding reagent wherefrom the formed complex would have migrated to a spatially distinct zone of the same matrix for detection by use

of an immobilised binding reagent.

16. Apart from the above considerations, which are already indicative of an inventive step, the claimed assay device is characterised by a number of additional features, which, although individually or in some combinations known from the prior art, are not found or suggested as a whole in any of the cited prior art documents.
17. No need is seen by the board to introduce in claim 1, as further mandatory feature, a pre-treatment with a glazing material (this being in the present set a feature of a dependent claim) as there is no evidence on file that the claimed test device does not work without such a pre-treatment.
18. In summary, for the reasons given above, the subject-matter of the claim 1, and thus that of dependent claims 2 to 21 and of claim 22, which concerns its use, involves an inventive step.

**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 on the basis of the respondent's main request, submitted in the oral proceedings, and a description to be adapted thereto.

The Registrar:

The Chairperson:

A. Townend

U. Kinkeldey