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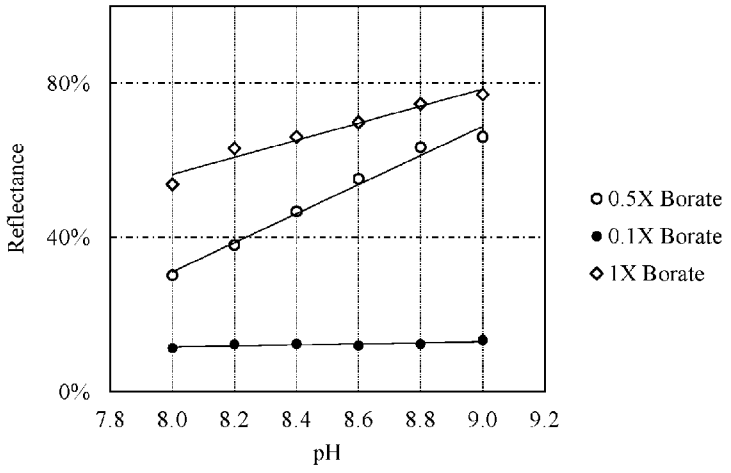
(54) Title: METHODS FOR ENHANCED DETECTION OF HEMOGLOBIN

FIG 2.



(57) Abstract: Described herein are methods for quantifying hemoglobin in fluids such as urine samples. The methods employ hemoglobin control compositions containing a low strength buffer, providing lower limits of detection and increased consistency over a wider range of assay conditions.

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METHODS FOR ENHANCED DETECTION OF HEMOGLOBIN

CLAIM OF PRIORITY

[0001] This patent application claims the benefit of priority to U.S. Provisional Application Serial No. 62/786,038, filed December 28, 2018, which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[0002] The determination of hemoglobin or blood level in a urine specimen is an important reportable parameter in the diagnosis of a patient's medical condition. The reported value of hemoglobin is used to help physicians prescribe further testing in order to provide therapies to their patients with educated judgment. However, hemoglobin in urine is not always easy to measure quantitatively from a specimen due to the sample condition of the specimen. The methods that clinicians rely on to measure hemoglobin are calibrated with calibrators and controls prior to measuring the specimen. The quantitative value of the hemoglobin in the calibrators and controls affects the outcome of the tested specimen. If a control composition provides a low calibration value that is too close to the limit of detection in a particular method, for example, "false negative" results are more likely to occur.

BRIEF SUMMARY OF THE INVENTION

[0003] Provided herein are methods for determining the quantity of hemoglobin in a urine sample. The methods include:

(i) contacting a first test strip with the urine sample, wherein the test strip comprises a test pad and a color correction pad;

(ii) measuring the color of the test pad after step (i) to obtain a first colorimetric value;

(iii) measuring the color of the color correction pad after step (i) to obtain a second colorimetric value;

(iv) comparing the first color value and the second color value to obtain a corrected color value;

(v) comparing the corrected color value to a known color value to quantify the hemoglobin in the urine sample;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide;

wherein the color correction comprises paper that is substantially free of impregnated chromogens or impregnated peroxides; and

wherein the known color value is determined by contacting a second test strip with a control composition comprising hemoglobin and a low strength buffer.

[0004] Also provided are methods for obtaining a color measurement for a hemoglobin control composition. The method includes:

(a) contacting a test strip with the hemoglobin control composition, wherein the test strip comprises a test pad and a color correction pad;

(b) measuring the color of the test pad after step (a) to obtain a first color value;

(c) measuring the color of the color correction pad after step (a) to obtain a second color value; and

(d) comparing the first color value and the second color value to obtain the color measurement for the hemoglobin control composition;

wherein the hemoglobin control composition comprises hemoglobin and a low strength buffer;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide; and

wherein the color correction pad is substantially free of impregnated chromogens or impregnated peroxides.

[0005] Also provided herein are solution compositions comprising hemoglobin and a low strength buffer. In some embodiments, the concentration of hemoglobin ranges from about 0.05 mg/dL to about 5 mg/dL. In some embodiments, the low strength buffer is a borate buffer having a concentration of less than 50 mM

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. 1 shows that that the sensitivity of hemoglobin quantitation by reflectance densitometry increases as the concentration of borate in the hemoglobin test compositions decreases. Measurements were made using compositions containing hemoglobin (0.14 mg/dL) and varying concentrations of sodium tetraborate.

[0007] FIG. 2 shows the reflectance value (%) of test pads treated with hemoglobin solutions containing 1X, 0.5X or 0.1X sodium tetraborate versus the pH of the hemoglobin

solution. The data demonstrate the unexpected pH independence at lower borate concentrations.

DETAILED DESCRIPTION OF THE INVENTION

[0008] The compositions and methods described herein were developed upon discovering that surprising improvements in hemoglobin quantitation and blood detection in urine samples can be obtained through the use of new hemoglobin standards. Urinalysis chemistry can be conducted with the standards to obtain lower limits of detection and higher data accuracy. As compared to the detection of other common urine analytes, hemoglobin chemistry and detection was found to be uniquely sensitive to conditions such as pH and buffer concentration. The methods and compositions described herein minimize such effects on hemoglobin detection without adversely affecting the chemistry used for detection of other analytes assays in parallel. In addition, the methods and compositions can be used for improved quality control in the manufacture of test strips and other consumables used for urine analysis.

Embodiments of the Invention

[0009] Provided herein is a method for determining the quantity of hemoglobin in a urine sample. The method includes:

(i) contacting a first test strip with the urine sample, wherein the test strip comprises a test pad and a color correction pad;

(ii) measuring the color of the test pad after step (i) to obtain a first colorimetric value;

(iii) measuring the color of the color correction pad after step (i) to obtain a second colorimetric value;

(iv) comparing the first color value and the second color value to obtain a corrected color value;

(v) comparing the corrected color value to a known color value to quantify the hemoglobin in the urine sample;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide;

wherein the color correction comprises paper that is substantially free of impregnated chromogens or impregnated peroxides; and

wherein the known color value is determined by contacting a second test strip with a control composition comprising hemoglobin and a low strength buffer.

[0010] In some embodiments, the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.05 mg/dL to about 5 mg/dL. The concentration of the hemoglobin can range, for example, from about 0.1 mg/dL to about 5 mg/dL, or from about 0.5 mg/dL to about 4 mg/dL, or from about 1 mg/dL to about 3 mg/dL. The concentration of hemoglobin can be about 0.1 mg/dL, 0.2 mg/dL, 0.3 mg/dL, 0.4 mg/dL, 0.5 mg/dL, 0.6 mg/dL, 0.7 mg/dL, 0.8 mg/dL, 0.9 mg/dL, 1.0 mg/dL, 1.1 mg/dL, 1.2 mg/dL, 1.3 mg/dL, 1.4 mg/dL, 1.5 mg/dL, 1.6 mg/dL, 1.7 mg/dL, 1.8 mg/dL, 1.9 mg/dL, or 2.0 mg/dL. In some embodiments, the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.5 mg/dL to about 5 mg/dL. In some embodiments, the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.1 mg/dL to about 2 mg/dL. In some embodiments, the concentration of hemoglobin in the hemoglobin control composition ranges from about 1 mg/dL to about 2 mg/dL. Hemoglobin from a number of organisms (*e.g.*, human hemoglobin, porcine hemoglobin, equine hemoglobin, bovine hemoglobin) is available from various commercial sources and can be used in the methods and compositions according to the present disclosure.

[0011] Typically, borate will be obtained and used as sodium tetraborate decahydrate (CAS No. 1303-96-4), although other forms such as anhydrous sodium tetraborate, sodium tetraborate pentahydrate, and boric acid can also be employed. In some embodiments, the concentration of the tetraborate in the hemoglobin control composition ranges from about 1 mM to about 45 mM. The tetraborate concentration can range, for example, from about 4 mM to about 6 mM, or from about 2 mM to about 8 mM, or from about 1 mM to about 10 mM. The tetraborate concentration can range from about 1 mM to about 5 mM, or from about 5 mM to about 10 mM, or from about 10 mM to about 15 mM, or from about 15 mM to about 20 mM, or from about 20 mM to about 25 mM, or from about 25 mM to about 30 mM, or from about 30 mM to about 35 mM, or from about 35 mM to about 40 mM, or from about 40 mM to about 45 mM. In some embodiments, the tetraborate concentration is about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mM. In some embodiments, the total concentration of borate ions in the hemoglobin control composition ranges from about 1 mM to about 200 mM (*e.g.*, 4-180 mM). In some embodiments, the total concentration of borate ions in the hemoglobin control composition ranges from about 15 mM to about 25 mM (*e.g.*, about 20 mM).

[0012] Other buffers can also be employed in the hemoglobin control composition in place of borate. Examples of such buffers include, but are not limited to, sodium bicarbonate, potassium bicarbonate, N-cyclohexyl-2-aminoethanesulfonic acid (CHES), sodium phosphate monobasic, sodium phosphate dibasic, potassium phosphate monobasic, potassium phosphate dibasic, taurine, N-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid (AMPSO), ammonium hydroxide, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BIS-Tris), barbital, glycine, piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), N-tris(hydroxymethyl)-methyl-4-aminobutanesulfonic acid (TABS), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), histidine, methylamine, ethanolamine. By keeping the concentration of the buffer in the hemoglobin control composition low (*e.g.*, below 50 mM), the pH of the reaction mixture can be controlled principally by buffering agents in the test pad to ensure appropriate reaction conditions for causing and detecting color change of the impregnated chromogen.

[0013] The hemoglobin control composition is formulated such that the pH of the reaction mixture following contact with the test pad is suitable for generating a color change in the presence of hemoglobin. In some embodiments, the pH of the hemoglobin control composition ranges from about 6.0 to about 9.4. For example, the pH of the hemoglobin control composition can range from about 6.0 to about 6.2, or from about 6.2 to about 6.4, or from about 6.4 to about 6.6, or from about 6.6 to about 6.8, or from about 6.8 to about 7.0., or from about 7.0 to about 7.2, or from about 7.2 to about 7.4, or from about 7.4 to about 7.6, or from about 7.6 to about 7.8, or from about 7.8 to about 8.0., or from about 8.0 to about 8.2, or from about 8.2 to about 8.4, or from about 8.4 to about 8.6, or from about 8.6 to about 8.8, or from about 8.8 to about 9.0, or from about 9.0 to about 9.2, or from about 9.2 to about 9.4. The pH of the hemoglobin control composition can range from about 6.0 to about 9.2, or from about 6.8 to about 9.2, or from about 7.4 to about 9.2, or from about 7.8 to about 9.2, or from about 8.0 to about 9.2. In some embodiments, the pH ranges from about 8.0 to about 9.0. Small amounts of an acid (*e.g.*, hydrochloric acid, sulfuric acid, or the like) or a base (*e.g.*, sodium hydroxide, potassium hydroxide, or the like) can be used to adjust the pH of the hemoglobin control composition to the desired level.

[0014] Test pads and color correction pads used in methods according to the present disclosure can be constructed from or contain any of a variety of materials. In some cases, a material used to fabricate a test pad can be selected based on any of a variety of factors,

including without limitation, bending resistance or stiffness, brightness (*e.g.*, the higher the reflectance, the brighter the appearance), bursting strength, burst factor, compressibility, elongation, gloss, grammage, hardness, moisture content, opacity, printability, print quality, ply bond, resiliency, Taber stiffness, surface strength, tearing resistance, tensile strength, thickness, water absorption, wettability, whiteness, and/or color. As a non-limiting example, a test pad may contain or be constructed from a filter paper having Grade MN 818 (weight: 180 g/m^2, thickness: 0.45 mm, filtration speed: 8 s/10 mL) or Grade MN 215 (weight: 145 g/m^2, thickness: 0.35 mm, migration distance: 85 mm/10 min) available from Macherey-Nagel. Such papers may exhibit smooth surfaces and/or high absorptivity. The size and shape of the test pads and color correction pads can be adjusted depending on factors such as the amount of the sample to be tested or the configuration of the instruments used for making color measurements. In some embodiments, for example, the test pad and/or the color correction pad can is a square after having a width ranging from about 0.15" to about 0.25" (*e.g.*, 0.2" or 0.51 cm). The test pads and color correction pads can be fixed to a suitable backing material, such as a Mylar strip or other material.

[0015] Test pads can be prepared to change color in the presence of hemoglobin (*e.g.*, hemoglobin present in a control composition or hemoglobin associated with blood present in a urine sample). Hemoglobin exhibits peroxidase activity, and color changes in the presence of hemoglobin can therefore be generated by allowing an organic peroxide to be cleaved by the hemoglobin in the presence of a chromogen. Reaction of the peroxide cleavage product with the chromogen produces the color change for measurement in the quantitation methods.

[0016] A number of chromogens and peroxides are suitable for use in the compositions and methods provided herein. By "impregnated," it is meant that the test pad is impregnated with chromogen, the peroxide, or other substance. Examples of suitable chromogens include, but are not limited to, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (also referred to as ABTS; CAS No. 30931-67-0), phenylene diamines such as *o*-phenylenediamine hydrochloride (also referred to as OPD; CAS No. 615-28-1), 3-amino-9-ethylcarbazole (also referred to as AEC; CAS No. 132-32-1), and benzidines such a 3,3′,5,5′-tetramethylbenzidine (also referred to as tetramethylbenzidine or TMB; CAS No. 54827-17-7), as well as chromogens described by Conyers *et al.* (*Analytical Biochem.* 1991, 192(1):207-211). Examples of suitable peroxides include, but are not limited to, cumene hydroperoxide (CAS No. 80-15-9), dicumyl peroxide (CAS No. 80-43-3), *tert*-butyl hydroperoxide (CAS No. 75-91-2), and benzoyl peroxide (CAS No. 94-36-0). In some embodiments, the impregnated

chromogen in the test pad is a benzidine. In some embodiments, the benzidine is tetramethylbenzidine or a salt thereof (*e.g.*, tetramethylbenzidine dihydrochloride). In some embodiments, the impregnated peroxide in the test pad is cumene hydroperoxide. The test pad will typically contain at least one molar equivalent of the impregnated peroxide, with respect to the amount of the impregnated chromogen in the test pad. The molar ratio of the impregnated peroxide to the impregnated chromogen may range, for example, from about 1:1 to about 50:1 (*e.g.*, from about 1:1 to about 25:1, or from about 5:1 to about 15:1, or from about 10:1 to about 15:1, or from about 10:1 to about 12:1). In some embodiments, the test pad comprises impregnated cumene hydroperoxide and impregnated tetramethylbenzidine in a molar ratio ranging from about 5:1 to about 15:1.

[0017] In some embodiments, the test pads and/or color correction pads also include one or more impregnated buffering agents. Examples of suitable buffers for impregnation in the pads include, but are not limited to, piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), N-(2-acetamido)iminodiacetic acid (ADA), N-(2-acetamido)-2-aminoethanesulfonic acid (ACES), citric acid, sodium phosphate monobasic, sodium phosphate dibasic, potassium phosphate monobasic, potassium phosphate dibasic, and the like. The test pads and/or color correction pads may further contain additional acids or bases, such as boric acid, hydrochloric acid, sulfuric acid, lithium hydroxide, sodium hydroxide, potassium hydroxide. The impregnated buffering agents and additional acids or bases, if present, will generally be employed in amounts that provide a pH suitable for generating a color change in the pads in presence of hemoglobin. In some embodiments, the impregnated buffering agents are present in the pad in amounts sufficient to provide a final pH ranging between about 5.5 and about 7.5 (*e.g.*, pH 6.2-7.5, or pH 6.4-7.3). In some embodiments, the pH of the pad prior to addition of hemoglobin control composition or test sample ranges from about 5.5 to about 6.5 (*e.g.*, pH 5.5-6.0, pH 5.7-5.9).

[0018] The test pad will be contacted with a urine sample, hemoglobin control composition, or other fluid under conditions sufficient for hemoglobin, if present, to react with the peroxide so as to cause a color change in the impregnated chromogen. For example, the color change reaction may be made to occur by contacting the test strip with a urine sample for a period of time ranging from a few seconds (*e.g.*, 5 seconds or less) to several minutes (*e.g.*, 15 minutes or more), or longer periods, at temperatures ranging between about 20 °C to about 40 °C (*e.g.*, around 20-37 °C, or around 20-25 °C). Test pads can be contacted with fluids under examination by dipping the pad, or a test strip containing the pad,

in the fluid. Alternatively, a volume of fluid may be placed on the surface of the test pad (*e.g.*, via pipette or syringe apparatus) in a manual fashion or an automated fashion. The color of the test pad may then be measured with or without removal of the fluid after the color change reaction. One or more washing steps (*e.g.*, with water, phosphate buffered saline, or other solution) may be conducted between the end of the color change reaction and the color measurement. The test pad may be wet, dry, or partially dried at the time of the color measurement. In some embodiments, color correction pads and test pads are treated with substantially identical conditions, subject to normal margins of error, before, during, and/or after the color change reaction period, and/or before, during, and/or the color measurement.

[0019] Color values can be determined used using known instrumentation and methodology including, for example, instruments and methods described in U.S. Pat. Nos. 7,623,240; 7,820,104; and 8,150,115, which are incorporated herein by reference in their entirety, as well as WO 2018/112438, which is incorporated herein by reference in its entirety.

[0020] In some embodiments, measuring the color of the test pad and the color correction pad is conducted via reflectance densitometry. A reflectance densitometry measurement can be made, for example, using apparatus containing an optical measuring unit having a measuring plane upon which test strips are to be placed; an illumination device for illuminating the measuring plane and the test strips; a planar image sensor; an optical system for providing an image of the test strip onto the planar image sensor; and an electronic evaluation unit for evaluating signals generated by the planar image sensor. The illumination device can include multiple light sources for illuminating the test strips in different colors, such that the electronic evaluation unit detects the coloring of test pads under different illumination conditions. The device can further include an adjustable reference surface that is movable between a first position in which it takes on the position of a test strip during measurement and a second position in which it cannot be imaged and measured.

[0021] In some embodiments, the device contains a housing, an insertion station for inserting a test strip wetted with the urine sample or other liquid to be tested; and optical measuring unit as described above, and a transport device for transporting the test strip from the insertion station to the optical measuring unit. In some embodiments, the transport device is configures to move the test strip from the insertion station to the optical measuring unit

with a desired reaction period for the chemistry occurring in the test strip. The transport device may include two or more transport sections that move the test strips at different transport speeds. The apparatus may also containing a sensor for detecting a test strip at the insertion station, and/or an indicator for indicating whether a test strip is present in one or more of the transport sections.

[0022] In some embodiments, the apparatus is configured to record test strip reflectance values captured over time. A plurality of chemical strips can be moved through a plurality of imaging positions at discrete points in time while a camera captures images of a field of view that includes the imaging positions and any of the chemistry strips therein. Image capture and determination of reflectance values by a processor can be time with the movement of the strips through the imaging positions. In some embodiments, the processor may fit reflectance values to a curve defined by $R(t)=A \exp[-B(t + t_0)]+C$, where A, B, C, and t_0 are fit parameters. The processor can then calculate the concentration of an analyte on the test pad by comparing the slope of the curve at $t= -t_0$ and with slope values for known analyte concentrations, and comparing the asymptotic value of R(t) with that for known analyte concentrations, thereby making the concentration determination.

[0023] Urine samples may contain colored substances that change the color of the test pads even in the absence of hemoglobin, chromogens, and peroxides. As such, pads may take on colors including, but not limited to, yellows (sometimes due to conditions such as polyuria and diabetes), dark yellows (*e.g.,* in concentrated urine specimens, or from B-complex vitamins, dehydration, bilirubin, acriflavine, and nitrofurantoin), orange-yellows (*e.g.,* from phenazopyridine and phenindine), yellow-greens (*e.g.,* from biliverdin), greens (*e.g.,* in the case of Pseudomonas infections), blue-greens (*e.g.,* from anitriphyline, methocarbamol, chlorets, indican, methylene blue, and phenol), pinks (*e.g.,* from red blood cells), reds (*e.g.,* from myoglobin, beets, and rifampin], purples (*e.g.,* from porphyrins), browns (*e.g.,* from homogentistic acid), and black (*e.g.,* from melanin, melanoma, phenolic derivatives, Argynol, methyl-Levodopa and Flagyl). In order to make certain that a color change measured on a test pad is due to the presence of hemoglobin in a test sample, a color value can be obtained from a color correction pad without assay reagents (*e.g.,* without TMB and/or without cumene hydroperoxide) and subtracted from the color value obtained for the test pad. In certain instances, low concentrations of TMB and related chromogens can increase measured reflectance values for red light due to absorbance in the blue and green. On the other hand, higher concentration of bilirubin can increase measured reflectance values for red light due to

red reflectance of yellow pigments. Higher red reflectance can lead to measured hemoglobin levels that skew lower than the actual level present in a sample or control composition. In some embodiments, subtracting red light reflectance from green light reflectance can improve the accuracy of the measured hemoglobin level. This can be particularly useful for the analysis of mixtures containing high levels of blood and bilirubin.

[0024] In some embodiments, measuring the color of the test pad and the color correction pad is conducted via reflectance densitometry. In some embodiments, the reflectance densitometry includes illuminating the test pad and the color correction pad with light having a wavelength ranging from about 400 nm to about 700 nm. In some embodiments, the light has a wavelength around 625 nm. In some embodiments, the reflectance densitometry further comprises recording an image of the test pad and an image of the color correction pad. In some embodiments, the image of the test pad and the image of the color correction pad are recorded using a complementary metal oxide semiconductor (CMOS) image sensor or a charge-coupled device (CCD) image sensor.

[0025] The relationship between test color saturation and absorbance follows the Beer-Lambert law, where absorbance of light is proportional to concentration; a darker color is more positive than a light color. Reflectance measurements, on the other hand, decrease as color saturation increases. As absorbance increases, reflectance decreases. Color density (D) is a function of the percentage of light reflected where $D = \log_{10}(1/R)$; 100% Reflectance = 0 Density. In some embodiments, the hemoglobin control composition provides a known color value as a reflectance value ranging from about 5% to about 60%. In some embodiments, obtaining a known color value within a predetermined range provides confirmation that the instrument used for the measurement is properly calibrated for accurate quantitation in test samples such as patient urine samples.

[0026] Also provided is a method for obtaining a color measurement for a hemoglobin control composition. The method includes:

(a) contacting a test strip with the hemoglobin control composition, wherein the test strip comprises a test pad and a color correction pad;

(b) measuring the color of the test pad after step (a) to obtain a first color value;

(c) measuring the color of the color correction pad after step (a) to obtain a second color value; and

(d) comparing the first color value and the second color value to obtain the color measurement for the hemoglobin control composition;

wherein the hemoglobin control composition comprises hemoglobin and a low strength buffer;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide; and

wherein the color correction pad is substantially free of impregnated chromogens or impregnated peroxides.

[0027] Also provided are solution compositions comprising hemoglobin and a low strength buffer for use in the methods described above. A solution composition can contain hemoglobin in amounts as described above (*e.g.*, 0.05 mg/dL to about 5 mg/dL, or from about 0.1 mg/dL to about 2 mg/dL, or from about 1 mg/dL to about 2 mg/dL) and buffer in the amounts described above (*e.g.*, borate less than 50 mM, *e.g.*, from about 1 mM to about 45 mM). In some embodiments, the pH of the solution composition ranges from about 6.0 to about 9.4 (*e.g.*, from about 7.8 to about 9.2). In some embodiments, the solution comprises further includes one or more control substances for urine analyte assays. Such control substances may include, but are not limited to, glucose and bilirubin.

Example

[0028] Solutions containing hemoglobin (0.14 mg/dL) and sodium tetraborate at various concentrations were prepared, with pH values ranging from 8.0-9.0 at each borate concentration. The solutions were kept in the refrigerator overnight prior to filtration with a 0.2 μm filtration unit. The solutions were used for testing with iChemVELOCITY Urine Chemistry Strips and Arkray test strips with hemoglobin test pads containing impregnated TMB and cumene hydroperoxide, and color changes on the test strips were assessed with an iChemVELOCITY Urine Chemistry System or Arkray AX 4280instrument for hemoglobin quantitation by reflectance densitometry. 8-10 μL of solution was dispensed onto the test pads, and the reactions were allowed to proceed for 30-120 seconds. Reflectance readings were determined automatically by the iChem Velocity instrument. The pH of the blood pads was determined by adding 10 microliters of hemoglobin solution to the pads and reading pH directly from the pad after pH stabilized (one minute) with a flat surface Exstick (Extech) pH meter with automatic temperature compensation and three point calibration (pH 4.0, 7.0, 10.0) at 23 °C. Each treatment measure was averaged for 3 to 6 strips/treatment.

[0029] As shown in FIG. 1, the sensitivity of the measurements increased as the concentration of borate decreased, as indicated by the lower reflectance values measured at lower borate concentrations. Unexpectedly, the measurements exhibited much less pH-dependent variation at lower borate concentration as shown in FIG. 2. This was surprising because less borate buffer was expected to provide more pH variation, and the opposite was observed. The pH independence at lower borate concentrations is advantageous because more consistent results can be obtained over a wider range of test conditions.

[0030] Similar borate solutions were also used to test standard compositions employed in assays for bilirubin, urobilinogen, ketones, ascorbic acid, protein, nitrite, and leukocytes. The results indicated that the reflectance values obtained for these analytes were not affected at even the most extreme range of pH and sodium borate concentration (pH 8.8-9 and Borate factor 1 vs. pH 8-8.2 Borate factor 0.1). Reflectance values obtained in glucose assays were reduced by a small amount (2-3%) across the range of pH and sodium borate concentration, which did not impact the quality of the assay results. The borate solutions did not alter the measured pH values. The conditions used to obtain improved hemoglobin results did not change or decrease the quality of data obtained for assay of other urine analytes. This is particularly advantageous for parallel quantitation of multiple analytes.

[0031] Although the foregoing has been described in some detail by way of illustration and example for purposes of clarity and understanding, one of skill in the art will appreciate that certain changes and modifications can be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for determining the quantity of hemoglobin in a urine sample, the method comprising:

(i) contacting a first test strip with the urine sample, wherein the test strip comprises a test pad and a color correction pad;

(ii) measuring the color of the test pad after step (i) to obtain a first colorimetric value;

(iii) measuring the color of the color correction pad after step (i) to obtain a second colorimetric value;

(iv) comparing the first color value and the second color value to obtain a corrected color value;

(v) comparing the corrected color value to a known color value to quantify the hemoglobin in the urine sample;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide;

wherein the color correction comprises paper that is substantially free of impregnated chromogens or impregnated peroxides; and

wherein the known color value is determined by contacting a second test strip with a control composition comprising hemoglobin and a low-strength buffer.

2. A method for obtaining a color measurement for a hemoglobin control composition, the method comprising:

(a) contacting a test strip with the hemoglobin control composition, wherein the test strip comprises a test pad and a color correction pad;

(b) measuring the color of the test pad after step (a) to obtain a first color value;

(c) measuring the color of the color correction pad after step (a) to obtain a second color value; and

(d) comparing the first color value and the second color value to obtain the color measurement for the hemoglobin control composition;

wherein the hemoglobin control composition comprises hemoglobin and a low-strength buffer;

wherein the test pad comprises an impregnated chromogen and an impregnated peroxide; and

wherein the color correction pad is substantially free of impregnated chromogens or impregnated peroxides.

3. The method of claim 1 or claim 2, wherein the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.05 mg/dL to about 5 mg/dL.

4. The method of claim 3, wherein the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.1 mg/dL to about 2 mg/dL.

5. The method of any one of claims 1-4, wherein the concentration of the low strength buffer is less than 50 mM.

6. The method of any one of claims 1-5, wherein the low-strength buffer is a borate buffer.

7. The method of claim 6, wherein the concentration of the borate in the hemoglobin control composition ranges from about 1 mM to about 45 mM.

8. The method of any one of claims 1-7, wherein the pH of the hemoglobin control composition ranges from about 6.0 to about 9.2.

9. The method of any one of claims 1-8, wherein the impregnated chromogen is a benzidine.

10. The method of claim 9, wherein the benzidine is tetramethylbenzidine or a salt thereof.

11. The method of any one of claims 1-10, wherein the impregnated peroxide is cumene hydroperoxide.

12. The method of any one of claims 1-11, wherein measuring the color of the test pad and the color correction pad is conducted via reflectance densitometry.

13. The method of claim 12, wherein the reflectance densitometry includes illuminating the test pad and the color correction pad with light having a wavelength ranging from about 400 nm to about 700 nm.

14. The method of claim 13, wherein the light has a wavelength around 625 nm.

15. The method of any one of claims 12-14, wherein the reflectance densitometry further comprises recording an image of the test pad and an image of the color correction pad.

16. The method of claim 15, wherein the image of the test pad and the image of the color correction pad are recorded using a complementary metal oxide semiconductor (CMOS) image sensor.

17. The method of any one of claims 1 and 3-16, wherein the known color value is a reflectance value ranging from about 5% to about 60%.

18. The method of any one of claims 2-16, wherein the color measurement for the hemoglobin control composition is a reflectance value ranging from about 5% to about 60%.

19. A solution composition comprising hemoglobin and a low strength buffer, wherein the concentration of hemoglobin ranges from about 0.05 mg/dL to about 5 mg/dL.

20. The solution composition of claim 19, wherein the concentration of the low strength buffer is less than 50 mM.

21. The solution composition of claim 19, wherein the concentration of hemoglobin in the hemoglobin control composition ranges from about 0.1 mg/dL to about 2 mg/dL.

22. The solution composition of any one of claims 19-21, wherein the low strength buffer is a borate buffer.

23. The solution composition of claim 22, wherein the concentration of the borate buffer ranges from about 1 mM to about 45 mM.

24. The solution composition of any one of claims 19-23, wherein the pH ranges from about 6.0 to about 9.2.

25. The solution composition of claim 24, wherein the pH ranges from about 7.8 to about 9.2.

26. The solution composition of any one of claims 19-25, further comprising glucose, bilirubin, or a combination thereof.

FIG 1.

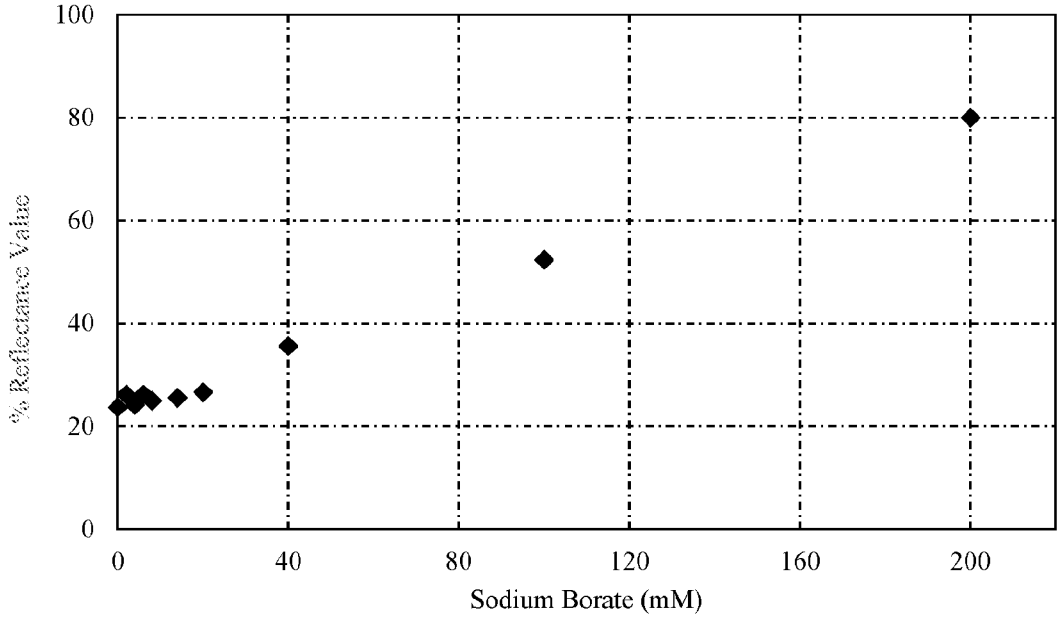
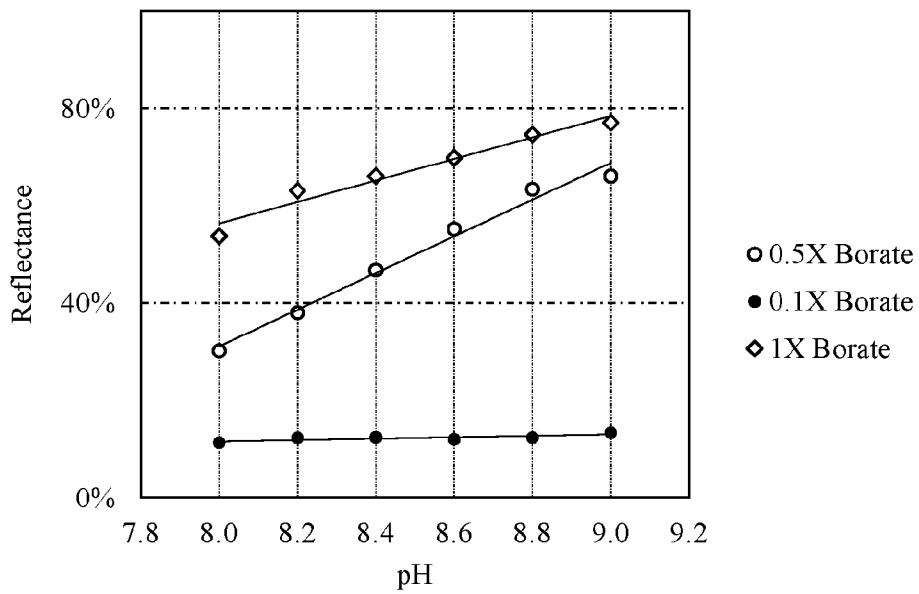

100
80
60
40
20
0
% Reflectance Value
0
40
80
120
160
200
Sodium Borate (mM)

FIG 2.


80%
40%
0%
Reflectance
7.8
8.0
8.2
8.4
8.6
8.8
9.0
9.2
pH
0.5X Borate
0.1X Borate
1X Borate

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/067904

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/52 G01N33/72 C12Q1/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 444 273 A1 (MILES INC [US]) 4 September 1991 (1991-09-04) the whole document -----	1-26
X	EP 0 444 263 A1 (MILES INC [US]) 4 September 1991 (1991-09-04) the whole document -----	1-26

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 April 2020	21/04/2020

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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