

Internal distribution code:

- (A) Publication in OJ
(B) To Chairmen and Members
(C) To Chairmen
(D) No distribution

**Datasheet for the decision
of 9 October 2008**

Case Number: T 1406/07 - 3.3.08

Application Number: 99113654.0

Publication Number: 0987551

IPC: G01N 33/558

Language of the proceedings: EN

Title of invention:

Method for the determination of analyte concentration in a lateral flow sandwich immunoassay exhibiting high-dose hook effect

Applicant:

Siemens Healthcare Diagnostics Inc.

Headword:

Sandwich immunoassay/SIEMENS HEALTHCARE

Relevant legal provisions:

EPC Art. 54, 56, 83, 84, 87, 123(2)

Relevant legal provisions (EPC 1973):

-

Keyword:

"Sole request - added matter (yes)"
"Clarity and sufficiency of disclosure (yes)"
"Novelty and inventive step (yes)"

Decisions cited:

T 0254/86, T 0931/95, T 0641/00

Catchword:

-



Case Number: T 1406/07 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 9 October 2008

Appellant: Siemens Healthcare Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591 (US)

Representative: Maier, Daniel Oliver
Siemens AG
Postfach 22 16 34
D-80506 München (DE)

Decision under appeal: Decision of the Examining Division of the
European Patent Office posted 12 March 2007
refusing European application No. 99113654.0
pursuant to Article 97(1) EPC 1973.

Composition of the Board:

Chairman: L. Galligani
Members: M. R. Vega Laso
C. Rennie-Smith

Summary of Facts and Submissions

- I. European Patent Application No. 99 113 654.0 was filed on 14 July 1999 claiming the priority of the earlier US application Serial Number 09/122,736 of 27 July 1998, and was published as EP 0 987 551 A1 with the title "Method for the determination of analyte concentration in a lateral flow sandwich immunoassay exhibiting high-dose hook effect".
- II. By a decision of the examining division posted on 12 March 2007, the application was refused under Article 97(1) EPC 1973. The examining division held that claims 1 to 7 filed at the oral proceedings on 7 February 2007 were neither clear nor supported by the description (Article 84 EPC 1973), and that, having regard to Articles 52(1) and 56 EPC 1973, the claimed invention did not constitute a patentable invention.

In the view of the examining division, the closest state of the art relevant to the assessment of inventive step was the known multiband immunostrip for the detection of C-reactive protein (CRP) disclosed in an article published in Labmedica April/May 1990 which was cited in the present patent application. Having regard to this prior art, the objective technical problem to be solved was formulated as "*how may the results from a known multiband immunostrip be analysed in order to overcome the hook effect?*" (see decision under appeal, point 4.3, last sentence of the first paragraph), and the solution proposed in the claims was held to consist in "*the use of mathematical processing to overcome the distortions caused by the hook effect*" (see point 4.4. of the decision under appeal). The

examining division finally concluded that "... , following this analysis, neither the problem [to be solved] nor the solution are of a technical nature or contribute to the technical character of the claimed subject matter. Therefore, no inventive step and hence invention, is present in the subject matter of the claims, contrary to Articles 52(1) and 56 EPC." (see point 4.5 of the decision under appeal).

III. The applicant (appellant) filed a notice of appeal against the decision of the examining division and, together with its statement setting out the grounds of appeal, submitted a copy of documents:

(1): R. Gambino, Labmedica, April/May 1990; and

(2): Preliminary instructions for use of the rapid CRP test dated 23 May 1997.

The appellant pursued as its sole request the set of claims which led to the refusal of the application. As a subsidiary request, oral proceedings under Article 116 EPC were requested.

IV. The examining division did not rectify its decision and the appeal was remitted to the boards of appeal (Article 109 EPC 1973).

V. By a letter dated 29 February 2008, the European Patent Office was notified of the transfer of the application to the present appellant.

VI. The appellant was summoned to oral proceedings. In a communication under Rule 11(1) of the Rules of

Procedure of the Boards of Appeal attached to the summons, the board expressed its provisional opinion on some of the issues to be discussed at oral proceedings, and gave the appellant the opportunity to submit comments and/or file amended claim requests.

- VII. No comments or claim requests were received within the time limit set by the board.
- VIII. Oral proceedings were held on 6 October 2008. During the proceedings, an amended set of claims (claims 1 to 5) was filed to replace the set of claims previously on file.
- IX. Amended claim 1 according to the sole request on file reads:

"1. A method for determining the concentration of an analyte in a fluid test medium which comprises:

- a) providing a strip of a porous material through which the test fluid suspected of containing the analyte can flow by capillarity which strip has 3 capture regions in which are immobilised antibodies specific to the first epitope of the analyte; and also providing labeled antibodies specific to a second epitope of the analyte which are able to flow through the strip along with the fluid test medium upon its application to the strip; and the strip having one collection region in which there is immobilized a collection means for the labeled antibody;

- b) applying the fluid test medium to the strip and allowing it to flow along the strip carrying the labeled antibodies with it to thereby contact the immobilized antibodies in the distinct capture regions and, when sufficient analyte is present in at least the first distinct capture region with which the fluid comes into contact as it flows along the strip, forming a sandwich of the immobilized antibody, analyte and labeled antibody in the distinct capture regions through which the fluid test medium carries analyte, the quantity of the sandwich formation being limited by the partial blocking of the immobilized antibody;
- c) quantitatively detecting the signal from the label on the labeled antibody in each of the distinct capture regions in which the sandwich has formed and in the collection region to obtain a pattern of signals which pattern is unique to the concentration of analyte in the fluid test medium; and
- d) mathematically combining the unique pattern of signals to create a monotonous dose-response curve to factor out the blocking of the binding between the immobilized antibody and the first epitope of the analyte."

Dependent claims 2 to 5 are directed to particular embodiments of the method of claim 1.

- X. The arguments put forward by the appellant, as far as they are relevant to this decision, may be summarized as follows:

Article 84 EPC

From the claims read in the context of the description the skilled person knew how to prepare a dose-response curve for a test strip with three capture regions and one collection region. The application provided an exemplary calculation using CRP as an analyte and gave a detailed description how to determine and use the mathematical formula which is part of the invention. It was obvious to the skilled person that obtaining this result was not particular to CRP, but would work in the same way for other analytes detectable by sandwich immunoassay. There was no reason to believe that CRP would behave differently with regard to the described "hook" effect than other analytes detectable by sandwich immunoassay.

Article 56 EPC

Document (2), a product description for the CRP immunostrip considered to be the closest prior art, described a semi-quantitative method in which it was merely determined whether in a given capture region a coloured line appeared or not, i.e. a qualitative determination. In contrast, the claimed method aimed at determining the concentration of an analyte by quantitative detection of the signal strength (e.g. by reflectance measurement).

The problem to be solved in view of the prior art was to provide a method which allowed a true quantitative determination of an analyte using a strip with three capture regions and one collection region. According to

the invention this problem was solved by making a quantitative signal strength determination for each capture region and the control region and then mathematically combining the pattern of signals to create a monotonous dose response curve. Since the prior art provided neither means for a quantitative signal detection nor a suggestion to use such means, the proposed solution was not obvious.

- XI. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 5 of the main request filed during the oral proceedings.

Reasons for the Decision

Article 123(2) EPC

1. The subject-matter of amended claim 1 is derivable from the disclosure in the passage starting on page 5, line 21 and ending on page 7, line 16 of the application as filed (see paragraph [0005] on page 3 of the published application), read together with the sentence bridging pages 10 and 11 of the application as filed ("*Typically, a maximum of 3 capture bands 3' will be incorporated into capture region 3 of the strip ...*"; see page 4, lines 9 to 11 of the published application), and the passage on page 11, lines 7 to 14 ("*Excess labeled antibody/analyte conjugate is captured in the collection band of area 4 [sic] by a collection means for labeled antibody [...] The collection band may [...] participate in the calculation of the analyte*

concentration." (see Figure 1 and page 4, lines 13 to 16 of the published application).

2. The additional feature in dependent claim 2 can be derived from claim 4 and the disclosure on page 11, lines 9 and 10 of the application as filed ("*... collection means for labeled antibody such as immobilized IgG ...*"; see page 4, lines 13 and 14 of the published application). Claims 3 to 5 have a basis in claims 5, 8 and 9 as originally filed. Thus, Article 123(2) EPC is complied with.

Article 84 EPC

3. In the decision under appeal, the examining division found that claims 1 and 4 then on file did not comply with Article 84 EPC. Since previous claim 4 is no longer included in the present set of claims, the examining division's objection of lack of support in respect of this claim (see point 3, lines 9 *et seq.* of the decision under appeal) does not need to be considered. Nor does the objection to claim 1 concerning the feature "*... numbers in the same range of magnitude as the signal of the collection region ...*", the meaning of which was held to be imprecise, because the feature in question is no longer present in the amended claim 1 presently on file.
4. As for the further finding of the examining division that "*the description provides support only for the determination of C-reactive protein (CRP) using the Labmedica immunostrip*" (see point 3 of the decision under appeal), the board does not concur. Whereas it is true that Example I describes a method for determining

the concentration of CRP, Example II and Example III - as far as it concerns a method using a strip with multiple capture bands - are not limited to the determination of CRP. The question whether or not the strip used in Example I is "*the Labmedica immuno-strip*" - which is not readily apparent from the example - does not seem to be relevant in this respect. Moreover, the board has no reason to believe that the claimed method, which is generally disclosed in the application, could not be applicable to any analyte bound by suitable antibodies in a sandwich immunoassay, with the aim of avoiding the hook effect.

5. Thus, the requirements of Article 84 EPC are considered to be met.

Article 83 EPC

6. Even though lack of sufficient disclosure was not among the grounds given by the examining division for the refusal of the application, in point 2.1 of the decision under appeal it was observed that the application as filed contained no specific technical teaching with regard to the collection region. However, the board notes that the function of the collection region is described on page 11, lines 7 to 10 of the application as filed (page 4, lines 13 to 17 of the published application), and that in Example I a specific example (polyclonal donkey antigoat antibody) is provided.
7. No further issues concerning sufficiency of disclosure were raised in the decision under appeal, and the board has no objections of its own. In particular, the board

considers that, in the absence of any evidence to the contrary, deriving an algorithm that combines the pattern of signals obtained for a given dose in such a way that a monotonous dose-response curve is created, could be done without undue burden of calculation work by using computational tools available at the priority date. Thus, the requirements of Article 83 EPC are considered to be met.

Article 87 EPC

8. The entitlement to the priority of the US application Serial Number 09/122,736 was not questioned by the examining division and the board sees no reason to do so of its own motion. Thus, the effective date for the purpose of assessing what is comprised in the state of the art is 27 July 1998.

Article 54 EPC

9. No objection of lack of novelty was raised by the examining division in its decision. With regard to the state of the art as it is apparent from the documents presently on file, the board is satisfied that the claimed invention is new.

Article 56 EPC

10. In the decision under appeal, the starting point for the assessment of inventive step was held to be the immunostrip for the detection of CRP described in document (1). However, since the examining division was unable to obtain a copy of the document in question, either from its own sources or from the present

appellant, the technical teaching of document (1) was assessed solely on the basis of the information provided in the application (see page 4, lines 30 to 40 of the published application).

11. Document (1), a copy of which was filed by the appellant together with its statement of grounds of appeal, is a short review written *"to emphasize the need for laboratories to make quantitative immunoassays for CRP available on both a stat and routine basis"* (see introductory section in the left column of the first page). After describing the physiological role and the synthesis of C-reactive protein (CRP) (see sections under the headings *"What is CRP?"* and *"What mediates synthesis of CRP?"* on the first page of the document), the review suggests possible applications of a CRP test to the diagnosis and/or monitoring of various disease conditions (see section *"What is the CRP test good for?"* on the second page of the document). The document gives, however, no technical details for any specific CRP immunoassay, let alone for an assay using a test strip with three capture regions and one collection region as defined in step a) of claim 1. Therefore, document (1) can hardly be regarded objectively as the *"most promising springboard"* towards the invention which was available to the skilled person (see T 254/86, OJ EPO 1989, 11; and further decisions cited in Chapter I.D.3.1 of *"Case Law of the Boards of Appeal of the European Patent Office"*, 5th edition 2006).

12. Rather, among the documents on file in appeal proceedings, the board regards document (2) as the closest prior art. This document, which was filed by

the appellant together with its statement of grounds of appeal, is dated "23.5.1997" (see top of each page), and its availability to the public before the priority date of the present application has not been disputed by the appellant. Thus, document (2) forms, *prima facie*, part of the state of the art relevant to the assessment of inventive step in respect of the claimed invention.

13. Document (2) describes a **semi**quantitative rapid test for the determination of CRP in plasma or serum using a dipstick. In the paragraph under the heading "Principle of test" on page 2/5 of the document, the test is said to be based on immunochromatography and to use monoclonal antibodies to human CRP which are immobilized on blue latex particles acting as the detecting label. In particular, in the carrier membrane on the dipstick "... *there are three CRP-specific antibody zones to which blue latex particles will bind if the sample contains CRP*". To perform the test, the bottom of the dipstick is dipped into the plasma or serum sample to be analyzed. When the sample is absorbed by capillarity, the liquid flow carries CRP contained in the sample and the latex particles provided in the dipstick to the "result area" (also called "reaction area") located upstream, where "*CRP will bind to the antibody zones and form none, one, two or three blue lines depending on the CRP content of the sample.*" The more CRP the sample contains the more blue lines become visible. An additional **red** line (the so-called "control line") is always formed upstream of the result area when the test is performed properly (see last paragraph under the heading "Principle of test" on page 2/5).

14. Concerning the interpretation of the results of the test (see page 3/5, last paragraph, and page 4/5, first paragraph), it is indicated in document (2) that:

"The appearance of the red control line confirms that the test is performed properly.

If in addition to the red control line

- there are no other lines, the sample contains less than 20 mg/l CRP*
- one blue line appears, the sample contains 20-40 mg/l CRP*
- two blue lines appear, the sample contains 40-80 mg/l CRP*
- three blue lines appear, the sample contains more than 80 mg/l CRP"*

In the second paragraph of page 5/5, it is further stated that "T[t]he red control line is in the upper part of the result area, blue lines indicating different concentrations of CRP become visible from the lower end of the result area, 0-3 lines depending on the concentration. If no red control becomes visible the test is invalid."

15. Hence, using the terminology of the present patent application, document (2) describes a method for determining the concentration of CRP which comprises providing a strip of porous material having three capture regions (designated "CRP-specific antibody zones" in document (2)), in which are immobilized antibodies specific to the analyte. It is also apparent from the passages quoted above, that in the test method described in document (2) labeled antibodies specific

to the analyte (described as "antibodies immobilized on blue latex particles" in document (2)) are provided, which are able to flow through the test strip along with the fluid test medium (plasma or serum in the method described in document (2)) upon its application to the test strip.

16. Furthermore, even though it is not expressly stated in document (2), a person skilled in the art may infer from its technical content that the two types of CRP-specific antibodies used in the rapid test described therein must bind to different epitopes, because otherwise binding of the labeled antibodies to CRP would interfere with the binding of CRP to the antibodies immobilized in the antibody zones. The fact that monoclonal antibodies are used in the test strip of document (2) strongly supports this conclusion.

17. The dipstick described in document (2) differs from the test strip in step a) of the method according to claim 1 in that it lacks a collection region in which there is immobilized a collection means for the labeled antibody. The additional control line described in document (2) cannot be regarded as a collection region for labeled antibodies, the purpose of the control line being - as clearly stated in document (2) - to indicate whether or not the test has been performed properly, ie. whether or not the sample flowed through the strip and contacted the CRP-specific antibody zones, before reaching the area of the control line located upstream to the antibody zones. The fact that, for the result of the CRP test to be considered valid, the control line must turn red - instead of turning blue like the CRP-specific antibody zones where labeled antibody

- bound to CRP is captured - indicates that blue labeled antibodies are not involved in the reaction.
18. Further differences from the closest prior art are found in steps c) and d) of the claimed method. Whereas semiquantitative determination of the analyte based on the appearance of none, one, two or three blue lines in the antibody zones of the test strip is described in document (2), claim 1 as presently on file requires that the signal from the label on the labeled antibodies is quantitatively detected in each of the capture regions **and in the collection region** (see claim 1, step c)). Moreover, step d) of the method according to claim 1, ie. mathematically combining the pattern of signals obtained for the different zones or regions of the test strip to create a monotonous dose response curve, is not derivable from document (2).
19. When formulating the objective technical problem and assessing the technical contribution of the invention to the art (see points 4.3 and 4.4 of the decision under appeal which are summarized in section II above), the examining division was limited to rely solely on the scarce pieces of information with respect to document (1) which are provided in the present patent application. Thus, the assessment of inventive step applying the "problem and solution approach" made by the examining division in the decision under appeal is based on assumptions as to the content of document (1) rather than on facts.
20. The board holds that, starting from document (2) as the closest prior art and having regard to the ascertained differences between the prior art and the claimed

method (see points 16 and 17 *supra*), the technical problem formulated in the patent application represents in fact the objective technical problem to be solved. It is stated in the description of the present patent application that an object of the invention is to avoid the "hook" effect, ie. a decrease in assay response at high analyte concentration leading to a multi-valued dose-response curve, by providing an assay method using an immunochromatographic strip *"whose efficacy is not affected by high analyte concentrations in the test sample and, accordingly, does not require sample dilution or reassaying of samples containing high analyte concentrations"* (see page 2, lines 41 to 44 of the published application).

21. Document (2) does not draw attention to possible ambiguous results when determining CRP in samples with a high analyte concentration, perhaps due to the fact that in the method of the prior art the serum or plasma sample is diluted before being applied to the dipstick (see step 2 under the heading "Performance of the test" on page 3/5), thus diminishing the risk of a "hook" effect. However, it was well-known at the relevant date that sandwich immunoassays like the CRP assay described in the document (2) may suffer from a high dose "hook" effect. Therefore, in the present case an inventive step cannot be based on the mere realization that, when using a test strip as described in document (2), quantitative determination of high analyte concentrations in a sample may be distorted by the "hook" effect.

22. Rather, the technical contribution of the claimed invention to the art is to be seen in the teaching that

a "hook" effect can be avoided by providing a test strip having three capture regions and a collection region in which collection means for labeled antibody are immobilized, and quantitatively detecting the signal from the label on the labeled antibody not only in the three capture regions, but also in the collection region, the signals being then mathematically combined to create a monotonous dose-response curve in which no "hook" appears.

23. None of the documents presently on file suggests the technical solution proposed in the claims. While it was argued in the decision under appeal that collection regions were a common feature of test strips (see point 2.1 of the decision), no evidence in this respect was put forward by the examining division. Neither did the examining division indicate any prior art document suggesting that, in order to avoid a "hook" effect, the signal from the label on the labeled antibody in each of the capture regions **and** the collection region of the test strip may be quantitatively detected, and the pattern of signals thereby obtained be mathematically combined to create a monotonous dose-response curve, as proposed in claim 1.
24. Hence, in view of the reasons given by the examining division for its finding of lack of inventive step and the evidence on file the board is not convinced that, with regard to the state of the art at the relevant date as presently on file, the claimed invention was obvious to a person skilled in the art. An inventive step must, therefore, be acknowledged.

Article 52(1) and (2) EPC

25. In the decision under appeal, the examining division acknowledged that steps a) to c) of the claimed method are of a technical nature; it found, however, that the technical contribution of the invention to the art resided solely in step d). Since in the view of the examining division this step consisted in a mathematical method which was excluded from being considered an invention by virtue of Article 52(2) EPC, the sole contribution of the invention was to be found in an area excluded from patentability "..., which even when combined with known technical features, does not display a technical characteristic." (see last paragraph on page 5 of the decision)
26. The board disagrees with this assessment. As stated above when assessing inventive step (see point 22 *supra*), the technical contribution of the claimed invention to the art is not restricted to the teaching of mathematically combining the pattern of signals obtained from the test strip, but also includes providing a collection region for labeled antibodies on the strip (see step a) in claim 1), and quantitatively detecting the signal from the label in each of the capture regions and the collection region (see step c)).
27. There is no doubt that these features are part of the technical contribution of the claimed invention to the art and - as the examining division implicitly acknowledged - have a technical character. In the board's view, the need for an algorithm to be used in step d) to mathematically combine the pattern of signals obtained from the test strip, does not

automatically convert the claimed method into a mathematical method excluded from patentability by virtue of Article 52(2) EPC, because the method as a whole, the objective technical problem solved and at least part of the features contributing to solve the technical problem, clearly have a technical character.

28. The board notes that, in the decisions of the boards of appeal cited by the examining division in this context (see T 931/95, OJ EPO 2001, 441; and T 641/00, OJ EPO 2003, 352), the deciding board did not question the technical character of the claimed invention as a whole, although in both cases the invention was defined by a mixture of technical and non-technical features. Rather, in the cited decisions the deciding board, when assessing inventive step, took into account only those features which contributed to the technical character of the invention, because in its view features making no such contribution could not support the presence of an inventive step. In both decisions, the board concluded that the claimed subject-matter did not involve an inventive step.

29. The relevant circumstances of the present case are, however, different from those in the cited decisions (see points 23 and 24 above). Having considered the specific circumstances of the present case apparent from this decision, the board is convinced that the claimed subject-matter constitutes an invention within the meaning of Article 52(1) EPC, and that the claims and the invention to which they relate fulfil the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 to 5 of the main request filed during the oral proceedings and a description and drawings to be adapted thereto.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani