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Datasheet for the decision of 16 April 2008

Case Number: T 1702/06 - 3.4.02

Application Number: 01941435.8

Publication Number: 1303746

IPC: G01N 21/03

Language of the proceedings: EN

Title of invention:
Analysis method and cuvette therefor

Applicant:
Migrata U.K. Limited

Opponent:
-

Headword:
-

Relevant legal provisions (EPC 1973):
EPC Art. 56

Keyword:
"Inventive step"

Decisions cited:
-

Catchword:
-

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Case Number: T 1702/06 - 3.4.02

D E C I S I O N
of the Technical Board of Appeal 3.4.02
of 16 April 2008

Appellant: Migrata U.K. Limited
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Representative: Andersson, Mikael Per Robert
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 22 June 2006 refusing European application No. 01941435.8 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: A. G. Klein
Members: F. J. Narganes-Quijano
B. Müller

Summary of Facts and Submissions

I. The appellant (applicant) lodged an appeal against the decision of the examining division to refuse European patent application No. 01941435.8 based on the International application No. PCT/SE01/01442 published under the International publication No. WO 02/01195.

In its decision the examining division referred, *inter alia*, to documents

D1: US-A-5674457
D2: US-A-5064282
D5: US-A-4185964

and held that the subject-matter of claim 1 then on file did not involve an inventive step (Article 56 EPC 1973) over the disclosure of documents D1 and D2. The examining division also noted that the subject-matter of independent claim 11 then on file did not involve an inventive step over the disclosure of documents D1 and D5.

II. With the statement of grounds of appeal the appellant contested the examining division's view on the issue of inventive step of the claimed subject-matter and requested oral proceedings on an auxiliary basis.

III. In a communication dated 23 January 2008 annexed to summons to attend oral proceedings scheduled for 16 April 2008 the Board expressed its provisional opinion on the appellant's case.

IV. In a further communication dated 3 March 2008 the Board drew the attention of the appellant to the following documents cited in the European Search Report issued on 26 February 2008 and drawn up in respect of a European patent application filed as a divisional application of the application in suit:

A1: US-A-5300779
A2: US-A-5686316
A3: WO-A-9918433.

V. In reply to the Board's communications, the appellant filed with the letters dated 17 March 2008 and 11 April 2008 sets of amended claims replacing the previous set of claims and submitted *inter alia* the following documents

B1: "An azide-methemoglobin method for hemoglobin determination in blood" G. Vanzetti, Journal of Laboratory and Clinical Medicine (US), Vol. 67 (1966), pages 116 to 126
B2: "A new approach to photometry of glycated hemoglobin in human blood" R. Deeg *et al.*, Clinical Chemistry, Vol. 30 (1984); pages 790 to 793.

VI. Oral proceedings took place before the Board on the scheduled date. During the oral proceedings the appellant submitted the following document

B3: "Visible and near infrared absorption spectra of human and animal haemoglobin" W. G. Zijlstra *et al.*, VSP Utrecht (2000); first page and pages 12, 25 and 26

and the Board introduced the following document into the proceedings:

A4: "New method for hemoglobin determination by using sodium lauryl sulfate (SLS)" I. Oshiro *et al.*, Clin. Biochem. Vol. 15 (1982) [XP008058143]; pages 83 to 88.

The appellant also submitted two sets of claims amended according to a main request and an auxiliary request labelled "auxiliary request 1" and amended pages 3, 7 and 8 of the description, and requested setting aside of the decision under appeal and the grant of a patent on the basis of one of the two requests.

At the end of the oral proceedings the Board announced its decision as recorded in the order.

VII. The main request includes an independent claim 11 reading as follows:

"Disposable microcuvette having at least one cavity for spectrophotometric determination of haemoglobin in undiluted whole blood, characterised in that the cavity includes a dried, non hygroscopic hemolysing agent provided that the cavity is essentially free from azide and nitrite."

Independent claims 1 and 11 amended according to the auxiliary request of the appellant read as follows:

"1. A method for quantitative haemoglobin determination in undiluted whole blood comprising the steps of:

introducing a sample of undiluted whole blood by capillary action into a disposable microcuvette having at least one cavity for receiving the sample, the cavity including an essentially non-hygroscopic hemolysing agent, selected from the group consisting of ionic and non-ionic surface active substances, in a dry form, whereby the hemolysing agent is dissolved, hemolyses the red blood cells and releases the haemoglobin contained in the blood cells;

performing a first absorption measurement at a wavelength range 490-520 nm directly on the hemolysed sample in the cuvette; and

further conducting a second absorption measurement at a wavelength range 850-910 nm to compensate for background interference,

wherein the period for hemolysing said undiluted whole blood is less than 40 seconds."

"11. Disposable microcuvette having at least one cavity for spectrophotometric determination of haemoglobin in undiluted whole blood, characterised in that the cavity includes a dried, non hygroscopic hemolysing agent provided that the cavity is essentially free from azide and nitrite, and any other additive, wherein the hemolysing agent is adapted to hemolyse undiluted whole blood in less than 40 seconds."

The auxiliary request includes dependent claims 2 to 10 and 12 referring back to claims 1 and 11, respectively.

VIII. The arguments submitted by the appellant in support of its requests can be summarized as follows:

The method of haemoglobin measurement of document D1 represents the closest prior art. The method is based on a modification of the azide-methaemoglobin method according to Vanzetti described in document B1. The haemoglobin is released by haemolysing the red blood cells with sodium deoxycholate, which replaces the detergent used in document B1; the haemoglobin is then oxidised by the sodium nitrite reagent to form methaemoglobin and subsequently reacted with the sodium azide reagent to form azide-methaemoglobin. Thus, the different haemoglobin variants present in the hemolysed blood are converted into one single stable product, namely azide-methaemoglobin, the concentration of which is then determined by absorbance measurements at 570 and at 880 nm.

The technical problem formulated by the examining division was to improve the method of document D1 such that accurate measurements can be obtained for blood samples of any origin and in particular from both smokers and non-smokers. This, however, is not correct. While in document D1 all the haemoglobin variants are converted into one single stable product and it is irrelevant whether the blood originates from smokers or from non-smokers, in the invention the measurement is carried out in the hemolysed product containing all haemoglobin variants and consequently the method of the invention is not to be used with blood from smokers.

The examining division also proposed as objective problem the toxicity of sodium nitrite and sodium

azide. These reagents, however, are present in a very small amount and always kept in the cuvette and they do not represent a substantial problem. In addition, the skilled person would consider alternative, less toxic reagents to solve the problem without abandoning the Vanzetti method as suggested by document B2 (paragraph bridging the two columns on page 792).

The improvement of the invention over the disclosure of document D1 is based on the fact that it is possible to abandon the Vanzetti method and still obtain accurate haemoglobin measurements by just hemolysing the erythrocytes and changing the absorbance measurements to about 490-520 nm without the use of sodium azide and sodium nitrite or the like, which are necessary reagents in the Vanzetti method to convert all haemoglobin variants present in a whole blood sample to one single compound. According to the invention, in view of the absorption spectra of the different haemoglobin variants and derivatives (document D2, figure 6 and document B3, figure 2.1), the fact of performing the first absorption measurement at about 490-520 nm - i.e. at the isobestic point improving the measurement accuracy and reducing the impact of the different haemoglobin variants in the measurement - directly on an unreacted haemolysed sample allows for a reliable measurement in a significantly shorter time (page 7 of the application, lines 17 to 31), which is a great advantage, especially when used in hospitals in checking the Hb-value of blood in donors. The objective problem is therefore reducing the time required to perform the analysis so as to speed up the measurement.

Document D2 involves a reusable cuvette that is cleaned before and after usage and thus cannot contain any dry matter. Moreover, in the method of this document the measurements are carried out at about 510 nm (where the different types of haemoglobin absorb about equally) and at about 650 nm, and the blood is haemolysed with a saponin, which is known to be hygroscopic and does not give reproducible results as shown in the application. In addition, the document requires a separate manual haemolysing step (sample stirred on a glass slide) before the treated sample enters the microcuvette and the procedure is time-consuming.

Document D5 concerns the problem of replacing an azide containing diluent by another diluent for preventing microbial growth. In addition, the lysing agent is used in an aqueous solution and not in a dry form in a microcuvette.

Document A1 teaches pre-treatment of a blood sample by haemolysis and requires filtration prior to introduction of the sample into a chamber for haemoglobin determination (example 6b). The procedure is therefore time-consuming and, in addition, the measurement is carried out in a reacted sample.

Document A2 requires transport of the sample through different chambers or cavities. The treatment of the sample is carried out in a different chamber as that used for the measurement and the procedure is time consuming.

Document A3 relies on the measurement on oxidised samples (page 20, lines 22 to 24) and on the use of saponin (example 5).

The method of document A4 is based on measurements carried out in a sample in which haemoglobin has already reacted, and not in a sample that has only been hemolysed. In addition, the disclosure of this documents relies on the use of diluted blood and of SLS in diluted form (page 83, second column, second paragraph), on absorption measurements at 539 nm, and on waiting periods of about 5 minutes before the measurement is carried out (page 85, last paragraph).

The prior art would not lead the skilled person to reduce the measurement time by abandoning the Vanzetti approach and removing the sodium azide and sodium nitrite reagents in document D1. In particular, the skilled person would never attempt to remove the sodium nitrite and azide reagents in the method of document D1 because these reagents are required in the Vanzetti method in order to give reliable results (application, page 6, last paragraph, and document B1, page 116, second paragraph). None of the documents suggests carrying out the measurement on all haemoglobin variants and compensating for the inaccuracies in this measurement by carrying out the measurement at a wavelength as claimed.

The cuvette according to the invention includes a cavity suitable itself for spectrophotometric determination of haemoglobin and the very same cavity includes the dried, non-hygroscopic haemolysing agent without azide and nitrite reagents. As indicated above,

the skilled person would never have considered removing these agents from the cuvette of document D1 in order to reduce the measurement time because they are essential in the modified Vanzetti method followed in the document.

Reasons for the Decision

1. The appeal is admissible.

2. *Main request - Claim 11*

2.1 Document D1 discloses a disposable microcuvette (Figures 1 to 4, abstract, column 1, lines 6 to 10 and column 2, lines 26 to 55) having a cavity for the spectrophotometric determination of haemoglobin in undiluted whole blood (column 2, lines 28 to 41 and column 3, lines 26 to 32). According to the single example of the document, the cavity includes a dried reagent composed of sodium desoxycholate, sodium nitrite and sodium azide (column 3, lines 17 to 27), the sodium desoxycholate constituting a non-hygroscopic hemolysing agent as specified in the present application (page 4, lines 22 to 25 and page 5, lines 9 to 22).

2.2 The disposable microcuvette defined in independent claim 11 differs from the microcuvette disclosed in document D1 only in that the cuvette is essentially free from azide and nitrite.

However, the broad formulation of claim 11 ("the cavity includes ... provided that the cavity is essentially

free from azide and nitrite") excludes explicitly azide and nitrite reagents but does not exclude the presence in the cavity of any other substance. In particular, claim 11 does not exclude the presence of any other non-azide, non-nitrite reagent of arbitrary characteristics compatible with the spectrophotometric determination of haemoglobin in undiluted whole blood. As a consequence, the mere fact of specifying the absence of azide and nitrite in the cavity of the claimed microcuvette does not achieve any technical effect over the microcuvette disclosed in document D1. In particular, any technical effect that might be alleged as resulting from the absence of nitrite and azide such as a reduction in the degree of toxicity of the device or an improvement in the speed or efficiency of the haemoglobin determination would not be supported by the claimed subject-matter as the latter encompasses variants involving the use of non-azide, non-nitrite reagents exhibiting a degree of toxicity even higher than that of azide and nitrite or leading to a slower or a less efficient haemoglobin determination.

It follows that the claimed subject-matter does not achieve any technical effect that could be taken into account in the formulation of the objective problem solved by the claimed microcuvette over that disclosed in document D1 according to the problem-solution approach. Consequently, the objective problem actually solved by the claimed subject-matter can only be seen in the provision of an alternative to the microcuvette of document D1.

The skilled person confronted with the problem of providing an alternative to the microcuvette of

document D1 would obviously consider, among other possibilities, the replacement of the reagents of the microcuvette of document D1 by other reagents having either similar or better characteristics, or possibly even worse characteristics, the latter possibility not being excluded by the claimed subject-matter. In particular, the skilled person would be aware that reagents such as sodium nitrite and sodium azide can be replaced by alternative reagents as illustrated by the disclosures of document D5 (column 2, lines 24 to 50 and column 3, line 34 *et seq*.), document A2 (column 13, lines 6 to 30), document A4 (abstract) and document B1 (abstract and two first paragraphs). The appellant itself has acknowledged in the discussion of the objective problem solved by the method according to the invention corresponding to claim 11 that it would be obvious to replace azide reagents by other less toxic reagents such as those disclosed in document B2 (abstract and paragraphs bridging the two columns on page 792).

Therefore, the skilled person confronted with the problem of finding an alternative to the microcuvette of document D1 would find in the prior art numerous possibilities leading to a microcuvette as claimed, i.e. a microcuvette free from azide and nitrite.

The different arguments submitted by the appellant during the appeal proceedings are not found convincing as none of these arguments is supported by the subject-matter actually claimed. In particular, the line of argument according to which the method of the invention relies on measurements directly carried out on an unreacted hemolysed sample is not pertinent as claim 11

encompasses microcuvettes containing non-azide, non-nitrite reagents that would immediately react with the hemolysed blood sample, thus rendering it impracticable to carry out measurements on an unreacted hemolysed sample.

2.3 It follows that the Board cannot see any inventive step within the meaning of Article 56 EPC 1973 in the mere fact of excluding azide and nitrite agents in a microcuvette of the type disclosed in document D1. Therefore, independent claim 11 of the main request does not define patentable subject-matter (Article 52(1) EPC) and consequently the main request of the appellant cannot be allowed.

3. *Auxiliary request*

3.1 *Amendments*

The Board is satisfied that the application documents amended according to the auxiliary request satisfy the formal requirements of the EPC. In particular,

- claim 1 is based on the combination of the features of claims 1, 6 and 12 as published together with the passage on page 7, lines 25 to 28 of the description as published, independent claim 11 is based on claim 13 as published together with the passages on page 4, lines 15 and 16 and page 8, lines 3 to 5 and on page 7, lines 25 to 28 of the description as published, and claims 2 to 10 and 12 are based on claims 2, 3, 5, 7 to 12 and 14 as published, respectively (Article 123(2) EPC), and

- the description has been brought into conformity with the invention as defined in the amended claims

(Article 84, second sentence and Rule 27(1)(c) EPC 1973).

3.2 *Claim 1 - Inventive step*

3.2.1 The Board concurs with the appellant's and with the examining division's view that the closest state of the art is represented by the disclosure of document D1. This document discloses a method for the quantitative determination of haemoglobin in undiluted whole blood by means of the disposable microcuvette referred to in point 2.1 above. According to the document, a sample of undiluted whole blood is introduced by capillarity into a cavity of the microcuvette including dried sodium desoxycholate (column 3, lines 14 to 34), i.e. a hemolysing agent having non-hygroscopic and surface-active characteristics (see description of the application, page 5, lines 9 to 22); the hemolysing agent is dissolved and hemolyses the red blood cells, thus releasing the haemoglobin contained in the blood cells. The determination of haemoglobin is then carried out on the basis of a first absorption measurement at a wavelength of 570 nm and a second absorption measurement at a wavelength of 880 nm to compensate for background interference (column 3, lines 27 to 34).

However, while in document D1 the first measurement is carried out in a blood sample that has been hemolysed and then reacted with reagents, namely with sodium azide and nitrite (column 3, lines 17 to 22), according to claim 1 the first measurement is carried out directly on the hemolysed sample. Furthermore, while in document D1 the first measurement is carried out at

570 nm, according to claim 1 this measurement is carried out in the range 490 to 520 nm.

The method of claim 1 also requires that the undiluted whole blood is hemolysed in a time period of less than 40 seconds. Document D1 is silent as to the hemolysing rate of the agents used in the microcuvette, and the question of whether or not the hemolysing rate in document D1 is intrinsically below 40 seconds does not affect the following assessment and the conclusion drawn below and can therefore be left open.

3.2.2 As submitted by the appellant, the fact of carrying out the first measurement directly on a sample that has been hemolysed in a time period of less than 40 seconds without requiring - as it is the case in document D1 - that the haemoglobin variants present in the hemolysed sample react with reagents allows for a faster determination of the haemoglobin content as supported by the description of the application (page 2, lines 24 to 26, page 3, lines 22 to 27 and page 7, lines 17 to 31). In the invention the measurement is therefore carried out on a sample comprising the different haemoglobin variants released after the hemolysing step and not on a reacted sample in which the haemoglobin variants have been converted into one single haemoglobin derivative. In addition, the adverse effects of carrying out the measurement in a sample containing different haemoglobin variants are compensated, at least in part, by carrying out the measurement at a wavelength in the range 490 to 520 nm and therefore at, or close to the isobestic point at 510 nm at which the impact of the different haemoglobin

variants in the measurement is relatively low (document D2, column 1, line 44 *et seq*. and Figure 6).

Other aspects considered during the proceedings do not appear to support further technical effects of the claimed invention. In particular, in the decision under appeal the examining division noted that the stronger presence of carboxyhaemoglobin in smokers had an impact on the absorbance measurement at 570 nm and that the measurement in the range 490 to 520 nm as claimed rendered possible the determination of haemoglobin irrespectively of whether the blood sample originated from smokers or non-smokers; however, as submitted by the appellant, the claimed method is not to be used with blood samples originating from smokers since the different haemoglobin composition would lead to deviations in the haemoglobin determination (page 6, line 22 to page 7, line 2 of the description of the application). The further aspect discussed during the proceedings and relating to the toxicity concerns associated with the use in document D1 of sodium nitrite and azide is not considered pertinent since, as pointed out by the appellant, this aspect would at the most lead to the replacement of these reagents by other less toxic reagents, but not to carrying out the measurement on an unreacted blood sample as required by the claimed subject-matter.

Consequently, the objective problem solved by the claimed method over that disclosed in document D1 can be seen in improving the speed in the quantitative determination of haemoglobin while allowing results that, although possibly less reliable, are still acceptable.

3.2.3 Documents D5, A1, A2 and A3 all concern the determination of haemoglobin in blood and all address explicitly or implicitly the problem of providing a rapid process of determination (document D5, column 1, lines 17 to 19 and paragraph bridging columns 3 and 4, document A1, column 1, lines 58 to 62, document A2, abstract and column 3, lines 36 to 40, and document A3, page 3, lines 2 to 5). However, none of these documents teaches or suggests solving the problem formulated in the former paragraph as claimed. In particular:

- Document D5 relies on spectrophotometric measurements carried out on a sample containing a haemoglobin complex resulting from the reaction of the hemolysed blood with appropriate reagents (column 2, lines 51 to 59 and paragraph bridging columns 3 and 4) and therefore the document teaches away from the claimed method requiring carrying out the measurements directly on a hemolysed blood sample.

- Document A1 discloses the determination of haemoglobin on the basis of the absorbance of a hemosylate resulting from treating a blood sample with sodium deoxycholate (column 29, lines 45 to 68). However, the document is silent as to the method of measurement of the absorbance and, in addition, requires the blood sample to be hemolysed and filtered before being introduced in a cuvette device where measurements are carried out (column 29, lines 62 to 68), thus teaching away from the claimed method and resulting in a time-consuming procedure contrary to the objective problem solved by the claimed invention.

- In document A2, the amount of glycated haemoglobin present in the erythrocytes of a whole blood sample (column 1, lines 9 to 30) is determined

according to an affinity binding method (abstract). The determination involves hemolysing the blood sample (column 6, lines 4 to 61), carrying out two absorbance measurements (paragraph bridging columns 3 and 4) and the use of stable glycated calibrators containing azidomethaemoglobin or the like (column 11, line 53 to column 13, line 29). However, the method only determines the relative amount of glycated haemoglobin in the blood sample (column 3, lines 65 to 67 and column 10, lines 46 to 54) and not the quantitative haemoglobin content as claimed. In addition, the document mentions absorbance measurements in the broad range 340 to 633 nm (column 8, lines 28 to 33) and at the specific wavelengths 553 and 628 nm (column 6, lines 27 to 31) and therefore the document does not direct the skilled person to carry out the measurements in the relatively narrow wavelength range 490-520 nm as required by claim 1.

- Document A3 (abstract) relies on the use of saponin, i.e. a lysing agent that - as contrarily required by the claimed subject-matter - is generally hygroscopic, or of an unspecified detergent (page 5, lines 33 and 34, page 20, lines 12 to 27 and example 5 on page 25) and, in addition, is silent as to the measurement wavelengths.

Documents D2, A4, B1 and B2 are all directed to the spectrophotometric determination of haemoglobin in blood. However, none of these documents addresses the objective problem formulated above. In addition, none of these documents discloses or suggests modifying the method disclosed in document D1 as claimed. In particular:

- Document D2 proposes carrying out the first measurement in the range 500 to 520 nm (column 6, lines 3 to 9) and in particular at 510 nm (column 4, line 21 *et seq*.), i.e. at wavelengths within the first of the claimed ranges; however, contrary to document D1 and to the claimed method, the document requires the time-consuming step of hemolysing the blood sample before being drawn into a reusable cuvette (column 5, lines 44 to 56) and, in addition, the reagent used is saponin (column 5, lines 45 to 48), i.e. a lysing agent that is generally hygroscopic.

- Document A4 (abstract), document B1 (abstract) and document B2 (abstract) all rely on spectrophotometric measurements carried out on a sample in which the haemoglobin has reacted and therefore all of them teach away from the claimed method for the same reasons indicated above with regard to document D5.

The remaining documents on file are less relevant.

3.2.4 In view of the above considerations, the Board concludes that the subject-matter of claim 1 of the auxiliary request is not rendered obvious by the prior art when starting with document D1 as the closest state of the art.

3.3 *Independent claim 11 - Inventive step*

3.3.1 Claim 11 of the auxiliary request is directed to a disposable microcuvette for the spectrometric determination of haemoglobin in undiluted whole blood. The Board also considers the disposable microcuvette disclosed in document D1 and referred to in point 2.1 above as representing the closest state of the art.

While in the microcuvette of document D1 the cavity for the spectrophotometric determination of haemoglobin in undiluted whole blood contains, in addition to a non-hygroscopic hemolysing agent in dried form, further additives, and in particular reagents constituted by sodium azide and nitrite (column 3, lines 17 to 22), the cavity of the claimed microcuvette includes the dried non-hygroscopic hemolysing agent and is essentially free from azide and nitrite and from any other additive.

As regards the claimed feature that the hemolysing agent is adapted to hemolyse the undiluted whole blood in less than 40 seconds, the same comments in the last paragraph of point 3.2.1 above also apply in the context of this claim.

3.3.2 In line with the submissions of the appellant and also with the considerations in point 3.2.2 above, the claimed microcuvette allows for an undiluted whole blood sample to be rapidly hemolysed in the cavity of the microcuvette without the haemoglobin variants released in the hemolysing process reacting with reagents as it is required in document D1, thus rendering possible a rapid determination of the haemoglobin content in the blood sample by carrying out a spectrophotometric measurement directly in the unreacted, hemolysed blood.

None of the documents on file discloses or suggests the claimed combination of features nor the technical advantages achieved therewith. In particular:

- Document D5 (paragraph bridging columns 3 and 4), document A4 (abstract), document B1 (abstract) and document B2 (abstract) all require carrying out measurements in a blood sample after the hemolysed blood has reacted with appropriate reagents as it is the case in document D1. Thus, all these documents teach away from removing from the microcuvette disclosed in document D1 the reagents required for reacting with the hemolysed sample.

- Document D2 discloses carrying out photometric absorbance measurements of haemoglobin in an undiluted blood sample drawn into a cavity in a microcuvette (abstract). However, the blood sample is hemolysed before being drawn into the microcuvette and, in addition, the lysing agent is saponin (column 5, lines 44 to 56), i.e. a substance that is generally hygroscopic. Thus, the document teaches away from hemolysing the blood sample in the cavity and by means of a non-hygroscopic hemolysing agent as claimed. Similar comments apply to document A3 (abstract) which also relies on the use of saponin (page 5, lines 33 and 34, page 20, lines 12 to 27 and example 5 on page 25) and to document A1 which, although using sodium deoxycholate as hemolysing agent (column 29, lines 45 to 68), requires the blood sample to be hemolysed and filtered before being introduced in a cuvette device where measurements are then carried out (column 30, lines 62 to 68, and column 16, lines 59 to 65 together with column 9, lines 17 to 21).

- Document A2 discloses a cuvette having different cavities or chambers in communication which each other for the determination of the relative amount of glycated haemoglobin in a blood sample (abstract and figure 1). The blood sample circulates between the

different chambers where it is hemolysed by means of agents such as saponin and sodium deoxycholate (column 6, lines 44 to 61) and subjected to absorbance measurements (paragraph bridging columns 3 and 4). The document also specifies reagents for the preparation of calibrators (column 11, line 52 *et seq*.). However, contrary to the claimed microcuvette in which the blood sample is hemolysed in the same measurement cavity, in document A2 the chamber in which the blood sample is hemolysed is different from the chamber in which the absorbance measurements are carried out (Figure 1 and the corresponding description).

The remaining documents on file are less relevant.

3.3.3 It follows from the above considerations that the subject-matter of independent claim 11 of the auxiliary request is not rendered obvious by the prior art when starting with document D1 as the closest state of the art.

3.4 Having regard to the above conclusions, the subject-matter of claims 1 and 11 involves an inventive step over the disclosure of document D1 as closest state of the art (Article 56 EPC 1973). The Board is also satisfied that no other conclusion would be drawn when starting from any other one of the documents on file as the closest state of the art. The same conclusion applies to dependent claims 2 to 10 and to dependent claim 12 by virtue of their dependence on claims 1 and 11, respectively.

3.5 The Board is also satisfied that the application documents amended according to the auxiliary request of

the appellant and the invention to which they relate meet the remaining requirements of the EPC within the meaning of Article 97(2) EPC. In view of these considerations, the Board concluded during the oral proceedings that the decision under appeal was to be set aside and a patent granted on the basis of the application documents amended according to the auxiliary request (Article 111(1) EPC 1973).

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 grant a patent in the following version:
   - description: pages 1, 2 and 4 to 6 as published and pages 3, 7 and 8 as filed in the oral proceedings of 16 April 2008,
   - claims: n° 1 to 12 of auxiliary request 1 filed in the oral proceedings of 16 April 2008, and
   - drawings: sheet 1/1 as published.

The Registrar: The Chairman:

N. Maslin A. G. Klein