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(71) Applicant: **SIEMENS HEALTHCARE DIAGNOSTICS INC.** [US/US]; 511 Benedict Avenue, Tarrytown, New York 10591 (US).

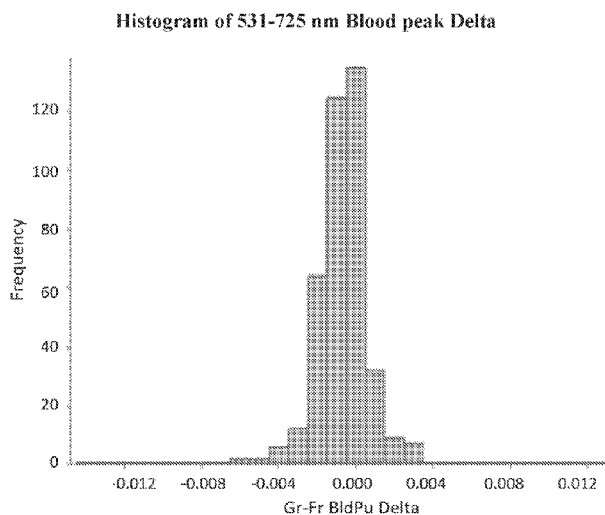
(72) Inventors: **KAUFFMANN, Aaron**; 221 Jefferson St., Elkhart, Indiana 46516 (US). **ZIMMERLE, Chris**; 58613 Glenriver Drive, Goshen, Indiana 46528 (US).

(74) Agent: **STEIN, Kevin** et al.; SIEMENS CORPORATION, Intellectual Property Dept., 3501 Quadrangle Blvd Ste 230, Orlando, Florida 32817 (US).

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(54) Title: METHODS OF BLOOD SAMPLE SUSPENSION



FR: Far Red = 710 to 850 nm wavelength. 725 nm was specifically used herein.
GR: Green = 495 to 570 nm wavelength. 531 nm was specifically used herein.

Figure 1

(57) Abstract: Disclosed herein are methods for rapid detection of suspension of a blood sample from a subject and methods for improving the processing time of a point of care blood analysis. Specifically, the disclosed methods provide a rapid detection of hemoglobin concentration, more specifically glycated hemoglobin A1c, and determination of sugar concentration in the blood sample from the subject. Further disclosed herein are methods for rapidly diagnosing diabetes in a subject.



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METHODS OF BLOOD SAMPLE SUSPENSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application number 62/801,927, filed February 6, 2019, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] Disclosed herein are methods for detection of suspension of a blood sample from a subject. Specifically, the disclosed methods provide a detection of hemoglobin concentration, more specifically glycated hemoglobin A1c, and determination of sugar concentration in the blood sample from the subject.

BACKGROUND

[0003] Hemoglobin present in red blood cells can be glycated by the non-enzymatic addition of a glucose molecule to the amino terminus of the β -chain of the hemoglobin. Once a hemoglobin molecule is glycated, it remains glycated, and an accumulation of glycated hemoglobin within a red cell reflects the average level of glucose to which the cell has been exposed during its life cycle. The level of glycated hemoglobin present in an individual's blood is thus proportional to the level of glucose in the blood and is an indicator of the individual's mean daily blood glucose concentration over the previous four weeks to three months.

[0004] Specifically, hemoglobin A1c (HbA1c) reflects the mean blood glucose concentration and is used as a risk parameter for diabetic complications and as a quality assurance indicator for the quality of diabetes care. Point-of-care (POC) instruments for HbA1c are widely used for the measurement of HbA1c. These instruments assist health care providers with decision-making for diagnosing, treating, and managing patient care and the swiftness for obtaining results minimize the delay associated with conventional laboratory measures and reduce the need for additional office visits to implement clinical decisions.

[0005] In general, a blood sample is mixed for several rounds before getting pulled out of the instrument's capillary. Typically, this process is relatively fast (less than 10 minutes) but under high hematocrit and long incubation times in the capillary, considerable mixing time is required. The current POC instruments mix more than necessary for most samples to compensate for the cases where a high amount of mixing is required. Rapid results

can increase clinical effectiveness and contribute to improved outcomes for patients, but it is imperative that the results provided by the device are accurate and reliable as faster results are only safe if they are accurate.

[0006] There is a need in the art for improved POC instruments for blood sample analysis that provide quick and reliable results which would benefit both the patients and health care providers. This invention addresses this need.

SUMMARY

[0007] Disclosed herein are methods for detecting and analyzing a blood sample.

[0008] In one aspect, provided herein are methods for performing a blood sugar assay. The methods comprise:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
- (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
- (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;
- (f) determining the absolute value (V_n) of the differential between $dhemo_{xn}$ and $dhemo_{yn}$;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint n + 1, wherein the timepoint n of step (c) becomes equal to n + 1, to generate $dhemo_{xn+1}$ and $dhemo_{yn+1}$;
- (h) determining the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$, wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$;
- (j) determining the absolute value (V_{n+1}) of the differential between $dhemo_{xn+1}$ and $dhemo_{yn+1}$;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N , wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is

- about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
- b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
- ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
 - (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
 - (m) assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and,
 - (n) providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0009] In some embodiments, the methods comprise analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration; assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and, providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0010] Methods for diagnosing diabetes in a subject also are provided. The methods comprise:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
- (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
- (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;

- (f) determining the absolute value (V_n) of the differential between dhemO_{xn} and dhemO_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemO_{xn+1} and dhemO_{yn+1} .
- (h) determining the differential between dhemO_{yn} and dhemO_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemO_{yn} and dhemO_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemO_{xn+1} and dhemO_{yn+1}
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

[0011] In some embodiments of the disclosed methods for diagnosing diabetes in a subject, the further analysis of the blood sample comprises the additional steps of : (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration; (m) determining the glucose concentration in the blood sample from the subject based upon the glycated hemoglobin concentration so detected; (n) providing an output result comprising the glycated hemoglobin concentration and the glucose

concentration in the blood sample from the subject; (o) diagnosing the subject with diabetes when the glucose concentration in the blood sample is above a reference concentration; and, (p) when diagnosed with diabetes, recommending a treatment of the subject, wherein the treatment comprises diet, exercise and/or medication. In some embodiments, the steps (a) to (o) of blood sample processing and the diagnosing of the subject are performed in less than 5 minutes. In other embodiments, the steps (a) to (o) of blood sample processing and the diagnosing of the subject are performed in about 3.5 minutes. Also provided herein are methods for improving the processing time of a point of care blood analysis. The methods comprise:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
- (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
- (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;
- (f) determining the absolute value (V_n) of the differential between $dhemo_{xn}$ and $dhemo_{yn}$;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate $dhemo_{xn+1}$ and $dhemo_{yn+1}$.
- (h) determining the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$, wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$;
- (j) determining the absolute value (V_{n+1}) of the differential between $dhemo_{xn+1}$ and $dhemo_{yn+1}$;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N , wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or

- b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
- (l) analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration; and,
- (m) providing in less than 5 min an output result comprising the hemoglobin concentration thereby improving the point of care blood analysis as compared to a standard point of care blood analysis.

[0012] In some embodiments of the various disclosed methods, the step of mixing to generate the hemolysate mixture comprises sonicating the blood sample and/or treating the blood sample with a lysis buffer. In some embodiments, the buffer comprises lithium thiocyanate, digitonin and/or potassium ferricyanide. In some embodiments, the buffer may comprise an anticoagulant selected from the group consisting of EDTA, heparin, citrate and fluoride/oxalate.

[0013] In some embodiments, the blood sample may be in a vessel such as, but not limited to, a capillary tube, a capillary plate or a capillary membrane.

[0014] In some embodiments, the wavelength x is within the range of at least one set of wavelengths selected from the group consisting of: (a) 380 nm to 455 nm; (b) 455 nm to 580 nm; and (c) 580 nm to 680 nm.

[0015] In some embodiments, the wavelength y is within the range of 700 nm to 2500 nm. In yet other embodiments, the wavelengths x and y correspond respectively to 531 nm and 725 nm.

[0016] In some embodiments of the disclosed methods, step (c) is performed at least 2 seconds after step (b).

[0017] In some embodiments, if the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample is replaced by another blood sample.

[0018] In some embodiments, the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.

[0019] According to some embodiments, the hemolysate is at least partially suspended.

[0020] In some embodiments, the blood sample is a human blood sample.

[0021] In further embodiments, the absolute value of (i) is about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$. In yet further embodiments, the absolute value of $(V_{n+1})-(V_n)$ is about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.

[0022] According to some embodiments, the hemoglobin or glycated hemoglobin concentration is at least about one selected from the group consisting of: 5 g/dL and 7g/dL.

[0023] In some embodiments, the glycated hemoglobin is hemoglobin A1c (HbA1c).

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Fig. 1 is a histogram depicting the distribution of blood absorbance between readings. An experimental dataset was collected with whole blood (14 g/dL, about 4.7% HbA1c). From this dataset the normal distribution of absorbance (531-725 nm) between Blood Pickup readings was obtained. The mean and standard deviation of the expected values were used for true/false criteria 3. FR: Far Red = 710 to 850 nm wavelength. 725 nm was specifically used herein; GR: Green = 495 to 570 nm wavelength. 531 nm was specifically used herein.

[0025] Fig. 2 is a histogram based on the data of Fig. 1: The same dataset from Figure 1 was used for determining the mean and standard deviation for true/false criteria 2.

[0026] Fig. 3 is a graph illustrating the percentage of bias with time saved.

[0027] Figs. 4A-4B are series of tables demonstrating how the data is processed with the presently disclosed methods. Fig. 4A is a table listing an exemplary dataset for given blood sample. Fig. 4B. is a table illustrating the various data processing steps for rapid analysis of a blood sample.

[0028] Fig. 5 is a diagram depicting the main steps of the methods disclosed herein. Mixing is performed to extract blood sample from capillary. Hemoglobin (Hb) is measured to determine if enough blood sample has been extracted. If yes, the assay can proceed without additional mixing. If no, the mixing operation and the absorbance measuring steps are repeated.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0029] The disclosed methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods are not limited to the specific methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods.

[0030] It is to be appreciated that certain features of the disclosed methods which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

Definitions

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

[0032] It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0033] As used herein, the singular forms “a,” “an,” and “the” include the plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise.

[0034] As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. As used herein when referring to a measurable value such as an amount, a concentration, a temporal duration, and the like, the term “about” is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[0035] As used herein, the terms “comprising,” “including,” “containing” and “characterized by” are exchangeable, inclusive, open-ended and do not exclude additional, unrecited elements or method steps. Any recitation herein of the term “comprising,” particularly in a description of components of a composition or in a description of elements

of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements.

[0036] As used herein, the term “consisting of” excludes any element, step, or ingredient not specified in the claim element.

[0037] By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

[0038] An “individual”, “patient” or “subject”, as these terms are used interchangeably herein, includes a member of any animal species including, but are not limited to, birds, humans and other primates, and other mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs. Preferably, the subject is a human.

[0039] As used herein, the terms “treatment” and “treating” refer to an approach for obtaining beneficial or desired results including, but not limited to, therapeutic benefit and/or a prophylactic benefit.

[0040] For example, the term treatment includes the administration of an agent prior to or following the onset of a disease or disorder thereby preventing or removing all signs of the disease or disorder. As another example, administration of the agent after clinical manifestation of the disease to combat the symptoms of the disease comprises “treatment” of the disease. This includes prevention of a disease such as diabetes.

[0041] By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying disorder. For prophylactic benefit, the compositions may be administered to a patient at risk of developing a particular disease, or to a patient reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made.

[0042] Throughout this disclosure, various aspects of the invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range and, when appropriate, partial integers of the numerical values within ranges. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4,

from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0043] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

Methods

[0044] Provided herein are methods for performing a blood sugar assay, for detecting suspension of a blood sample, and methods for improving the processing time of a point of care blood analysis. The methods comprise the following steps:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .
- (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein yn and $yn+1$ relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemo_{xn+1} and dhemo_{yn+1} ;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N , wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is

- about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
- b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
- ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
- (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
- (m) assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and,
- (n) providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0045] In some embodiments, each blood pickup read is compared to the prior blood pickup read. For instance, $dhemo_{yn}$ from a given timepoint $t1$ is compared to $dhemo_{yn+1}$ from a consecutive timepoint $t2$.

[0046] In some embodiments, the methods comprise analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration; assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected and, providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0047] In the disclosed methods for improving the processing time of a point of care blood analysis, these methods comprise analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration; and, providing in less than 5 min an output result comprising the hemoglobin concentration thereby improving the point of care blood analysis as compared to a standard point of care blood analysis.

[0048] In some embodiments, step (c) is performed at least 2 seconds after step (b).

[0049] If the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample may be replaced by another blood sample.

[0050] In some embodiments, the steps (c) to (e) are repeated about 60, about 50, about 40, about 30, about 20, about 10, about 5 times or less. In some embodiments, the timepoints n range from n equals 1 to 29.

[0051] In some embodiments, the time t is about 30 seconds, about 40 seconds, about 50 seconds, about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds, about 120 seconds, about 130 seconds, about 140 seconds, about 150 seconds, about 160 seconds, about 170 seconds, about 180 seconds, about 190 seconds, about 200 seconds or more. In some embodiments, the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.

[0052] In some embodiments, the absolute value of step (i) and/or $(V_{n+1})-(V_n)$ is any absolute value that ranges: between 0 and 0.006; between 0 and 0.0055; between 0 and 0.005; between 0 and 0.0045; between 0 and 0.004; between 0 and 0.0035; between 0 and 0.003; between 0 and 0.0025; between 0 and 0.002; between 0 and 0.0015; between 0 and 0.001; between 0 and 0.0005; or between 0 and 0.0001. For example, the absolute value in step (i) may be about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$ and/or the absolute value in step (j) may be about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.

[0053] In some embodiments, the analysis is performed in less than 5 minutes.

[0054] Further provided herein are methods for detection of a glycated hemoglobin in a blood sample and methods for performing a blood sugar assay. These methods comprise similar steps as the methods and embodiments listed herein above. Specifically, such methods comprise the following steps:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
- (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
- (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;
- (f) determining the absolute value (V_n) of the differential between $dhemo_{xn}$ and $dhemo_{yn}$;

- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemox_{n+1} and dhemoy_{n+1} .
- (h) determining the differential between dhemoy_n and dhemoy_{n+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemoy_n and dhemoy_{n+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemox_{n+1} and dhemoy_{n+1}
- (k) assessing the absolute values V_n , $(V_{n+1}) - (V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1}) - (V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1}) - (V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

[0055] In addition to steps (a) through (k), the disclosed methods for performing a blood sugar assay comprise analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration; assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and, providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0056] In some embodiments, step (c) is performed at least 2 seconds after step (b).

[0057] If the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample may be replaced by another blood sample.

[0058] In some embodiments, the steps (c) to (e) are repeated about 60, about 50, about 40, about 30, about 20, about 10, about 5 times or less. In some embodiments, the timepoints n range from n equals 1 to 29.

[0059] In some embodiments, the time t is about 30 seconds, about 40 seconds, about 50 seconds, about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds, about 120 seconds, about 130 seconds, about 140 seconds, about 150 seconds, about 160 seconds, about 170 seconds, about 180 seconds, about 190 seconds, about 200 seconds or more. In some embodiments, the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.

[0060] In some embodiments, the absolute value of step (i) and/or $(V_{n+1})-(V_n)$ is any absolute value that ranges: between 0 and 0.006; between 0 and 0.0055; between 0 and 0.005; between 0 and 0.0045; between 0 and 0.004; between 0 and 0.0035; between 0 and 0.003; between 0 and 0.0025; between 0 and 0.002; between 0 and 0.0015; between 0 and 0.001; between 0 and 0.0005; or between 0 and 0.0001. For example, the absolute value in step (i) may be about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$ and/or the absolute value in $(V_{n+1})-(V_n)$ may be about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.

[0061] Further provided herein are methods for diagnosing diabetes in a subject. Such methods comprise similar steps and embodiments as the methods described above herein. Specifically, such methods comprise the following steps:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .

- (h) determining the differential between dhemO_{y_n} and $\text{dhemO}_{y_{n+1}}$, wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemO_{y_n} and $\text{dhemO}_{y_{n+1}}$;
- (j) determining the absolute value (V_{n+1}) of the differential between $\text{dhemO}_{x_{n+1}}$ and $\text{dhemO}_{y_{n+1}}$
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N , wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

[0062] In some embodiments, step (c) is performed at least 2 seconds after step (b).

[0063] If the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample may be replaced by another blood sample.

[0064] In some embodiments, the step (g) is repeated about 60, about 50, about 40, about 30, about 20, about 10, about 5 times or less. In some embodiments, the timepoints n range from n equals 1 to 29.

[0065] In some embodiments, the time t is about 30 seconds, about 40 seconds, about 50 seconds, about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds, about 120 seconds, about 130 seconds, about 140

seconds, about 150 seconds, about 160 seconds, about 170 seconds, about 180 seconds, about 190 seconds, about 200 seconds or more. In some embodiments, the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.

[0066] In some embodiments, the absolute value of step (i) and/or $(V_{n+1})-(V_n)$ is any absolute value that ranges: between 0 and 0.006; between 0 and 0.0055; between 0 and 0.005; between 0 and 0.0045; between 0 and 0.004; between 0 and 0.0035; between 0 and 0.003; between 0 and 0.0025; between 0 and 0.002; between 0 and 0.0015; between 0 and 0.001; between 0 and 0.0005; or between 0 and 0.0001. For example, the absolute value in step (i) may be about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$ and/or the absolute value in $(V_{n+1})-(V_n)$ may be about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.

[0067] Additionally, in some embodiments of the disclosed methods for diagnosing diabetes in a subject, the further analysis of the blood sample comprises: analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration; determining the glucose concentration in the blood sample from the subject based upon the glycated hemoglobin concentration so detected; diagnosing the subject with diabetes when the glucose concentration in the blood sample is above a reference concentration; and when diagnosed with diabetes, recommending a treatment of the subject, wherein the treatment comprises diet, exercise and/or medication. In some embodiments, the steps (a) to (o) of the blood sample processing and the diagnosing of the subject are performed in less than 5 minutes. In other embodiments, the steps (a) to (o) of the blood sample processing and the diagnosing of the subject are performed in about 3.5 minutes. In some embodiments, by following the methods provided herein, the time necessary for processing the blood sample, determining the glucose concentration in the blood sample and diagnosing the subject is shortened as compared to other methods currently known and used by a skilled artisan, such as but not limited to the In2it™ analyzer (Bio-Rad) or the DCA Vantage® analyzer (Siemens Healthineers). Methods known in the art for measuring blood sugar (glucose) concentration also include methods based on an immunoassay, high performance liquid chromatography (HPLC) and borate affinity chromatography.

[0068] It is known in the art that when blood sugar concentration rises, a glucose moiety conjugates with hemoglobin. This hemoglobin-glucose conjugate is referred to as glycated hemoglobin. There are three different forms of glycated hemoglobin: HbA1a, HbA1b and HbA1c. HbA1c is the most common form and is the one mainly used in blood

assays. For a given subject, the glycated hemoglobin value is in equilibrium with a value of blood sugar over the past two to three months. HbA1c provides valuable information for evaluating average blood glucose levels over a period of two to three months and has particular clinical utility because its value is minimally affected by short, large fluctuations in blood glucose concentration. The glycated hemoglobin value is checked and used as an index for glycemia and/or glycosuria management.

[0069] The glycated hemoglobin has a structure in which the glucose is conjugated to the N-terminal valine in the β -chain hemoglobin in the blood, and only a glucose moiety must be specifically detected and measured to distinguish it from the hemoglobin. To quantitatively measure the glycated hemoglobin value, a fraction of an amount of glycated hemoglobin on the basis of an amount of total hemoglobin is given. In some embodiments, the hemoglobin comprises hemoglobin A, hemoglobin A1c, hemoglobin C, hemoglobin E, hemoglobin D, hemoglobin F, and hemoglobin S, or any combination thereof. In other embodiments, the hemoglobin is a glycated hemoglobin. In further embodiments, the glycated hemoglobin is hemoglobin A1c (HbA1c).

[0070] The amount of total hemoglobin generally has a range of 13 to 17g/dL for adult men, a range of 12 to 15g/dL for adult women, and a range of 11 to 14g/dL for infants. When the glycated hemoglobin value is 6.0%, it may be expressed as about 12g/dL on average for a two to three-month blood sugar. The American Diabetes Association (ADA) recommends that the glycated hemoglobin value be maintained to 6.5% or less, and a normal range thereof is 4.0 to 5.7%. In some embodiments, the hemoglobin or glycated hemoglobin concentration is about at least 7g/dL. In other embodiments, the hemoglobin concentration ranges from about 7g/dL to about 21 g/dL. In yet other embodiments, the absolute value of step (f), as described in the methods above herein, indicates a hemoglobin or glycated hemoglobin concentration, at ± 0.1 g/dL, of at least one selected from the group consisting of: 1g/dL; 1.5 g/dL; 2g/dL; 2.5 g/dL; 3 g/dL; 3.5 g/dL; 4g/dL; 4.5 g/dL; 5 g/dL; 5.5 g/dL; 6g/dL; 6.5 g/dL; 7 g/dL; 7.5 g/dL; 8g/dL; 8.5 g/dL; 9 g/dL; 9.5 g/dL; 10g/dL; 10.5 g/dL; 11 g/dL; 11.5 g/dL; 12g/dL; 12.5 g/dL; 13 g/dL; 13.5 g/dL; 14g/dL; 14.5 g/dL; 15 g/dL; 15.5 g/dL; 16 g/dL; 16.5 g/dL; 17g/dL; 17.5 g/dL; 18 g/dL; 18.5 g/dL; 19g/dL; 19.5 g/dL; 20 g/dL; 20.5 g/dL; 21g/dL; 21.5 g/dL; 22 g/dL; 22.5 g/dL; 23g/dL; 23.5 g/dL; 24 g/dL; 24.5 g/dL; 25g/dL; 25.5 g/dL; 26 g/dL; 26.5 g/dL; 27g/dL; 27.5 g/dL; 28g/dL; 28.5 g/dL; 29 g/dL; 29.5 g/dL; 30g/dL; 31.5 g/dL; 32 g/dL; 32.5 g/dL; 33g/dL; 33.5 g/dL; 34 g/dL; 3.5 g/dL; 35g/dL; and more than 35g/dL. In yet other embodiments, the absolute value of step (f)

indicates a hemoglobin or glycated hemoglobin concentration of at least about 5 g/dL +/- 0.1 g/dL.

[0071] The amount of hemoglobin can be measured using a light reflective characteristic. A hemoglobin measuring instrument preferably measures the amount of hemoglobin using a light emitting device such as a light emitting diode and a light receiving device such as a photodiode. For instance, the measurement of the hemoglobin value (e.g. HbA1c value) can be performed by using a spectrophotometer that measures the absorbance with a wavelength corresponding to hemoglobin. This measurement can be done at different absorbances (referred in some instances herein as hemo_x and/or hemo_y). In some embodiments, the wavelength x is within the range of at least one set of wavelengths selected from the group consisting of: (a) 380 nm to 455 nm; (b) 455 nm to 580 nm; and (c) 580 nm to 680 nm. An example of the wavelength corresponding to HbA1c is a wavelength in a range of 415 to 430 nm. In other embodiments, the wavelength y is within the range of 700 nm to 2500 nm. In yet other embodiments, the wavelengths x and y correspond respectively to 531 nm and 725 nm.

[0072] The measurement of glycated hemoglobin requires determining a background or a baseline value. In some embodiments, this value is computed based upon the absorbance value of the buffer that is mixed with the blood sample. The measurement of the buffer's absorbance in the absence of blood is done at similar wavelengths as the ones used for hemoglobin (referred in some instances herein as buffer_x and/or buffer_y).

[0073] In some embodiments, the blood sample is in a vessel, such as but not limited to, a capillary tube, a capillary plate or a capillary membrane, collectively referred to herein as a "capillary".

[0074] The blood sample may be a human blood sample. The blood sample may also be from other primates, other vertebrates and other mammals including, but not limited to, commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

[0075] In some embodiments of the various disclosed methods, the step of mixing to generate the hemolysate mixture comprises sonicating the blood sample and/or treating the blood sample with a lysis buffer. There is no particular limitation required for generating a hemolysate and treating the blood sample for the described methods. Various methods known in the art could be used for treating a blood sample. In some instances, the blood sample is treated under mildly acidic conditions with a pH between 3 to 6.9. Examples of buffers used under these conditions include aliphatic carboxylate buffers or borate buffers. Examples of aliphatic carboxylate buffers include monocarboxylate and dicarboxylate buffers, and more

specifically, succinate, malonate, fumarate, maleate, glutarate or acetate buffers. In some embodiments, the buffer comprises lithium thiocyanate, digitonin and/or potassium ferricyanide. The buffer used in the blood assay for the described methods may include, for example, about 8.1% w/v lithium thiocyanate and about 0.01% digitonin in 200 mM glycine buffer. Variations in the concentrations and/or buffer components can be implemented by a skilled artisan such as the use of alternates to glycine. These alternates include, but are not limited to, Bis-Tris Propane, Trizma, Bicine, HEPBS, TAPS, AMPD, TABS, AMPSO, CHES, CAPSO, and AMP. The buffer may also comprise an anticoagulant selected from the group consisting of EDTA, heparin, citrate and fluoride/oxalate.

[0076] In accordance with some embodiments of the methods described herein, the hemolysate may be at least partially suspended or fully suspended.

ILLUSTRATIVE EMBODIMENTS

[0077] Provided here are illustrative embodiments of the disclosed technology. These embodiments are illustrative only and do not limit the scope of the present disclosure or of the claims attached hereto.

[0078] Embodiment 1. A method for detection of suspension of a blood sample, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} ;
- (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;

- (j) determining the absolute value (V_{n+1}) of the differential between $dhemo_{xn+1}$ and $dhemo_{yn+1}$
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$, is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$, is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

[0079] Embodiment 2. A method for improving the processing time of a point of care blood analysis, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
- (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
- (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;
- (f) determining the absolute value (V_n) of the differential between $dhemo_{xn}$ and $dhemo_{yn}$;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate $dhemo_{xn+1}$ and $dhemo_{yn+1}$.

- (h) determining the differential between dhem_{Oyn} and $\text{dhem}_{\text{Oyn}+1}$, wherein yn and $\text{yn}+1$ relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhem_{Oyn} and $\text{dhem}_{\text{Oyn}+1}$;
- (j) determining the absolute value (V_{n+1}) of the differential between $\text{dhem}_{\text{Oxn}+1}$ and $\text{dhem}_{\text{Oyn}+1}$;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N , wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$, is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$, is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
- (l) analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration; and,
- (m) providing in less than 5 min an output result comprising the hemoglobin concentration thereby improving the point of care blood analysis as compared to a standard point of care blood analysis.

[0080] Embodiment 3. A method for detection of a glycated hemoglobin in a blood sample, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;

- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhem_{oxn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhem_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhem_{oxn} and dhem_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhem_{oxn+1} and dhem_{yn+1} ;
- (h) determining the differential between dhem_{yn} and dhem_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhem_{yn} and dhem_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhem_{oxn+1} and dhem_{yn+1} ;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,

- i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
- ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

[0081] Embodiment 4. A method for performing a blood sugar assay, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .
- (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemo_{xn+1} and dhemo_{yn+1} ;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and

- a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
- (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
- (m) assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and,
- (n) providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample.

[0082] Embodiment 5. A method for diagnosing diabetes in a subject, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .
- (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemo_{xn+1} and dhemo_{yn+1} ;
- (k) assessing the absolute values V_n , $(V_{n+1}) - (V_n)$, and V_N , wherein,

- i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
- ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
- (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
- (m) determining glucose concentration in the blood sample from the subject based upon the glycated hemoglobin concentration so detected;
- (n) providing an output result comprising the glycated hemoglobin concentration and the glucose concentration in the blood sample from the subject;
- (o) diagnosing the subject with diabetes when the glucose concentration in the blood sample is above a reference concentration; and,
- (p) when diagnosed with diabetes, recommending a treatment of the subject, wherein the treatment comprises diet, exercise and/or medication.

[0083] Embodiment 6. The method of embodiment 5, wherein the steps (a) to (n) of blood sample processing of embodiment 4 are performed in less than 5 minutes.

[0084] Embodiment 7. The method of embodiment 5, wherein the steps (a) to (n) of blood sample processing of embodiment 4 are performed in about 3.5 minutes.

[0085] Embodiment 8. The method of any one of embodiments 1-5, wherein the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.

[0086] Embodiment 9. The method of any one of embodiments 1-5, wherein the hemolysate mixture comprises sonicating the blood sample and/or treating the blood sample with a lysis buffer.

[0087] Embodiment 10. The method of any one of embodiments 1-5, wherein the buffer comprises lithium thiocyanate, digitonin and/or potassium ferricyanide.

[0088] Embodiment 11. The method of any one of embodiments 1-5, wherein the buffer comprises an anticoagulant selected from the group consisting of EDTA, heparin, citrate and fluoride/oxalate.

[0089] Embodiment 12. The method of any one of embodiments 1-5, wherein the blood sample is in a capillary tube, a capillary plate or a capillary membrane.

[0090] Embodiment 13. The method of any one of embodiments 1-5, wherein the wavelength x is within the range of at least one set of wavelengths selected from the group consisting of:

- a. 380 nm to 455 nm;
- b. 455 nm to 580 nm; and
- c. 580 nm to 680 nm.

[0091] Embodiment 14. The method of any one of embodiments 1-5, wherein the wavelength y is within the range of 700 nm to 2500 nm.

[0092] Embodiment 15. The method of any one of embodiments 1-5, wherein the wavelengths x and y correspond respectively to 531 nm and 725 nm.

[0093] Embodiment 16. The method of any one of embodiments 1-5, wherein step (c) is performed at least 2 seconds after step (b).

[0094] Embodiment 17. The method of any one of embodiments 1-5, wherein, if the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample is replaced by another blood sample.

[0095] Embodiment 18. The method of any one of embodiments 1-5, wherein step (g) is repeated 30 times or less.

[0096] Embodiment 19. The method of any one of embodiments 1-5, wherein the hemolysate is at least partially suspended.

[0097] Embodiment 20. The method of any one of embodiments 1-5, wherein the blood sample is a human blood sample.

[0098] Embodiment 21. The method of any one of embodiments 1-5, wherein the absolute value of (i) is about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$.

[0099] Embodiment 22. The method of any one of embodiments 1-5, wherein the absolute value of $(V_{n+1})-(V_n)$ is about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.

[0100] Embodiment 23. The method of any one of embodiments 1-5, wherein the hemoglobin concentration or glycated hemoglobin concentration is at least about 5g/dL.

[0101] Embodiment 24. The method of any one of embodiments 1-5, wherein the hemoglobin concentration or glycated hemoglobin concentration is about at least about 7g/dL.

[0102] Embodiment 25. The method of any one of embodiments 1 and 2, wherein the hemoglobin comprises hemoglobin A, hemoglobin A1c, hemoglobin C, hemoglobin E, hemoglobin D, hemoglobin F, and hemoglobin S.

[0103] Embodiment 26. The method of any one of embodiments 1 and 2, wherein the hemoglobin is a glycated hemoglobin.

[0104] Embodiment 27. The method of embodiment 26, wherein the glycated hemoglobin is hemoglobin A1c (HbA1c).

[0105] Embodiment 28. The method of any one of embodiments 3-5, wherein the glycated hemoglobin is hemoglobin A1c (HbA1c).

EXAMPLES

[0106] The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

[0107] Blood is drawn into a capillary tube from a fingerstick or tube of venous blood. Excess blood is wiped from the capillary tube. The capillary tube is inserted into a sample cartridge. The cartridge is placed in a Siemens DCA Vantage[®] analyzer. Buffer (8.1% w/v lithium thiocyanate and 0.01% digitonin in 200 mM glycine) absorbance is read at 531 nm and at 725 nm. The differential (Δ_{buffer}) between the buffer pickup reads is computed. The blood sample is mixed with the buffer to generate a hemolysate. The absorbance value of the hemolysate at 531 nm (hemo_{531}) and at 725 nm (hemo_{725}) at a first timepoint is computed. The differential between hemo_{531} and hemo_{725} (Δ_{hemo}) is computed. The absolute value of the differential between Δ_{hemo} and Δ_{buffer} [$|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$] is computed as Criteria 1. The absorbance at timepoint n of the hemolysate at wavelength 531 (hemo_{531n}) and at wavelength 725 (hemo_{725n}) is measured. The absolute value of the differential between the blood pickup reads of the two timepoints at 725 nm [$|\text{hemo}_{725} - \text{hemo}_{725n}|$] is computed as Criteria 2. The

absolute value of the differential between the 531 nm to 725 nm change in two blood pickup reads taken at different timepoints from the start of mixing is computed as Criteria 3.

[0108] If the criteria are evaluated at less than 60 seconds from the start of mixing of the blood sample with the buffer, and: $|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$ is indicative of a hemoglobin concentration of at least about 5 g/dL $\pm$ 0.1 g/dL (Criteria 1); $|\text{hemo}_{725} - \text{hemo}_{725n}|$ is about zero (Criteria 2); and the absolute value of the differential between the 531 nm to 725 nm change in the two blood pickup reads is about zero (Criteria 3), the blood sample is sufficiently mixed with the buffer for further analysis.

[0109] If the criteria are evaluated at less than 60 seconds from the start of mixing of the blood sample with the buffer, and: $|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$ is not indicative of a hemoglobin concentration of at least about 5 g/dL $\pm$ 0.1 g/dL (Criteria 1); $|\text{hemo}_{725} - \text{hemo}_{725n}|$ is not about zero (Criteria 2); or the absolute value of the differential between the 531 nm to 725 nm change in the two blood pickup reads is not about zero (Criteria 3), the hemolysate is mixed further and the following steps are repeated. The absorbance value of the hemolysate at 531 nm (hemo_{531}) and at 725 nm (hemo_{725}) at a first timepoint is computed as Criteria 1. The differential between hemo_{531} and hemo_{725} (Δ_{hemo}) is computed. The absolute value of the differential between Δ_{hemo} and Δ_{buffer} $|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$ is computed. The absorbance at timepoint n of the hemolysate at wavelength 531 (hemo_{531n}) and at wavelength 725 (hemo_{725n}) is measured. The absolute value of the differential between the blood pickup reads of the two timepoints at 725 nm $|\text{hemo}_{725} - \text{hemo}_{725n}|$ is computed as Criteria 2. The absolute value of the differential between the 531 nm to 725 nm change in two blood pickup reads taken at different timepoints from the start of mixing is computed as Criteria 3, wherein in this repeated step, the first blood pickup read is the first blood pickup read of the overall assay.

[0110] If the criteria are evaluated at 60 seconds or more from the start of mixing of the blood sample with the buffer, and: $|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$ is indicative of a hemoglobin concentration of at least about 5 g/dL $\pm$ 0.1 g/dL (Criteria 1), the blood sample is sufficiently mixed with the buffer for further analysis.

[0111] If the criteria are evaluated at 60 seconds or more from the start of mixing of the blood sample with the buffer, and: $|\Delta_{\text{hemo}} - \Delta_{\text{buffer}}|$ is not indicative of a hemoglobin concentration of at least about 5 g/dL $\pm$ 0.1 g/dL (Criteria 1), the blood sample is not sufficiently mixed with the buffer for further analysis.

[0112] The distribution of blood absorbance between readings is shown in Fig. 1. An experimental dataset was collected with whole blood (14 g/dL, about 4.7% HbA1c). From

this dataset the normal distribution of absorbance (531-725 nm) between Blood Pickup readings was obtained. The mean and standard deviation of the expected values were used for true/false criteria 3.

[0113] The histogram depicted in Fig. 2 is based on the data of Fig. 1: The same dataset from Figure 1 was used for determining the mean and standard deviation for true/false criteria 2.

[0114] The dataset from Fig. 1 and Fig. 2 was used for determining the impact of using hemoglobin values collected when the 3 criteria were met (or criteria 1 after final Blood Pickup reading). No hemoglobin correction was used and only the first absorbance values were used for determining hemoglobin concentration. This explains why the percentage coefficient of variation (%CV) is centered on 2.5 rather than 2.0.

[0115] The percentage of bias with time saved is shown in Fig. 3. When one standard deviation was used for criteria 2-3 there was an average time savings of 26 seconds. There was minimal to no impact on the %CV observed and the percentage bias (%Bias) appeared to be a linear correlation to time mixing (Fig. 3).

[0116] The tables from Figs. 4A-4B provide an experimental demonstration of how a dataset (Fig. 4A) is processed using the presently disclosed methods. The various steps of data processing, including the main “True/False” tested criteria, are listed in Fig. 4B.

[0117] In sum, the presently disclosed methods are adaptive methods as they rely on measuring hemoglobin and using this measure as a reference signal to determine if enough of the blood sample has come out of the capillary of the instrument. These adaptive methods allow the assay to be completed faster for most samples. For the samples that may have resulted in an error, the disclosed methods provide the ability to mix longer while resulting in a longer assay time which prevents an error and a resampling. The overall protocol for these methods is illustrated in the diagram of Fig. 5.

[0118] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

[0119] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

What is claimed is:

1. A method for performing a blood sugar assay, the method comprising:
 - (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
 - (b) measuring the absorbance value of the buffer at a wavelength x ($buffer_x$) and the absorbance value of the buffer at a wavelength y ($buffer_y$);
 - (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x ($hemo_{xn}$) and at a wavelength y ($hemo_{yn}$);
 - (d) determining the differential ($dhemo_{xn}$) between $hemo_{xn}$ and $buffer_x$;
 - (e) determining the differential ($dhemo_{yn}$) between $hemo_{yn}$ and $buffer_y$;
 - (f) determining the absolute value (V_n) of the differential between $dhemo_{xn}$ and $dhemo_{yn}$;
 - (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate $dhemo_{xn+1}$ and $dhemo_{yn+1}$.
 - (h) determining the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$, wherein y_n and y_{n+1} relate to two consecutive measurements;
 - (i) determining the absolute value (V_N) of the differential between $dhemo_{yn}$ and $dhemo_{yn+1}$;
 - (j) determining the absolute value (V_{n+1}) of the differential between $dhemo_{xn+1}$ and $dhemo_{yn+1}$;
 - (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and

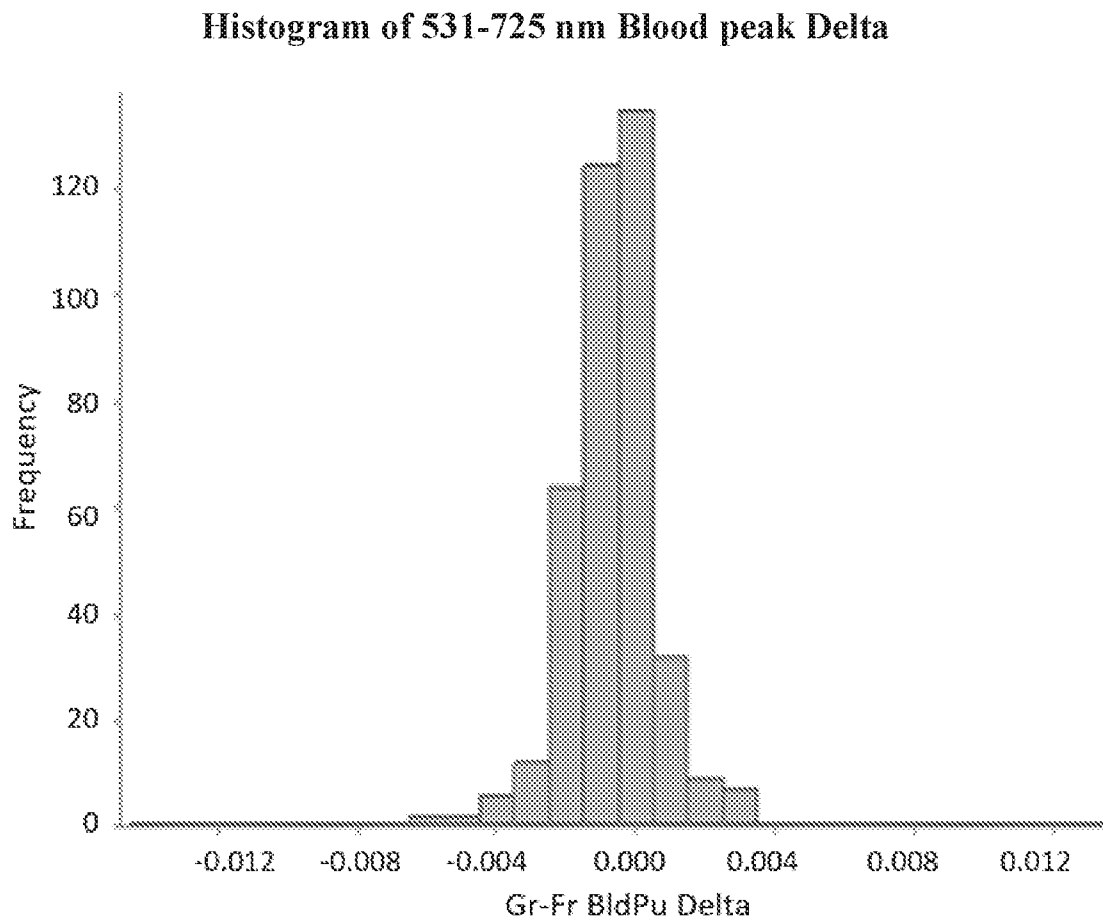
- a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
 - (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
 - (m) assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected; and,
 - (n) providing an output result for the blood sugar assay, the output comprising the glycated hemoglobin concentration and the level of sugar in the blood sample
2. A method for diagnosing diabetes in a subject, the method comprising:
- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
 - (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
 - (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
 - (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
 - (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
 - (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
 - (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .
 - (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
 - (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;
 - (j) determining the absolute value (V_{n+1}) of the differential between dhemo_{xn+1} and dhemo_{yn+1} ;
 - (k) assessing the absolute values V_n , $(V_{n+1}) - (V_n)$, and V_N , wherein,

- i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$ is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value V_n is not indicative of a glycated hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
 - (l) analyzing the sufficiently mixed blood sample to detect the glycated hemoglobin concentration;
 - (m) determining glucose concentration in the blood sample from the subject based upon the glycated hemoglobin concentration so detected;
 - (n) providing an output result comprising the glycated hemoglobin concentration and the glucose concentration in the blood sample from the subject;
 - (o) diagnosing the subject with diabetes when the glucose concentration in the blood sample is above a reference concentration; and,
 - (p) when diagnosed with diabetes, recommending a treatment of the subject, wherein the treatment comprises diet, exercise and/or medication.
3. The method of claim 2, wherein the steps (a) to (n) are performed in less than 5 minutes or in about 3.5 minutes.
 4. A method for improving the processing time of a point of care blood analysis, the method comprising:

- (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer;
- (b) measuring the absorbance value of the buffer at a wavelength x (buffer_x) and the absorbance value of the buffer at a wavelength y (buffer_y);
- (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemo_{xn}) and at a wavelength y (hemo_{yn});
- (d) determining the differential (dhemo_{xn}) between hemo_{xn} and buffer_x ;
- (e) determining the differential (dhemo_{yn}) between hemo_{yn} and buffer_y ;
- (f) determining the absolute value (V_n) of the differential between dhemo_{xn} and dhemo_{yn} ;
- (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemo_{xn+1} and dhemo_{yn+1} .
- (h) determining the differential between dhemo_{yn} and dhemo_{yn+1} , wherein y_n and y_{n+1} relate to two consecutive measurements;
- (i) determining the absolute value (V_N) of the differential between dhemo_{yn} and dhemo_{yn+1} ;
- (j) determining the absolute value (V_{n+1}) of the differential between dhemo_{xn+1} and dhemo_{yn+1} ;
- (k) assessing the absolute values V_n , $(V_{n+1})-(V_n)$, and V_N ,
wherein,
 - i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value $(V_{n+1})-(V_n)$, is about zero, and the absolute value V_N is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or
 - b. the absolute value of V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of $(V_{n+1})-(V_n)$ is not about zero, or the absolute value of V_N is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated;
 - ii. if step (k) is performed at time t from the start of the mixing and
 - a. the absolute value V_n is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or

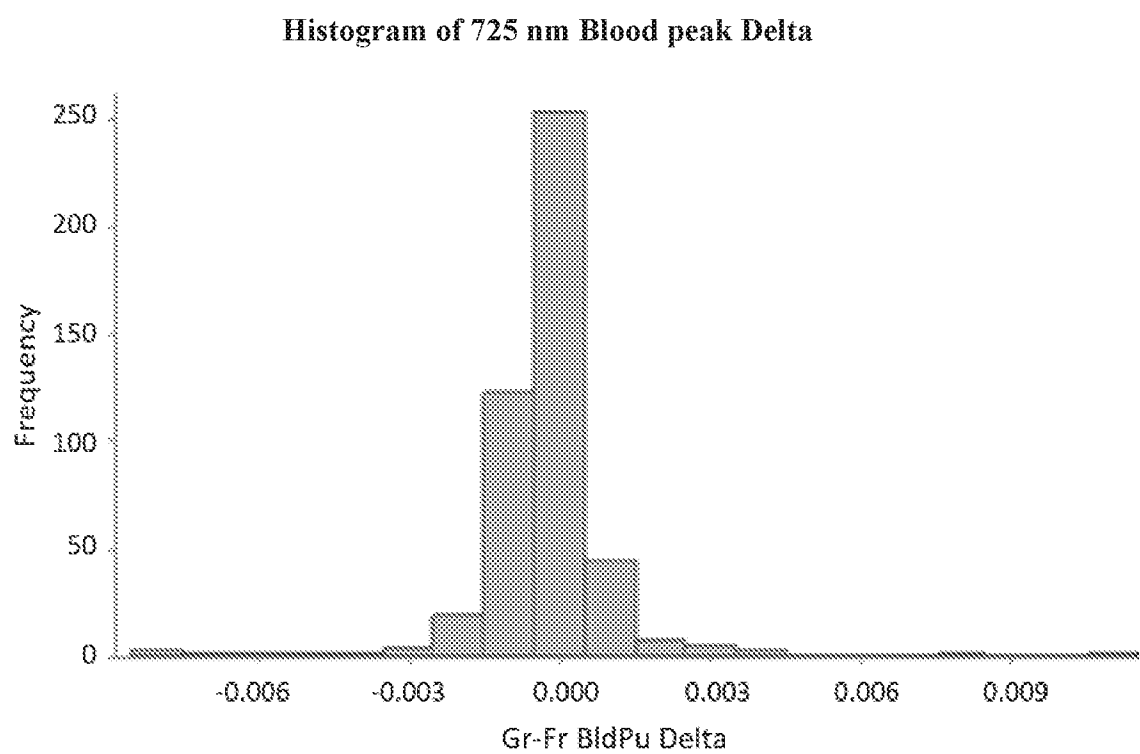
- b. the absolute value V_n is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis;
 - (l) analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration; and,
 - (m) providing in less than 5 min an output result comprising the hemoglobin concentration thereby improving the point of care blood analysis as compared to a standard point of care blood analysis.
5. The method of any one of claims 1-4, wherein the mixing of the blood sample comprises sonicating the blood sample and/or treating the blood sample with a lysis buffer.
6. The method of any one of claims 1-5, wherein the buffer comprises lithium thiocyanate, digitonin, potassium ferricyanide and/or an anticoagulant selected from the group consisting of EDTA, heparin, citrate and fluoride/oxalate.
7. The method any one of claims 1-6, wherein the blood sample is in a capillary tube, a capillary plate or a capillary membrane.
8. The method of any one of claims 1-7, wherein the wavelength x is within the range of at least one set of wavelengths selected from the group consisting of:
- a. 380 nm to 455 nm;
 - b. 455 nm to 580 nm; and
 - c. 580 nm to 680 nm; and,
- wherein the wavelength y is within the range of 700 nm to 2500 nm.
9. The method of any one of claims 1-8, wherein the wavelengths x and y correspond respectively to 531 nm and 725 nm.
10. The method of any one of claims 1-9, wherein step (c) is performed at least 2 seconds after step (d).

11. The method of any one of claims 1-10, wherein, if the blood sample is indicated to be not sufficiently mixed in step (k)(ii)(b), the blood sample is replaced by another blood sample.
12. The method of any one of claims 1-11, wherein the time t is about 60 seconds, about 70 seconds, about 80 seconds, about 90 seconds, about 100 seconds, about 110 seconds or about 120 seconds.
13. The method of any one of claims 1-12, wherein the hemolysate is at least partially suspended.
14. The method of any one of claims 1-13, wherein the blood sample is a human blood sample.
15. The method of any one of claims 1-14, wherein the absolute value of (i) is about the absolute value of (0 ± 0.002) or $(-0.0002619 \pm 0.001349)$.
16. The method of any one of claims 1-15, wherein the absolute value of $(V_{n+1})-(V_n)$ is about the absolute value of (0 ± 0.002) or $(-0.0006418 \pm 0.001758)$.
17. The method of any one of claims 1-16, wherein the hemoglobin concentration or the glycated hemoglobin concentration is at least about one selected from the group consisting of: 5g/dL and 7g/dL.
18. The method of any one of claims 1-2 and 4-17, wherein the glycated hemoglobin is hemoglobin A1c (HbA1c).



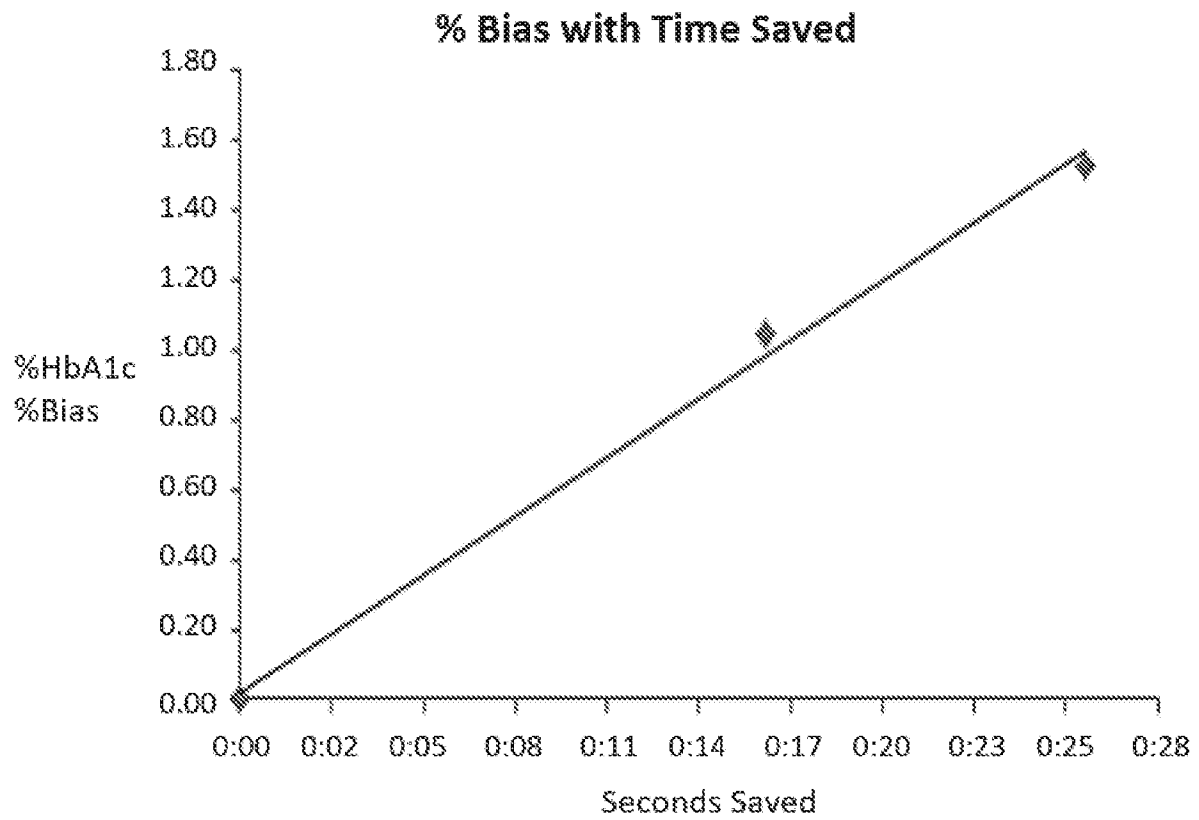
FR: Far Red = 710 to 850 nm wavelength. 725 nm was specifically used herein.
GR: Green = 495 to 570 nm wavelength. 531 nm was specifically used herein.

Figure 1



FR: Far Red = 710 to 850 nm wavelength. 725 nm was specifically used herein.

Figure 2

**Figure 3**

Exemplary dataset used for a hemoglobin test

	B	C	D	E
1	Data Stream Source			
2	Instrument	Data line from the instrument data stream	Absorbance reading tag	Absorbance reading value
3	B40011	15	G-buf1	0.048487633
4		16	FR-buf1	0.052213006
5		18	G-buf2	0.0504168
6		19	FR-buf2	0.053907012
7		21	G-buf3	0.052543731
8		22	FR-buf3	0.055540524
9		24	G-buf4	0.054115538
10		25	FR-buf4	0.056742482
11		27	G-buf5	0.055063553
12		28	FR-buf5	0.05750333
13		30	G-buf6	0.055730086
14		31	FR-buf6	0.05799888
15		33	G-buf7	0.056351184
16		34	FR-buf7	0.058550188
17		36	G-buf8	0.056715757
18		37	FR-buf8	0.058931038
19		39	G-buf9	0.057084104
20		40	FR-buf9	0.059140996
21		42	G-buf10	0.057471106
22		43	FR-buf10	0.05959808
23		58	G-pkup1a	0.199109788
24		59	FR-pkup1a	0.075320727
25		61	G-pkup1b	0.196003083
26		62	FR-pkup1b	0.073047705
27		64	G-pkup1c	0.194103767
28		65	FR-pkup1c	0.073233243
29		67	G-pkup1d	0.193491715
30		68	FR-pkup1d	0.073240954
31		71	G-pkup1e	0.193760993
32		72	FR-pkup1e	0.072618857
33		80	G-hb1	0.190926024
34		81	FR-hb1	0.070782429
35		83	G-hb2	0.190988718
36		84	FR-hb2	0.07081719
37		86	G-hb3	0.190978605
38		87	FR-hb3	0.070805433
39		89	G-hb4	0.19098265
40		90	FR-hb4	0.070916888
41		92	G-hb5	0.191028496
42		93	FR-hb5	0.070896435
43		95	G-hb6	0.190884233
44		96	FR-hb6	0.07082946
45		98	G-hb7	0.190855926
46		99	FR-hb7	0.070727737
47		101	G-hb8	0.190810772
48		102	FR-hb8	0.070666409
49		104	G-hb9	0.190876819
50		105	FR-hb9	0.070743071
51		107	G-hb10	0.190996808
52		108	FR-hb10	0.070724671

FR: Far Red = 710 to 850 nm wavelength. 725 nm was specifically used herein.

G: Green = 495 to 570 nm wavelength. 531 nm was specifically used herein.

Hb = Hemoglobin; Buf = Buffer; Pkup = Peak up read

Figure 4A

Figure 4B

	G	H	I	J	K	L	M	N	O
	Value tags pulled from column E for TRUE/FALSE criteria	Absorbance values from column F for TRUE/FALSE criteria	TRUE/FALSE criteria 1 (Is absorbance high enough above signal noise)	TRUE/FALSE criteria 2 (Is lysis complete)	TRUE/FALSE criteria 3 (Has hemoglobin stopped coming out of the capillary)	Are criteria 1-3 TRUE or is only criteria 1 TRUE for the "Hb Read"	What time was the first passing criteria met?	What was the Hb absorbance at the first passing criteria?	What is the [Hb] to be used when calculating %HbA1c?
2									
3	Gr Buffer	0.0543							
4	Fr Buffer	0.0570							
5	PkUp 1a	0.1264	TRUE	N/A	N/A	N/A	N/A	N/A	N/A
6	PkUp 1b	0.1255	TRUE	FALSE	TRUE	FALSE	0	0	0
7	PkUp 1c	0.1234	TRUE	TRUE	FALSE	FALSE	0	0	0
8	PkUp 1d	0.1228	TRUE	TRUE	TRUE	TRUE	4	0.1228	15.018
9	PkUp 1e	0.1237	TRUE	TRUE	FALSE	FALSE	0	0	0
10	Hb Read	0.1227	TRUE	FALSE	TRUE	TRUE	0	0	0
11	Example of the coding by column using "PkUp 1e"	531nm - 725nm absorbance =(E31-H3)-(E32-H4)	=IF(H9>0.0586, TRUE, FALSE)	=AND(((E32-E30)>-E30)>-0.0009355), ((E32-E30)<E30)<0.0006745))	=AND(((E31-E29)-(E32-E30))<-0.0006418 +0.000618), ((E31-E29)-(E32-E30))>-0.0006418 -0.000618))	=IF(AND (K9,J9,I9), TRUE, FALSE)	=IF(AND (L9=TRUE, L8=FALSE, L7=FALSE, L6=FALSE), 4.25,0)	=IF(AND (L9=TRUE, L8=FALSE, L7=FALSE, L6=FALSE), H9,0)	= 111.29 * SUM(N5:N10) + 1.3439

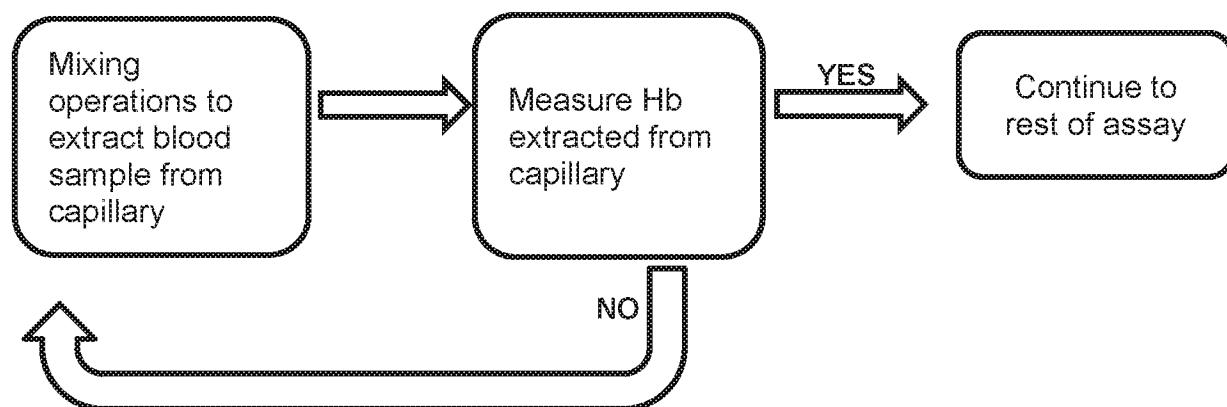


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/16348

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 5/00; A61B 5/145 (2020.01)

CPC - A61B 5/14532; A61B 5/7275; G16H 50/20; G16H 50/30; A61B 5/743

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,194,218 B1 (Rieders et al) 27 February 2001 (27.02.2001), entire document esp col 3 ln 6-20, col 5 ln 13-18, col 10 ln 6-17	1 and 5/1
Y	US 5,674,699 A (Saunders et al) 7 October 1997 (07.10.1997), entire document esp col 8 ln 46-63, col 20 ln 18-35	1 and 5/1
A	Wikipedia.com "Assay" 10 December 2018 (10.12.2018), entire document esp pg 3	1 and 5/1
A	WO 2013/059293 A1 (OHVIX Corporation) 25 April 2013 (25.04.2013), entire document	1 and 5/1

☐ 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

"D" document cited by the applicant in the international application

"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 another 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

13 April 2020

Date of mailing of the international search report

16 JUN 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-1300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/16348

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.: 6-18
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1 and 5/1, directed to a method for performing a blood sugar assay

Group II: Claims 2, 3, 5/(2,3), directed to a method for diagnosing diabetes in a subject

Group III: Claims 4 and 5/4, directed to a method for improving the processing time of a point of care blood analysis.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

*****See Supplemental Box below*****

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 additional fees, this Authority did not invite payment of additional fees.
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.:
1 and 5/1

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/US 20/16348

Box III Lack of Unity

Special Technical Features:

Group I requires a method for performing a blood sugar assay, not required by Groups II or III.

Group II requires a method for diagnosing diabetes in a subject comprising diagnosing the subject with diabetes when the glucose concentration in the blood sample is above a reference concentration; and, when diagnosed with diabetes, recommending a treatment of the subject, wherein the treatment comprises diet, exercise and/or medication, not required by Groups I or III.

Group III requires a method for improving the processing time of a point of care blood analysis, wherein hemoglobin is measured and reported and providing in less than 5 min an output result comprising the hemoglobin concentration thereby improving the point of care blood analysis as compared to a standard point of care blood analysis, not required by Groups I and II.

Common technical features:

Groups I-III share the feature of a method comprising (a) generating a hemolysate mixture by mixing a blood sample from a subject with a buffer; (b) measuring the absorbance value of the buffer at a wavelength x (buffer x) and the absorbance value of the buffer at a wavelength y (buffer y); (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemox n) and at a wavelength y (hemOyn); (d) determining the differential (dhemox n) between hemox n and buffer x ; (e) determining the differential (dhemOyn) between hemOyn and buffer y ; (f) determining the absolute value (Vn) of the differential between dhemox n and dhemOyn; (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemox $n+1$ and dhemOyn $+1$. (h) determining the differential between dhemOyn and dhemOyn $+1$, wherein yn and yn $+1$ relate to two consecutive measurements; (i) determining the absolute value (VN) of the differential between dhemOyn and dhemOyn $+1$; (j) determining the absolute value (Vn+1) of the differential between dhemox $n+1$ and dhemOyn $+1$ (k) assessing the absolute values Vn, (Vn+1)-(Vn), and VN, wherein, i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and a. the absolute value Vn is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value (Vn+1)-(Vn) is about zero, and the absolute value VN is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or b. the absolute value of Vn is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of (Vn+1)-(Vn) is not about zero, or the absolute value of VN is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated; ii. if step (k) is performed at time t from the start of the mixing and a. the absolute value Vn is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or b. the absolute value Vn is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis; (1) analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration; (n) providing an output result for the blood sugar assay, the output comprising the hemoglobin concentration.

These shared technical features, however, do not provide a contribution over the prior art, as being obvious over US 6,194,218 B1 to Rieders et al (hereinafter Rieders) in view of US 5,674,699 A to Saunders et al (hereinafter Saunders).

Rieders discloses a method comprising (a) generating a hemolysate mixture by mixing a blood sample from a subject with a Buffer (col 5 ln 13-18 .. According to a preferred embodiment of the invention, 2 ml of buffer is combined with a 25 ul whole blood Sample for assay.); (c) measuring the absorbance value of the hemolysate mixture at a timepoint n at a wavelength x (hemox n) and at a wavelength y (hemOyn) (col 3 ln 6-12 .. measuring the spectrophotometric absorbance of the Sample at wavelengths 528.3 nm (A528.3) and 557.8 nm (A557.8);.. it is well understood a timepoint exists for the measurement); (f) determining the absolute value (Vn) of the differential between the absorbance measurements at the two wavelengths (col 3 ln 15-20 A528.3 - A557.8 is assessed, it is well understood the absolute value is the result obtained). Rieders further discloses (1) analyzing the sufficiently mixed blood sample to detect the hemoglobin concentration (col 10 ln 6-17 .. Whole blood was purged with oxygen to ensure that 100% of the hemoglobin was in the form of oxyhemoglobin. The blood was then added to a solution of 0.4% w/v ammonium hydroxide at a dilution of approximately 100:1 (or where the absorbance at 576 nm is about 1.0)..The determined value, 529.3, was then used for the hemoglobin determinations below..). Rieders does not disclose the steps of (g) repeating steps (c) to (e) for one additional consecutive timepoint $n + 1$, wherein the timepoint n of step (c) becomes equal to $n + 1$, to generate dhemox $n+1$ and dhemOyn $+1$. (h) determining the differential between dhemOyn and dhemOyn $+1$, wherein yn and yn $+1$ relate to two consecutive measurements; (i) determining the absolute value (VN) of the differential between dhemOyn and dhemOyn $+1$; (j) determining the absolute value (Vn+1) of the differential between dhemox $n+1$ and dhemOyn $+1$, as Rieders does not take multiple timepoints and thus does not monitor how the assay evolves with time, which these measurements would accomplish. It would have been obvious to one skilled in the art to assess the time differentials of the measured quantity by taking timepoints and performing routine calculations in order to see how the quantity is changing with time, because timepoints are routine in assays (see Wikipedia.com Assay in citations pg 3 section Assay Types based on the nature of the assay process at bottom of the page .. Time and number of measurements taken.. Depending on whether an assay just looks at a single time point or timed readings taken at multiple time points, an assay may be: A kinetic assay, in which measurements are performed multiple times over a fixed time interval. Kinetic assay results may be visualized numerically (for example, as a slope parameter representing the rate of signal change over time), or graphically (for example, as a plot of the signal measured at each time point). For kinetic assays, both the magnitude and shape of the measured response over information time provide important information..). Rieders does not specifically disclose the steps of (k) assessing the absolute values Vn, (Vn+1)-(Vn), and VN, wherein, i. if step (k) is performed at less than a time t from the start of the mixing in step (a) and a. the absolute value Vn is indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value (Vn+1)-(Vn) is about zero, and the absolute value VN is about zero, the blood sample is sufficiently mixed with the buffer for further analysis; or b. the absolute value of Vn is not indicative of a hemoglobin concentration of at least about 5 g/dL, the absolute value of (Vn+1)-(Vn) is not about zero, or the absolute value of VN is not about zero, the hemolysate is mixed further and steps (g) to (j) are repeated; ii. if step (k) is performed at time t from the start of the mixing and a. the absolute value Vn is indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is sufficiently mixed with the buffer for further analysis; or b. the absolute value Vn is not indicative of a hemoglobin concentration of at least about 5 g/dL, the blood sample is not sufficiently mixed with the buffer for further analysis.

*****see supplemental box below*****

Box III Lack of Unity

It would have been obvious to one skilled in the art to optimize the mixing time using routine experimentation and to do this by assessing that the time differentials of absorption, i.e. concentration, are as close to zero as possible and also by ensuring that the measurement of something, such as hemoglobin, is not an unexpected value by setting a threshold ahead of the tests, also using routine experimentation, such as 5 g/dL (see instant specification para [0070] .. The amount of total hemoglobin generally has a range of 13 to 17 g/dL for adult men, a range of 12 to 15 g/dL for adult women, and a range of 11 to 14 g/dL for infants. When the glycated hemoglobin value is 6.0%, it may be expressed as about 12 g/dL on average for a two to three-month blood sugar. The American Diabetes Association (ADA) recommends that the glycated hemoglobin value be maintained to 6.5% or less, and a normal range thereof is 4.0 to 5.7%..). Rieders does not specifically disclose (b) measuring the absorbance value of the buffer at a wavelength x (bufferx) and the absorbance value of the buffer at a wavelength y (buffery); (d) determining the differential (dhemoxn) between hemoxn and bufferx; (e) determining the differential (dhemoyn) between hemoyxn and buffery; (m) assessing the level of sugar in the blood sample based upon the hemoglobin concentration so detected; and, (n) providing an output result for the blood sugar assay, the output comprising the hemoglobin concentration and the level of sugar in the blood sample. Rieders measurements focus on methemoglobin (abstract .. blood samples are stabilized for methemoglobin determination..). Saunders discloses a method of measuring glycated hemoglobin (col 20 In 18-23 .. In our assay for glycohemoglobin, our preferred marker is unglycated hemoglobin..) comprising measuring absorbance at two wavelengths (col 20 In 24-35 .. Subtracting the reading at 630 nm from the readings of 560 nm, the following is obtained..). Saunders does not specifically disclose (b) measuring the absorbance value of the buffer at a wavelength x (bufferx) and the absorbance value of the buffer at a wavelength y (buffery); (d) determining the differential (dhemoxn) between hemoxn and bufferx; (e) determining the differential (dhemoyn) between hemoyxn and buffery. Saunders discloses wherein the readings are corrected by some blank (col 20 In 35 -46 blanks are measured and subtracted from the readings). Saunders does not specifically disclose using the buffer as a blank, Saunders discloses using a liquid with similar properties to the sample being measured (col 8 In 46-63 .. For the purpose of the present invention, a blank is a control solution in an at least partially transparent container as similar as possible to that used to receive and examine the sample. Preferably, if the sample is blood, the control solution is a saline buffer. If the sample is pretreated to prepare it for analysis, the blank is treated in the same way. For example, if an anti-coagulant is added to a blood sample, it is also added to the blank. The same particulate reagent is added to both the sample and to the blank. Both the sample and the blank are centrifuged or allowed to settle so as to form particle-rich and particle-free zones. The blank, of course, is free of both analyte and the other species endogenous to the sample, but is otherwise as similar as practical to the sample..). It would have been obvious to one skilled in the art to utilize the buffer added to the blood sample as the blank to correct all readings from because it could impact the reading of the sample and can be corrected for by blanking. Saunders does not specifically disclose (n) providing an output result for the blood sugar assay, the output comprising the hemoglobin concentration and the level of sugar in the blood sample. It would have been obvious to one skilled in the art to report the results of the assays disclosed by Saunders, glycated hemoglobin and the related sugar measurement, i.e. glucose.

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II share the technical feature of measuring glycated hemoglobin and assessing the level of sugar in the blood sample based upon the glycated hemoglobin concentration so detected.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by Saunders.

Saunders discloses measuring glycated hemoglobin (col 20 In 62-66 ..Glycohemoglobin by Optical Absorbance In this example, a direct assay for glycohemoglobin..) (m) assessing the level of sugar in the blood sample based upon the hemoglobin concentration so detected (col 20 In 62-66 ..Glycohemoglobin by Optical Absorbance In this example, a direct assay for glycohemoglobin.. col 1 In 34-40 .. measurement of glycated hemoglobin is a clinically accepted means of assessing glycemic control in diabetics. GHb values reflect blood glucose levels over the circulatory life of the erythrocyte (about 120 days).. Note: Glycohemoglobin is glycated hemoglobin and glucose is a sugar which can be assessed by measuring glycated hemoglobin, which is what this method does).

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note: claims 6-18 were determined to be unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a) and were not included in any group.