



- (51) **International Patent Classification:**
A61B 5/145 (2006.01) *G01N 33/72* (2006.01)
- (21) **International Application Number:**
PCT/US2013/035849
- (22) **International Filing Date:**
9 April 2013 (09.04.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/686,670 9 April 2012 (09.04.2012) US
- (71) **Applicant:** MEC DYNAMICS CORPORATION
[US/US]; 90 Rose Orchard Way, San Jose, CA 95134 (US).
- (72) **Inventors; and**
- (71) **Applicants :** MPOCK, Emmanuel [CM/US]; c/o Mec Dynamics Corporation, 90 Rose Orchard Way, San Jose, CA 95134 (US). MANGAN, Wilma [US/US]; c/o Mec Dynamics Corporation, 90 Rose Orchard Way, San Jose, CA 95134 (US).
- (74) **Agents:** FARMER-KOPPENOL, Pauline et al.; Fenwick & West LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (US).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) **Title:** MEASUREMENT OF TOTAL HEMOGLOBIN IN WHOLE BLOOD

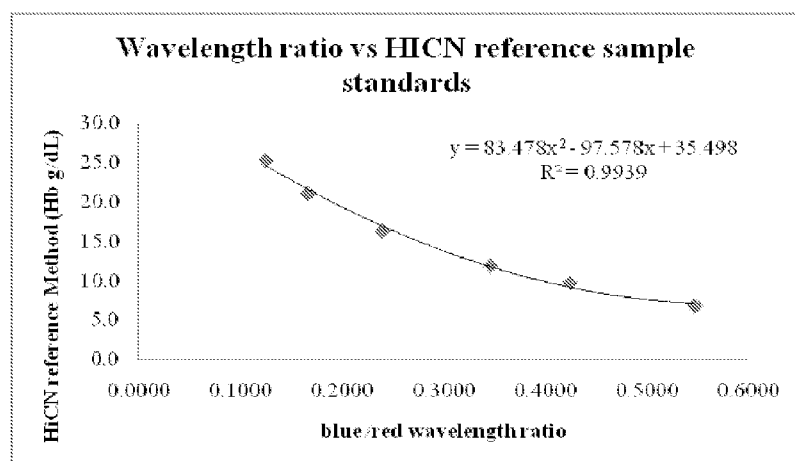


FIG.3

(57) **Abstract:** A method for determining total hemoglobin concentration in a blood sample comprising spectrophotometric analysis of a blood sample at two wavelengths and determining a ratio of the detected radiation at a first wavelength to the detected radiation at a second wavelength; and determining a concentration of total hemoglobin in the blood sample based on the ratio.

MEASUREMENT OF TOTAL HEMOGLOBIN IN WHOLE BLOOD

Inventors: Emmanuel Mpock and Wilma Mangan

Related Applications

[0001] This application claims the benefit of the earlier filing date of U.S. Provisional Application 61/686,670 filed April 9, 2012 which is hereby incorporated by reference for all purposes.

Field

[0002] The disclosure relates the direct determination of total hemoglobin concentration in whole blood without the need for transforming the hemoglobin through secondary reactions.

Background

[0003] Traditional total hemoglobin measurements have generally been done indirectly, requiring significant sample quantities from a venous draw, extensive sample preparation and the use of toxic and dangerous chemicals. The assay reagents are typically used to lyse the blood cells to release the hemoglobin molecules to which accompanying molecules react with and bind unto the hemoglobin molecules to form a stable compound that is optically detectable, and whose concentrations are proportional to the amount of hemoglobin in the sample. The need for reagents adds to the cost of the assay and makes it less accessible in the developing world. Certain reagents also require specific storage conditions which makes them less useful in areas where climate control is rare. Thus there is a need for a method to determine total hemoglobin content in modified or unmodified forms even in very small blood samples.

Brief Description of the Drawings

- [0004] FIG. 1 illustrates sample holders according to two embodiments.
- [0005] FIG. 2 illustrates measurements along a capillary according to one embodiment.
- [0006] FIG. 3 illustrates a calibration curve according to one embodiment.
- [0007] FIG. 4 illustrates linearity of the response.

Detailed Description

[0008] Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below. It must be noted that, as used in

the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0009] All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

[0010] Described herein is a convenient and inexpensive method for quantifying total hemoglobin directly and rapidly in a system designed for use at the point of care using a very small blood sample. The sample requires no addition of reagents to accomplish the measurement. The sample is exposed to light at a plurality of wavelengths. Absorbance, transmission or reflectance measurements are made and the ratio of the measurements at two wavelengths indicate the amount of total hemoglobin.

Sample Preparation

[0011] A small amount of blood is drawn into a sample holder. Only a few drops are needed. The sample holder can be any holder normally used to collect blood samples provided that the holder material allows transmission of the wavelengths of light used for analysis. Example sample holders include glass capillaries (open on both ends) as well as test strips incorporating a capillary channel that is open to the atmosphere at both ends of the channel. When one end of the capillary is touched to a drop of blood, the blood is drawn into the channel. The sample holder may be uncoated and need not contain any reagents. No reagents (such as lysing agents) need to be added to the sample. It should be noted however that the disclosed method can be used with samples where a lysing agent has been added to the blood sample. The sample is then analyzed spectrophotometrically.

[0012] Example sample holders of various shapes are shown in FIG. 1. The substrates holding the capillary channel can be cylindrical, rectangular or polymorphic.

Sample Analysis

[0013] The sample is exposed to light at at least two wavelengths. The selected wavelengths should be sufficiently separated on the spectrum but this does not mean that the edges of the ranges of the two wavelengths cannot overlap. While not wishing to be bound by any particular theory, it is believed that the use of multiple wavelengths avoids error due to hemoglobin variants and side reactions. In one embodiment, the sample is exposed to both blue light as well as red light. In some embodiments the blue light has wavelengths spanning 390 to 520 nm, or 390 to 495 nm, or any subrange of any of the foregoing. In some embodiments, the blue light has a wavelength of between 390 nm and 520 nm or a plurality of wavelengths selected from wavelengths of 390 nm to 520 nm. In some embodiments, the

red light has wavelengths spanning 500 to 700 nm, or 570 to 750 nm, or 620 to 750 nm or any subrange of any of the foregoing. In some embodiments, the red light has a wavelength of between 500 nm and 700 nm or a plurality of wavelengths selected from wavelengths of 500 nm to 700 nm. As shown in FIG. 2, the measurements can be made along any portion of the capillary channel provided that the substrate at that portion allows transmission of the wavelengths used for analysis.

[0014] The reflectance of the light off of the sample is detected or alternatively, the transmission of light through the sample of light is detected. In yet another alternative, the absorbance of light by the sample is determined. Standard spectrophotometric techniques can be used to make the spectrophotometric measurements. For ease of detection of the multiple wavelengths, the reflected or transmitted light is detected by a detector array such as a charge coupled device (CCD) or photodiode array. The sample is exposed to light for an extended period of time during which several measurements are taken. The sample may be exposed from 5-20 seconds, from 8-15 seconds or about 10 seconds. Measurements are taken every second, every half second or every quarter second. In some embodiments, the measurements are taken at several positions along the capillary channel.

[0015] The measurements for each color (or wavelength) of light are averaged and then the ratio is taken of the average of one color (wavelength) to the average of the other color (wavelength). If measurements are taken at multiple positions along the capillary channel, all measurements, regardless of position, for each color (wavelength) are averaged. For example if the measurements are transmission measurements and the light is blue light and red light, the ratio is determined as follows: (average of all transmission measurements of blue light)/(average of all transmission measurements of red light). This ratio correlates to the total hemoglobin concentration in the sample. After creation of a calibration curve, the ratio of optical measurements for a sample is used to determine the total hemoglobin concentration for the sample.

[0016] When larger samples are available, this method can be used with larger sample holders and spectrophotometers that hold larger volumes.

[0017] In some embodiments, the sample is exposed to many wavelengths of light and the two wavelengths used for analysis are those that are measured spectrophotometrically.

EXAMPLES

[0018] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure

accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1 - Determination of Calibration Curve

[0019] Haemoglobincyanide (HiCN) references were prepared at 6.8, 9.7, 11.9, 16.3, 21.0 and 25.2 g/dL. The standards were prepared and the calibration curve calculated using the standard World Health Organization (WHO) method (“Haemoglobinometry” Chap 7 in the *Blood and Safety and Clinical Technology, Guidelines on Standard Operating Procedures for Haematology* published by WHO). The transmission of red light (from 500 to 700 nm) and blue light (from 390 to 520 nm) through the sample was measured. For each sample the transmission of each color was measured 20 times at ½ second intervals. The ratio of blue to red was determined for each measurement and then the average ratio was determined. The last 5 measurements for each sample are shown below in Table 1. Spectrophotometric analysis was performed by the Avie™ A1c available from MEC Dynamics in San Jose, CA.

Table 1

Sample run		Avg(with sample)		WB	Avg
	Ref. HiCN	Red trans-mission	Blue trans-mission	blu/red	
1	6.8	56487	30843	0.5460	0.5464
		56437	30858	0.5468	
		56737	30977	0.5460	
		56437	30858	0.5468	
		56487	30873	0.5466	
2	9.7	55363	23460	0.4237	0.4239
		55124	23366	0.4239	
		55172	23383	0.4238	
		55124	23374	0.4240	
		55124	23374	0.4240	
3	11.9	53826	18615	0.3458	0.3458
		54054	18686	0.3457	
		53826	18615	0.3458	
		53826	18610	0.3457	
		53826	18615	0.3458	
4	16.3	51821	12427	0.2398	0.2398
		51821	12424	0.2397	
		52032	12473	0.2397	
		51779	12427	0.2400	
		52032	12478	0.2398	

Sample run		Avg(with sample)		WB	Avg
	Ref. HiCN	Red trans-mission	Blue trans-mission	blu/red	
5	21.0	50393	8396	0.1666	0.1666
		50354	8396	0.1667	
		50393	8397	0.1666	
		50393	8395	0.1666	
		50393	8396	0.1666	
6	25.2	49459	6188	0.1251	0.1251
		49268	6162	0.1251	
		49230	6164	0.1252	
		49230	6163	0.1252	
		49459	6189	0.1251	

[0020] The resulting calibration curve is shown as FIG. 3.

Example 2 – Linearity of Response

[0021] HiCN references from 5 to 23 g/dL were prepared by serial dilution and their concentration determined using the measurement procedure described in Example 1. FIG. 4 illustrates the linearity:

Example 3 – Accuracy of Response

[0022] Fifty whole blood samples were tested using both the procedure in Example 1 as well as using a Hemocue™ Hb 201 available from HemoCue AB of Angelholm Sweden. FIG. 5 illustrates excellent accuracy for the disclosed method as compared to HemoCue™ Hb 201.

Example 4 – Precision of Response

[0023] To assess the precision of the disclosed method, samples having low, mid and high hemoglobin concentrations were tested 20 times each using the method in Example 1. The results in Table 2 illustrate the precision of the disclosed method.

Replicate	Low Hb Total Hb g/dL	Mid Hb Total Hb g/dL	High Hb Total Hb g/dL
1	6.3	13.9	18.6
2	6.1	13.8	18.5
3	6.0	13.5	18.6
4	6.2	13.8	18.3
5	6.5	13.8	18.6
6	6.3	13.7	18.7
7	6.1	14.1	18.6
8	6.4	14.3	18.6
9	6.0	14.2	18.7
10	6.3	14.0	18.6

Replicate	Low Hb Total Hb g/dL	Mid Hb Total Hb g/dL	High Hb Total Hb g/dL
11	6.2	13.9	18.7
12	6.2	14.2	18.5
13	6.3	14.5	18.7
14	6.2	14.1	18.9
15	6.1	13.9	18.7
16	6.1	14.0	18.8
17	6.2	14.2	19.1
18	5.8	14.1	18.7
19	6.0	14.0	18.9
20	6.1	14.2	18.9
Avg	6.2	14.0	18.7
SD	0.2	0.2	0.2
%CV	2.6	1.7	0.9

[0024] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0025] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the invention as defined in the appended claims.

CLAIMS

1. A method for determining total hemoglobin concentration in a blood sample comprising:
 - directing radiation having a first wavelength and radiation having a second wavelength through a blood sample;
 - detecting the radiation at the first wavelength and the radiation at the second wavelength after passing through the blood sample;
 - determining a ratio of the detected radiation at the first wavelength to the detected radiation at the second wavelength; and
 - determining a concentration of total hemoglobin in the blood sample based on the ratio.
2. The method of claim 1 wherein the radiation having a first wavelength is blue light.
3. The method of claim 1 wherein the radiation having a first wavelength comprises radiation having wavelengths of 390 nm to 520 nm.
4. The method of claim 1 wherein the radiation having a first wavelength comprises radiation having a wavelength of between 390 nm and 520 nm.
5. The method of claim 1 wherein the radiation having a first wavelength comprises radiation having a plurality of wavelengths selected from wavelengths of 390 nm to 520 nm.
5. The method of claim 1 wherein the radiation having a second wavelength is red light.
6. The method of claim 1 wherein the radiation having a second wavelength comprises radiation having wavelengths of 500 nm to 700 nm.
7. The method of claim 1 wherein the radiation having a second wavelength comprises radiation having a wavelength of between 500 nm and 700 nm.
8. The method of claim 1 wherein the radiation having a second wavelength comprises radiation having a plurality of wavelengths selected from wavelengths of 500 nm to 700 nm.
9. The method of any previous claim wherein:
 - detecting the radiation at the first wavelength and the radiation at the second wavelength comprises detecting a plurality of signals generated by the radiation at

the first wavelength and detecting a second plurality of signals generated by the radiation at the second wavelength; and

determining the ratio of the detected radiation comprises determining an average of the plurality of signals and determining a second average of the second plurality of signals and determining a ratio of the average to the second average.

10. The method of any previous claim wherein determining a concentration of total hemoglobin in the blood sample based on the ratio comprises preparing a calibration curve of standard total hemoglobin solutions and determining the concentration of total hemoglobin in the blood sample based on the calibration curve.
11. The method of any previous claim wherein the blood sample has not been contacted by a lysing reagent.
12. The method of any previous claim wherein red blood cells in the blood sample remain whole.

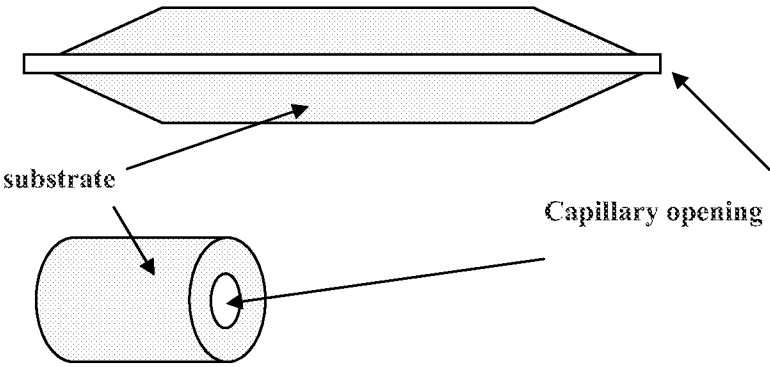


FIG. 1

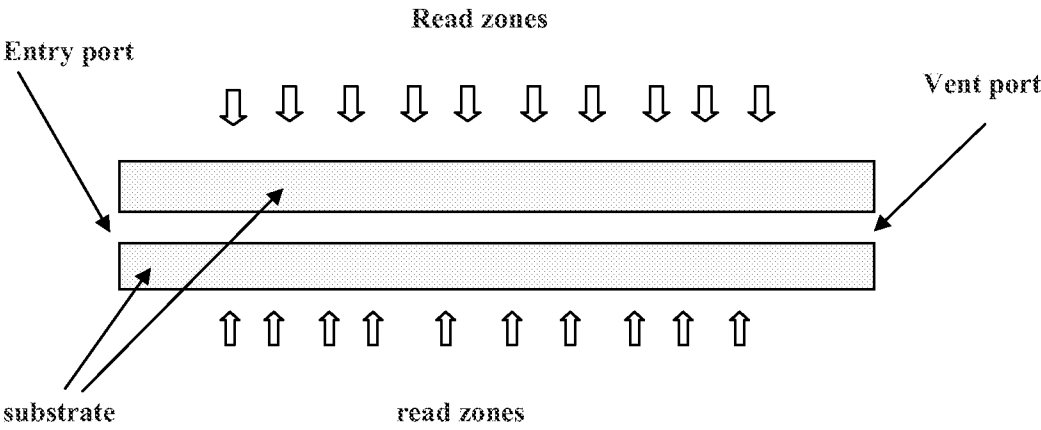


FIG. 2

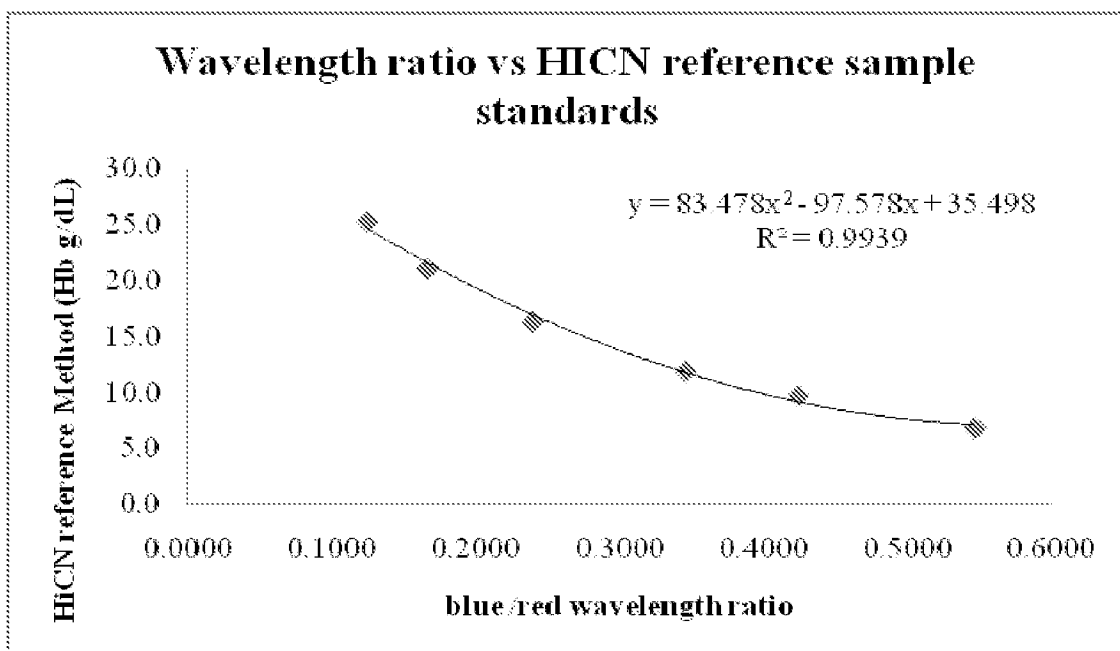


FIG.3

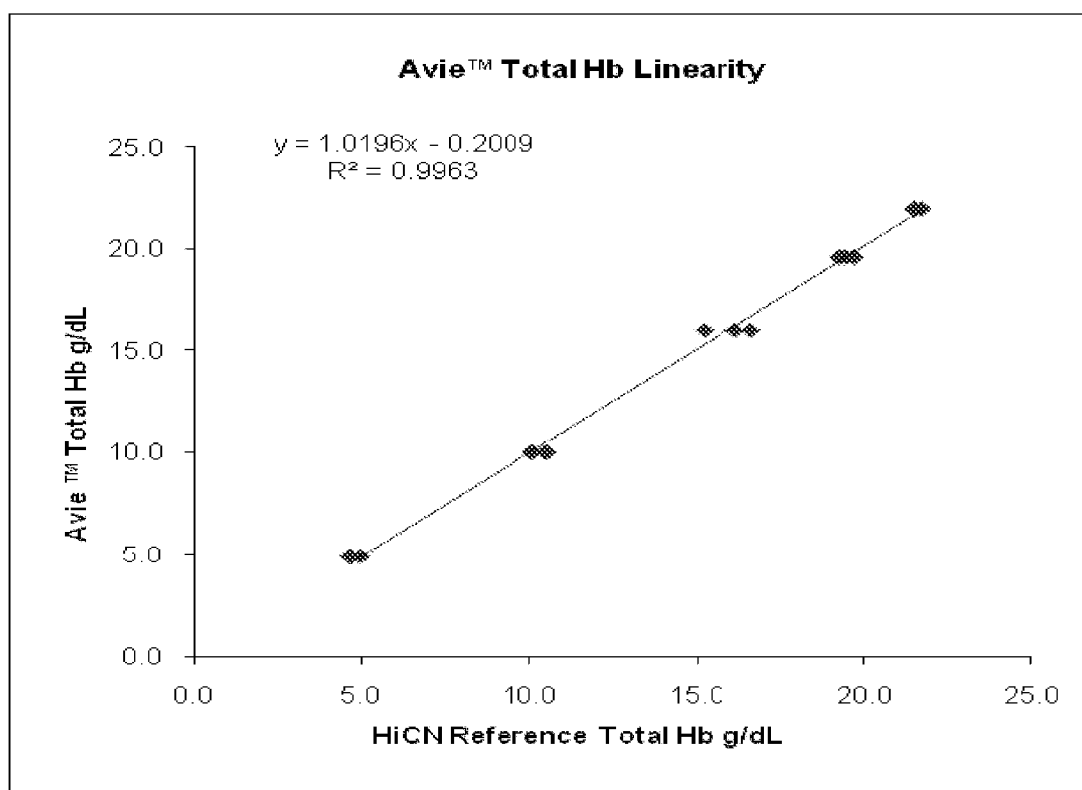


FIG. 4

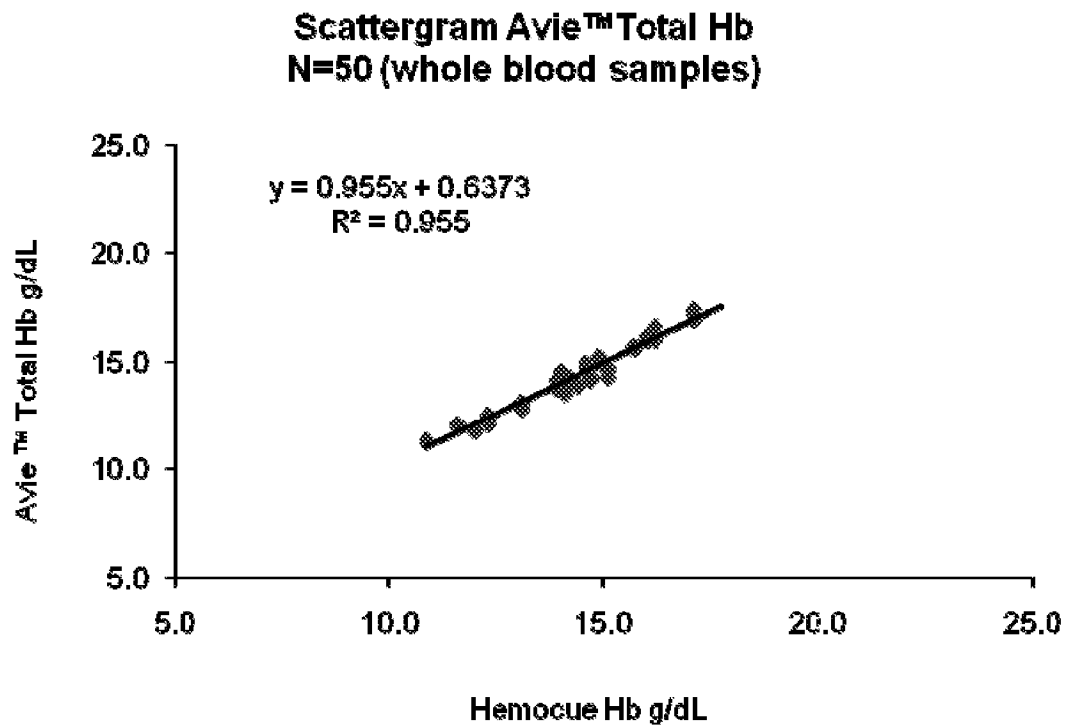


FIG. 5

A. CLASSIFICATION OF SUBJECT MATTER**A61B 5/145(2006.01)i, G01N 33/72(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61B 5/145; A61B 5/14; A61B 6/00; A61B 5/00; G01N 33/72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: hemoglobin, blood, radiation, wavelength

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009-0054767 A1 (NICHOLAS ALEXANDER TELISCHAK et al.) 26 February 2009 See abstract, paragraphs [0022]-[0075] and figures 1-5.	1-9
A	JP 2006-0061308 A (HITACHI LTD.) 09 March 2006 See abstract, paragraphs [0009]-[0038] and figures 1-4.	1-9
A	JP 10-0501141 A (NONINVASIVE MEDICAL TECHNOLOGY CORPORATION) 03 February 1998 See abstract, pages 7-13,18,19 and figures 1-1B.	1-9
A	US 2006-0063990 A1 (OK-KYUNG CHO et al.) 23 March 2006 See abstract, paragraphs [0045]-[0067] and figures 2-4.	1-9
A	US 4981138 A (LAWRENCE I. DECKELBAUM et al.) 01 January 1991 See abstract, column 2, lines 15-67, column 4, lines 1-67, column 5, lines 1-58 and figures 1-3. For the claim 5(1), the claim was renumbered by this authority because the claim 5 was found twice (PCT Rule 6.1(b)).	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

24 September 2013 (24.09.2013)

Date of mailing of the international search report

25 September 2013 (25.09.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City,
302-701, Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

KIM Tae Hoon

Telephone No. +82-42-481-8407



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/035849

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-12
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/035849

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009-0054767 A1	26/02/2009	US 7996068 B2	09/08/2011
JP 2006-061308 A	09/03/2006	CN 1739449 A	01/03/2006
		CN 1739449 C0	01/03/2006
		EP 1629766 A1	01/03/2006
		JP 3884036 B2	21/02/2007
		US 2006-0063990 A1	23/03/2006
JP 10-501141A	03/02/1998	CA 2160252 A1	27/10/1994
		CA 2160252 C	22/06/2004
		EP 0693900 A1	12/11/2003
		EP 0693900 B1	17/03/2004
		JP 3625475 B2	02/03/2005
		KR 10-0294294 B1	17/09/2001
		WO 94-23643 A1	27/10/1994
US 2006-0063990 A1	23/03/2006	CN 1739449 A	01/03/2006
		CN 1739449 C0	01/03/2006
		EP 1629766 A1	01/03/2006
		JP 2006-061308 A	09/03/2006
		JP 3884036 B2	21/02/2007
US 04981138 A	01/01/1991	WO 90-00035 A1	11/01/1990