(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
04 February 2021 (04.02.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/019553 A1

(51) International Patent Classification:
*G01N 21/25* (2006.01) *G01N 33/72* (2006.01)
*G01N 33/487* (2006.01)

(21) International Application Number:
PCT/IN2020/050617

(22) International Filing Date:
17 July 2020 (17.07.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
201931030430 27 July 2019 (27.07.2019) IN

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(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, ST, 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, KM, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report (Art. 21(3))*
— *with amended claims and statement (Art. 19(1))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: SMARTPHONE BASED BLOOD HEMOGLOBIN ESTIMATION SYSTEM.

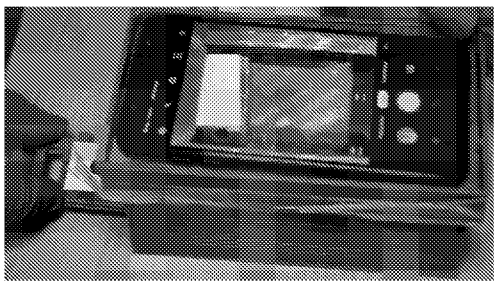

Fig. 2a

(57) Abstract: The present invention discloses a point of care rapid blood hemoglobin estimation system. The present system comprises a paper based microfluidic strip to contain blood sample and a camera based detection unit for cooperatively disposing with respect to said paper based microfluidic strip to capture its image while containing the blood sample. The camera based detection unit preferably includes a smartphone which is specifically configured to analyze the captured image in real time by using colorimetric analysis and instantly compute hemoglobin level in the blood sample by calibration technique.

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**TITLE: SMARTPHONE BASED BLOOD HEMOGLOBIN ESTIMATION SYSTEM.**

**FIELD OF THE INVENTION:**

The present invention relates point-of-care (POC) diagnostic system. More specifically the present invention is directed to develop a low cost Smartphone based point-of-care blood hemoglobin estimating system for non-invasive, fast and accurate detection of the blood hemoglobin level.

**BACKGROUND OF THE INVENTION:**

Anemia, a blood type disorder, is considered as one of the most prevalent diseases which has affected almost one-third of the world population. As per the GBD report (Global burden of Disease) in 2013, more than 183000 deaths were reported due to anemia. However, with early diagnosis blood hemoglobin level and subsequent start of on time treatment can save millions of life by providing proper nutrition's and dietary iron supplements.

Therefore, an affordable, user-friendly, portable, point-of-care (POC) diagnostic device for detection of the blood hemoglobin level was an utmost necessity to fulfill the need of the common people in the resource limited settings. A significant effort has already been made to address this issue by developing various techniques and methods of POC diagnostic devices. Over the past few years, several literatures have been published and patents have been filed on POC hemoglobin detection.

In the recent years, a significant progress has been made to develop low-cost, portable, user-friendly POC hemoglobin (Hgb) estimation device. The detection approach can be broadly classified into two categories: optical (spectrophotometry, reflectance photometry etc.) detection technique and electrochemical sensing. In a different approach, a non-invasive method for Hgb detection from the image of finger nails using smartphone app has been reported by Mannino et al *(Ref: RG Mannino, DR Myers, EA Tyburski, C Caruso, J*

*Boudreaux, T Leong, G D Clifford and W A Lam. Smartphone app for non-invesive detection of anemia using only patient-source photos. Nature Communications. 2018; 9:4924).*

Recently, a 3D printed POC tool has been developed by Knowlton et al. *(Ref: S Knowlton, A Joshi, P Syrrist, AF Coskun and S Tasoglu. 3D-printed smartphone-based point of care tool for fluorescence-and magnetophoresis-based cytometry. Lab Chip. 2017; 17:2839).* for fluorescence based detection where they have used a smartphone for image acquisition and processing. However, this work is reported for density-based cell sorting and subsequently fluorescence imaging for clinical assay. A new chemical method has been developed by Goyal et al.*(Ref: MM Goyal and A Basak. Estimation of plasma haemoglobin by a modified kinetic method using O-tolidine. Indian Journal of Clinical Biochemistry. 2009;24(1):36-41)* for colorimetric detection of plasma hemoglobin using o-tolidine.

Using optical absorbance, total Hgb detection from whole blood is reported in the US patent no. 6831733 B2. A disposable but not biodegradable polymeric transparent cuvette is used in which blood is filled up by the action of capillary force. The detection method is based on the light transmittance through the unlysed whole blood. A paper-based Hgb detection technique using spectrophotometer is discussed in the US patent no. 20120257188A. Sodium deoxycholate (SDOC) in PBS buffer treated paper was used to determine blood hemoglobin concentration. Blood color-based qualitative Hgb detection is mentioned in the patent number US703319. In this patent, blood was absorbed in paper and wait till its humid glossiness is gone. Then the natural blood color is compared to the color scale developed by the inventor. A light reflecting method is used to develop an Hgb detection device in the US patent no. US4057394. The device comprises of an opaque light reflecting matrix with a refracting index which is different from the refractive index of blood. Another invention in the patent no.US5773301 proposes Hgb detection using optical sensor by measuring with two different wavelengths. A capillary channel is used to hold the whole blood for determining total hemoglobin. US patent no. 20120257188A1 disclosed

estimation of blood hemoglobin on paper medium using spectrophotometric technique by measuring the light transmission at specified wavelength. The paper medium is chemically treated to lyse the blood prior to measure the

In another invention (US0192512A1), paper strip has been used to measure blood hemoglobin using reflectance spectroscopy. A large number of chemicals are used as a lysing agent of the RBC to extract the hemoglobin into the plasma solution. Yan et al. (US0257188A1) has used spectrophotometer for measurement of blood hem;oglobin on a chemically treated paper medium to lyse the blood sample.

However, most of these reported POC hemoglobin (Hgb) estimation devices are suffering from their inherent limitations like complex detection protocol, expensive disposable or non-disposable strips, provides qualitative result etc. As a result, most of these lab prototypes never reached to the market for commercialization. Thus there has been a need for developing improved cost effective and fast POC hemoglobin (Hgb) estimation system which would address the limitations of the existing POC hemoglobin (Hgb) estimation devices.

**OBJECT OF THE INVENTION:**

It is thus the basic object of the present invention is to develop a low cost point-of-care blood hemoglobin estimating system for non-invasive, fast and accurate detection of the blood hemoglobin level.

Another object of the present invention is to develop a low cost point-of-care blood hemoglobin estimating system which would be adapted to detect the blood hemoglobin level without using complex and costly spectroscopic instruments.

A still further object of the present invention is to develop a smartphone based point-of-care blood hemoglobin estimating system which would be adapted to detect the blood hemoglobin level from blood sample in real time.

Yet another object of the present invention is to develop a low cost point-of-care blood hemoglobin estimating system which would be easy to fabricate.

**SUMMARY OF THE INVENTION:**

Thus according to the basic aspect of the present invention there is provided a point of care rapid blood hemoglobin estimation system comprising

a paper based microfluidic strip to contain blood sample; and

a camera based detection unit for cooperatively disposing with respect to said paper based microfluidic strip to capture its image while containing the blood sample;

said camera based detection unit is configured to analyze the captured image in real time by using colorimetric analysis and instantly compute hemoglobin level in the blood sample by calibration technique.

In a preferred embodiment of the present point of care rapid blood hemoglobin estimation system, the paper based microfluidic strip comprises

a porous paper substrate with microfluidic fluid transport ability; and

atleast one reaction spot within said paper substrate having immobilized reagent for reaction with the blood sample.

In a preferred embodiment of the present point of care rapid blood hemoglobin estimation system, the paper substrate is coated with hydrophobic material;

said hydrophobic coating on the paper substrate is configured to guide the blood sample which is drop casted anywhere on the hydrophobic coating towards the reaction spot on the paper substrate.

In a preferred embodiment of the present point of care rapid blood hemoglobin estimation system, the paper based microfluidic strip includes said porous paper substrate preferably Whatman(R) filter paper with multiple reaction spots thereon, whereby outside of the reaction spots on the Whatman(R) filter paper is ink printed to acts as the hydrophobic coating and guide the blood sample flow toward anyone of the reaction spots.

In a preferred embodiment of the present point of care rapid blood hemoglobin estimation system, the camera based detection unit includes smartphone with camera and embedded application for analyzing the captured image of the paper based microfluidic strip with the reagent reacted blood sample on the reaction spot in real time by using colorimetric analysis including conversion of the captured image into grayscale version and thereby instantly computing the hemoglobin level or the concentration of the hemoglobin by calibration technique.

In a preferred embodiment, the present blood hemoglobin estimation system comprises an enclosure for cooperative integration of the paper based microfluidic strip with the smartphone based detection unit.

In a preferred embodiment of the present blood hemoglobin estimation system, the enclosure includes

a slot in its bottom wall for entry of the paper based microfluidic strip within the enclosure;

light source preferably LEDs are fitted in the enclosure to illuminate the paper based microfluidic strip;

enclosure top to accommodate the Smartphone ensuring the camera of the Smartphone appears before an imaging window defined in roof of the enclosure.

In a preferred embodiment of the present blood hemoglobin estimation system, the slot on the wall of the enclosure is contagious to the roof window of the enclosure such that the paper based microfluidic strip entered in enclosure through the wall slot is disposed below the roof window and image of the strip can be captured by the Smartphone camera.

The present blood hemoglobin estimation system is adapted to accurately estimate hemoglobin level within the range of 3.5 g/dL to 18 g/dL with sensitivity 0.1 g/dL.

**BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS:**

Fig. 1 shows paper based test strip used for hemoglobin detection involving the present system.

Fig. 2a, 2b and 2c shows a preferred embodiment of the present integrated system for colorimetric detection of the hemoglobin from the blood sample involving the paper based test strip.

Fig. 2d shows the step-wise hemoglobin detection flow chart with the present system for hemoglobin detection.

Fig. 3 shows calibration curve produced using standard cymethemoglobin sample.

Fig 4 shows clinical validation of POC colorimetric anemia test results from the present system with the results obtained from the pathological test.

**DETAILED DESCRIPTION OF THE INVENTION WITH REFERENCE TO THE ACCOMPANYING DRAWINGS:**

As stated hereinbefore, the present invention discloses a rapid, affordable, simple, portable non-invasive blood hemoglobin estimation system. The present system basically comprises a paper based microfluidic strip to contain blood sample and a smartphone based detection unit for real time colorimetric analysis of the blood sample contained in the paper based strip to determine the hemoglobin level in the contained blood sample.

Reference in this context invited from the accompanying figure 1 which shows paper based microfluidic strip. As shown in the figure 1, the paper based microfluidic strip includes highly porous paper substrate having good microfluidic fluid transport ability. The paper substrate includes atleast one reaction spot having a minute amount of reagent immobilized therein. This reagent preferably includes Glacial Acetic Acid, O-Tolidine, Triton x-100, Sodium Acetate, Hydrogen peroxide, Ethanol and DI Water mixed in a particular ratio to make a single solution.

The entire paper substrate excluding the reaction spot portion is coated hydrophobic material. The hydrophobic coating on the paper substrate guides the blood sample which is drop casted on the paper substrate towards the reaction spot.

In the present invention, Whatman(R) filter paper is used as the microfluidic strip as it has very high porosity and good quality fibres help in faster wicking and thus facilitating fast chemical reaction in the reaction spot between the blood sample and the immobilized reagent.

The hydrophobic coating on the Whatman(R) filter paper may be done by using wax, epoxy resin, titanium oxide etc. The paper based microfluidic strip embodiment as shown in the Fig. 1 includes the Whatman(R) filter paper (1) having multiple white reaction spots (2). The outside black area (3) of the Whatman(R) filter paper (1) is ink printed by an inkjet printer. This black area (3) is the hydrophobic coating which acts as barrier to guide the blood sample flow. The diameter of the reaction spot is 7mm.

Reference is next invited from the accompanying Fig. 2a, 2b and 2c which shows integration of the paper based microfluidic strip with the smartphone based detection unit. As shown in the referred figures, the above integration is done by rectangular enclosure (4). Wall of the enclosure (4) includes a slot at its bottom for entry of the paper based microfluidic strip within the enclosure (4). Light source preferably LEDs are fitted in the enclosure (4) to illuminate the paper based microfluidic strip. The Smartphone is placed on top of the enclosure (4) ensuring the camera of the Smartphone appears before an imaging window defined in roof of the enclosure (4). As shown in the above referred figures, the wall slot on the enclosure (4) is being contagious to the roof window of the enclosure (4) so that, the paper based microfluidic strip once entered in enclosure (4) through the wall slot it is disposed below the roof window and image of the strip can be captured by the Smartphone camera. The accompanying Fig. 2d shows the step-wise hemoglobin detection flow chart with the present system for hemoglobin detection.

The Smartphone used in the present system embodies a specific application for analyzing the captured image of the paper based microfluidic strip with the reagent reacted blood sample on the reaction spot in real time by using

colorimetric analysis. The application is configured to convert the captured image into grayscale version and thereby instantly compute the hemoglobin level or the concentration of the hemoglobin by calibration technique. The calibration curve for detection of the hemoglobin level is shown in the accompanying Fig. 3.

In regression statistical analysis, $R^2$ is the coefficient of determination signifies the correlation between dependent variable and independent variables. If $R^2$ is towards 1, then the relation between independent and dependent variable is very strong. In case of $R^2$ towards zero indicates there is a weak relation between the independent and dependent variables. In the present calibration curve $R^2$ is 0.9785 means strong correlation between hemoglobin concentrations with grayscale intensity.

The above Smartphone based detection of the hemoglobin level is capable enough to accurately estimate hemoglobin level within the range of 3.5 g/dL to 18 g/dL with sensitivity 0.1 g/dL.

The present system has also been validated with more than 70 blood samples collected from different patients covering a wide spectrum of hemoglobin data and subsequently, compared with standard pathological results measured using a hematology analyzer. The present system estimates are correlated with the pathological gold standard estimates of Hgb levels ($r = 0.909$), and the present system test method yielded similar sensitivity and specificity for detecting mild anemia ($n = 19$) (<11 g/dl) (sensitivity: 94.7 %, specificity: 100 %) and for severe anemia ($n = 6$) (<7 g/dl) (sensitivity: 100 %, specificity: 100 %). The estimated Hgb levels are, within 1.0 g/dl from the pathological estimate, for 95 % of the blood samples. Results demonstrate the elevated efficacy and viability of this POC colorimetric diagnostic test, in comparison to the state-of-the-art complex and expensive diagnostic tests for anemia detection.

It is thus estimable Hgb levels of the present system is well covering the entire range of high anemic to healthy condition. The advantageous aspects of the system of the present invention can be summarized as hereunder:

i. The present system can reproduce gold standard pathological results from a very simple detection protocol with a minute amount of reagent and ultra low-cost paper strips;

ii. The detection process of the present system is almost instrument free, requires only a Smartphone;

iii. The system does not require continuous power source. The LED of 3D enclosure can run for longer duration with a rechargeable pencil battery;

iv. The smartphone application is very simple to operate. Therefore no skilled or semi-skilled personnel is required to test;

v. The fabrication procedure of paper strip is very simple only to take print on Whatman filter paper followed by heating on the hot plate. Therefore, the fabrication cost is reducing significantly.

**CLAIMS:**

1. A point of care rapid blood hemoglobin estimation system comprising

a paper based microfluidic strip to contain blood sample; and

a camera based detection unit for cooperatively disposing with respect to said paper based microfluidic strip to capture its image while containing the blood sample;

said camera based detection unit is configured to analyze the captured image in real time by using colorimetric analysis and instantly compute hemoglobin level in the blood sample by calibration technique.

2. The point of care rapid blood hemoglobin estimation system as claimed in claim 1, wherein the paper based microfluidic strip comprises

a porous paper substrate with microfluidic fluid transport ability; and

atleast one reaction spot within said paper substrate having immobilized reagent for reaction with the blood sample.

3. The point of care rapid blood hemoglobin estimation system as claimed in claim 1 or 2, wherein the paper substrate is coated with hydrophobic material;

said hydrophobic coating on the paper substrate is configured to guide the blood sample which is drop casted anywhere on the hydrophobic coating towards the reaction spot on the paper substrate.

4. The point of care rapid blood hemoglobin estimation system as claimed in anyone of claims 1 to 3, wherein the paper based microfluidic strip includes said porous paper substrate preferably Whatman(R) filter paper with multiple reaction spots thereon, whereby outside of the reaction spots on the Whatman(R) filter paper is ink printed to acts as the hydrophobic coating and guide the blood sample flow toward anyone of the reaction spots.

5. The point of care rapid blood hemoglobin estimation system as claimed in anyone of claims 1 to 4, wherein the camera based detection unit includes smartphone with camera and embedded application for analyzing the captured image of the paper based microfluidic strip with the reagent reacted blood sample on the reaction spot in real time by using colorimetric analysis including conversion of the captured image into grayscale version and thereby instantly computing the hemoglobin level or the concentration of the hemoglobin by calibration technique.

6. The blood hemoglobin estimation system as claimed in anyone of claims 1 to 5 comprises an enclosure for cooperative integration of the paper based microfluidic strip with the smartphone based detection unit.

7. The blood hemoglobin estimation system as claimed in anyone of claims 1 to 6, wherein the enclosure includes

a slot in its bottom wall for entry of the paper based microfluidic strip within the enclosure;

light source preferably LEDs are fitted in the enclosure to illuminate the paper based microfluidic strip;

enclosure top to accommodate the Smartphone ensuring the camera of the Smartphone appears before an imaging window defined in roof of the enclosure.

8. The blood hemoglobin estimation system as claimed in anyone of claims 1 to 7, wherein the slot on the wall of the enclosure is contagious to the roof window of the enclosure such that the paper based microfluidic strip entered in enclosure through the wall slot is disposed below the roof window and image of the strip can be captured by the Smartphone camera.

9. The blood hemoglobin estimation system as claimed in anyone of claims 1 to 8, is adapted to accurately estimate hemoglobin level within the range of 3.5 g/dL to 18 g/dL with sensitivity 0.1 g/dL.

AMENDED CLAIMS
received by the International Bureau on 26 December 2020 (26. 12. 2020)

1. A point of care rapid blood hemoglobin estimation system comprising

an enclosure for cooperative integration of (i) a paper based microfluidic strip including atleast one reaction spot having immobilized reagent for reaction with blood sample within a porous paper based microfluidic strip with (ii) a smartphone based detection unit;

said enclosure including:

a wall of said enclosure having a slot in its bottom for entry of said paper based microfluidic strip within said enclosure;

light source including LEDs fitted in the enclosure to illuminate the paper based microfluidic strip when within the enclosure;

said enclosure having a roof providing an imaging window and accommodating said smartphone based detection unit including smartphone camera and embedded application ensuring that the camera of the Smartphone appears before said imaging window defined in said roof of the enclosure.

said slot in the wall of the enclosure being contagious to the said roof window of the enclosure such that any said paper based microfluidic strip entered in enclosure through said slot is disposed below the roof window and image of the strip can be captured by the said Smartphone camera;

said smartphone based detection unit including said smartphone camera and embedded application enabling analyzing the captured image of the paper based microfluidic strip with the reagent reacted blood sample on the reaction spot in real time by using colorimetric analysis including conversion of the captured image into grayscale version and thereby instantly computing the hemoglobin

level or the concentration of the hemoglobin by calibration technique involving correlation between hemoglobin concentrations with said grayscale intensity.

2. The point of care rapid blood hemoglobin estimation system as claimed in claim 1, wherein the paper substrate is coated with hydrophobic material;

said hydrophobic coating on the paper substrate is configured to guide the blood sample which is drop casted anywhere on the hydrophobic coating towards the reaction spot on the paper substrate.

3. The point of care rapid blood hemoglobin estimation system as claimed in claim 1 or 2, wherein the paper based microfluidic strip includes said porous paper substrate preferably Whatman(R) filter paper with multiple reaction spots thereon, whereby outside of the reaction spots on the Whatman(R) filter paper is ink printed to acts as the hydrophobic coating and guide the blood sample flow toward anyone of the reaction spots.

4. The blood hemoglobin estimation system as claimed in anyone of claims 1 to 3, is adapted to accurately estimate hemoglobin level within the range of 3.5 g/dL to 18 g/dL with sensitivity 0.1 g/dL.

# STATEMENT UNDER ARTICLE 19 (1)

Amended claims are directed to further clarify and qualify inventive aspects of the present invention residing in the development of the point of care rapid blood hemoglobin estimation system. The point of care rapid blood hemoglobin estimation system includes an enclosure which integrates (i) a paper based microfluidic strip having atleast one reaction spot having immobilized reagent for reaction with blood sample within a porous paper based microfluidic strip with (ii) a smartphone based detection unit for capturing the image of the paper based microfluidic strip by the smartphone camera and analyzing the captured image of the paper based microfluidic strip with the reagent reacted blood sample on the reaction spot in real time by using colorimetric analysis executed by the smartphone application. The enclosure includes a wall having a slot in its bottom for entry of the paper based microfluidic strip within the enclosure, a light source including LEDs fitted in the enclosure to illuminate the paper based microfluidic strip when within the enclosure. The enclosure roof includes an imaging window and accommodates the smartphone including smartphone camera ensuring that the camera of the Smartphone appears before the imaging window defined in the roof. The slot in the wall of the enclosure is contagious to the roof window of the enclosure such that any said paper based microfluidic strip entered in enclosure through said slot is disposed below the roof window and image of the strip can be captured by the smartphone camera for analyzing by using colorimetric analysis including conversion of the captured image into grayscale version and thereby instantly computing the hemoglobin level or the concentration of the hemoglobin by calibration technique involving correlation between hemoglobin concentrations with said grayscale intensity.

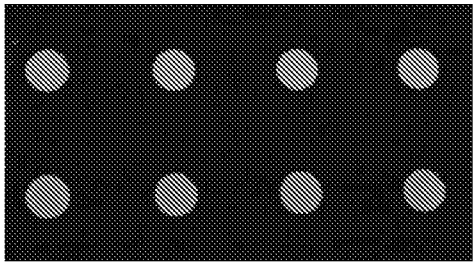

Fig. 1

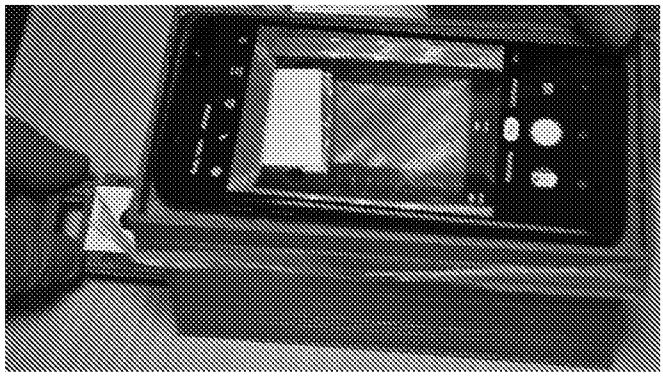

Fig. 2a

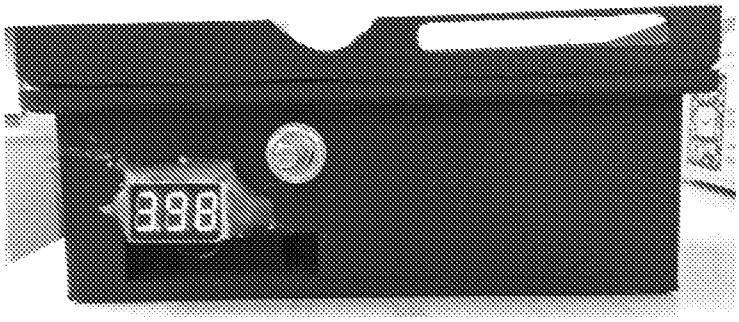


Fig. 2b

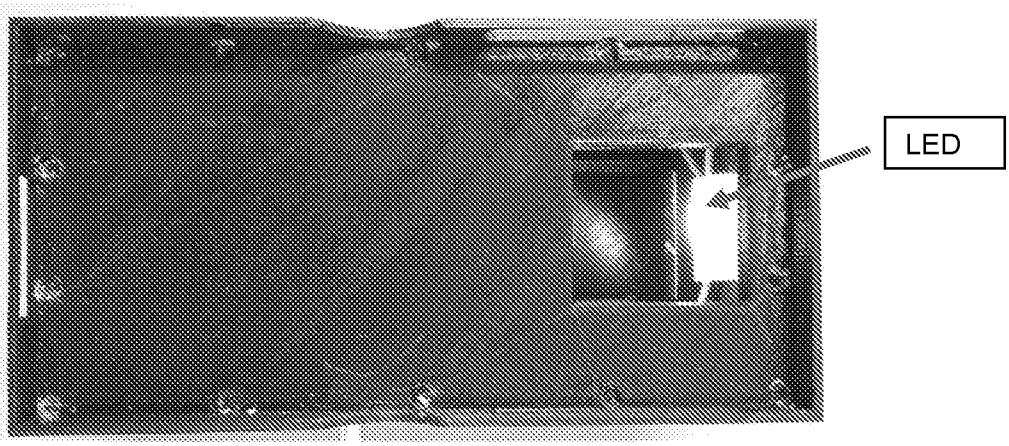
LED

Fig. 2c

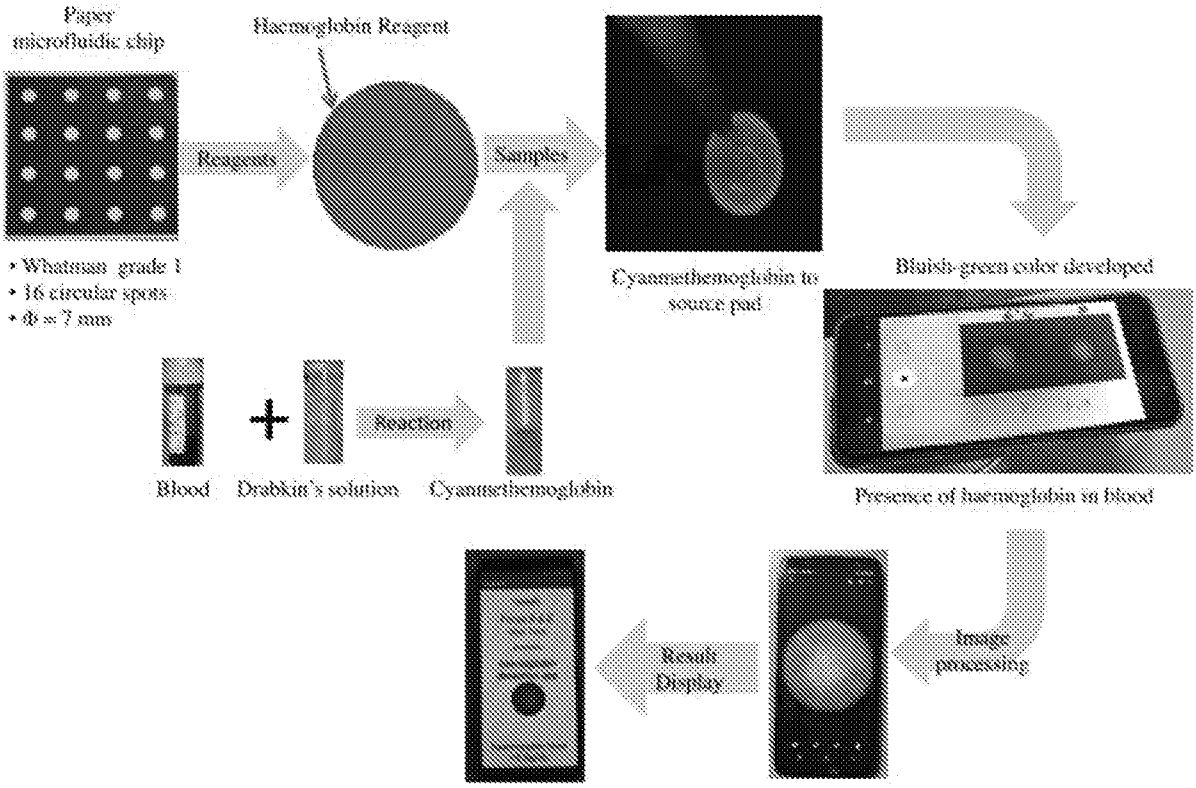
Paper microfluidic chip
Haemoglobin Reagent
Reagents
Samples
• Whatman grade 1
• 16 circular spots
• Φ = 7 mm
Cyanmethemoglobin to source pad
Bluish-green color developed
Blood
Drabkin's solution
Reaction
Cyanmethemoglobin
Presence of haemoglobin in blood
Image processing
Result Display

Fig. 2d

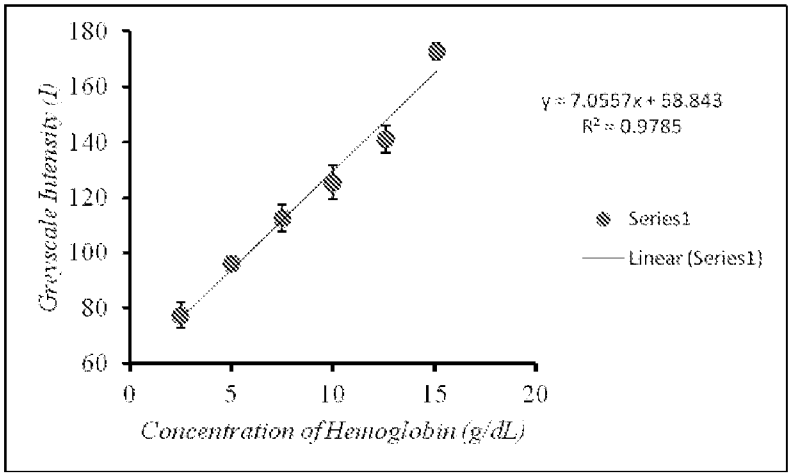
180
160
140
120
100
80
60
0
5
10
15
20
Greyscale Intensity (I)
Concentration of Hemoglobin (g/dL)
y = 7.0557x + 58.843
R² = 0.9785
Series1
Linear (Series1)

Fig. 3

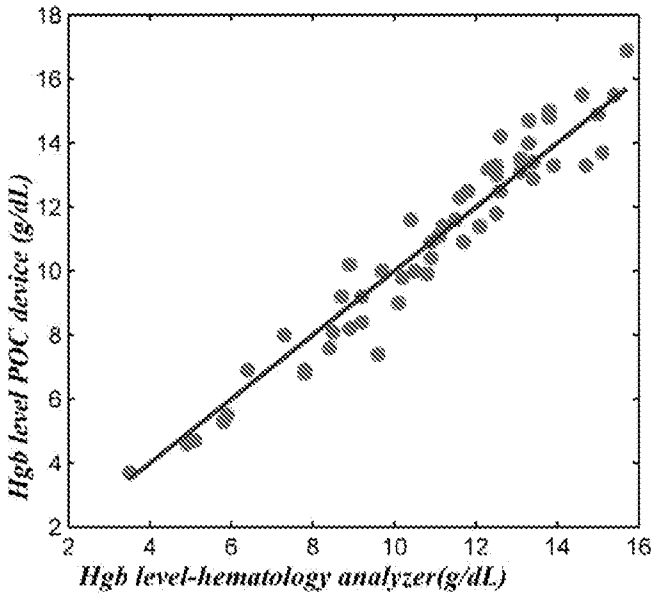
18
16
14
12
10
8
6
4
2
2
4
6
8
10
12
14
16
Hgb level POC device (g/dL)
Hgb level-hematology analyzer(g/dL)

Fig. 4

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2020/050617

A. CLASSIFICATION OF SUBJECT MATTER
G01N21/25,G01N33/487,G01N33/49,G01N33/72 Version=2020.01

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)

G01N

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

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

DATABASES: TotalPatent One, IPO Internal Database, WIPO PATENTSCOPE
KEYWORDS: BLOOD SAMPLE, MICROFLUIDIC, CAMERA, HEMOGLOBIN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US20170023556A1 (ADMINISTRATORS OF THE TULANE EDUCATION FUND) 26th January 2017 (26-01-2017) Abstract,,paragraps[0009],[0022],[0032],[0076], Figs. 2A and 6. | 1-9 |
| A | US10175162B2 (XEROX CORP) 8th January 2019 (08-01-2019) The whole document | 1-9 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"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 | Date of mailing of the international search report |
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| 10-11-2020 | 10-11-2020 |
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Form PCT/ISA/210 (second sheet) (July 2019)

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Information on patent family members

International application No.
PCT/IN2020/050617

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