



(51) International Patent Classification:

G01N 33/48 (2006.01)

G01N 33/62 (2006.01)

G01N 33/50 (2006.01)

(21) International Application Number:

PCT/US2020/036000

(22) International Filing Date:

03 June 2020 (03.06.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/862,477

17 June 2019 (17.06.2019)

US

(71) Applicants: **DIGNITY HEALTH** [US/US]; 185 Berry Street, Suite 300, San Francisco, CA 94017 (US). **ARIZONA BOARD OF REGENTS ON BEHALF OF ARIZONA STATE UNIVERSITY** [US/US]; 1475 N. Scottsdale Rd., Ste 200, Scottsdale, AZ 85257 (US).

(72) Inventors: **GARCIA, Antonio**; 642 W. Morelos Street, Chandler, AZ 85224 (US). **LIEBERMAN, Abraham**; 240 W. Thomas Rd, Ste. 301, Phoenix, AZ 85013 (US).

(74) Agent: **SIDORIN, Yakov**; Quarles & Brady LLP, One South Church Ave, Suite 1800, Tucson, AZ 85701 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

(54) Title: SALIVARY UREA NITROGEN RAPID DETECTION

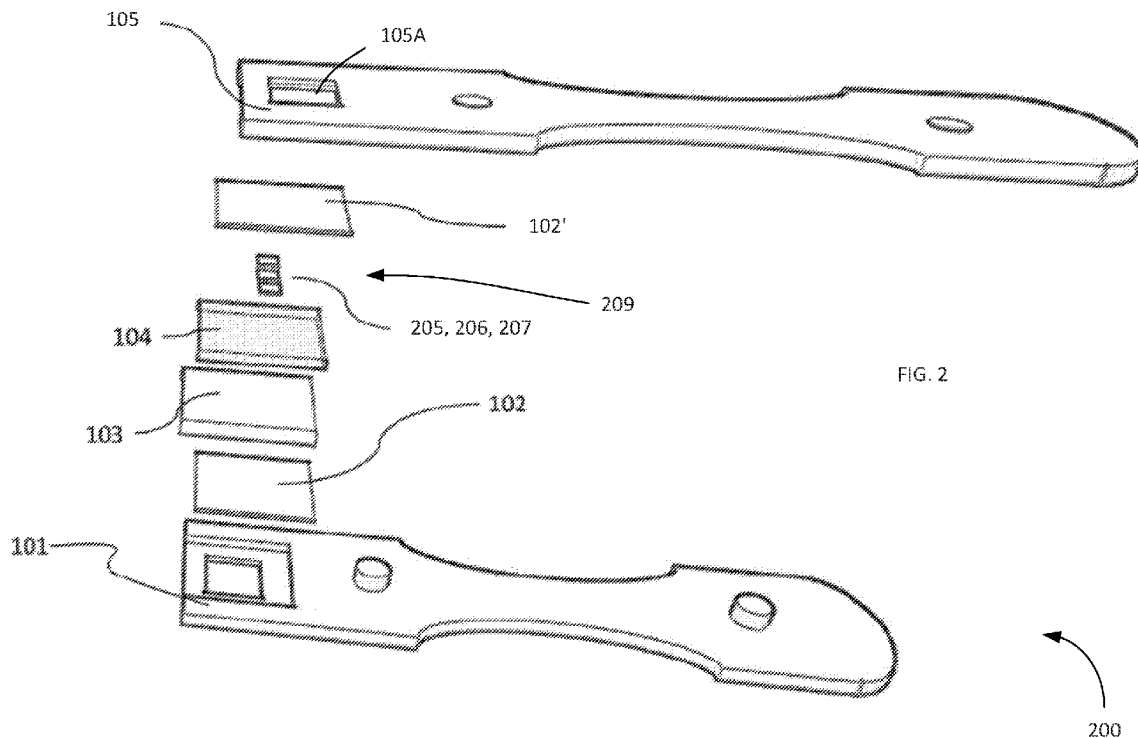


FIG. 2

(57) Abstract: Systems and methods for the rapid detection of at least two different target molecules using the same sample and same processing area. For example, a system comprises a porous pad, which is impregnated with a solution containing at least one chemical agent and contains an unfilled capillary matrix, and a gas sensor configured to detect at least two different gases, wherein the two different gases are indicative of the at least two different target molecules.

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

SALIVARY UREA NITROGEN RAPID DETECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority from the US Provisional Patent Application No. 62/862,477 filed on June 17, 2019, the disclosure of which is incorporated herein by reference.

RELATED ART

[0002] It is important and medically relevant to be able to identify patients whose blood pressure may drop to levels requiring hospitalization before such patients exhibit orthostatic hypotension (as orthostatic hypotension could lead to potentially unsafe situations or require urgent medical attention and/or hospitalization). If such patients are properly identified while sufficient time and resources remain for these patients to regain blood volume through properly formulated liquid imbibition and maintaining proper hydration, the desired patient outcome would be the minimization of hospitalizations for these groups of patients and therefore an improved quality of lives of the patients. Measuring the state of hydration of a person could also be used in outdoor or sports activities to monitor changes in urea concentration in saliva before any serious conditions occur. Another area of applications of checking for proper health maintenance procedures could be in care facilities.

[0003] Interest in non- or low-level invasive monitoring continued to grow in many bio- and/or medical technologies. A dehydrated state is an interesting and complex physiological condition that has been addressed in research literature via saliva and sweat testing and in cosmetics - with the use of skin-impedance monitoring devices. Saliva and sweat testing device electronics are still at the stages of research and development, and no accurate, rapid, and inexpensive means of determining key indicators such as urea in saliva are available to-date. Moreover, wearable or non-invasive hydration meters configured to operate based on measuring the skin impedance or other means continue facing various challenges that primarily stem from interpreting the result of a local measurement as being a true indicator of a systemic condition such as dehydration. A confounding issue here is likely present due to the body's control mechanisms directed to maintain homeostasis leading to a series of physiologically measurable changes such as skin dryness, which may or may not be directly relevant as a relevant measure if a person becomes dehydrated.

[0004] There remains a need, therefore, in a low cost and rapid test methodologies that are adequate and/or sufficient for a professions to make a decision as to whether a person is dehydrated or maintains normal level of hydration.

SUMMARY

[0005] Embodiments of the current technology relate to systems and methods for measuring a concentration of a target molecule from a biological sample.

[0006] Embodiments provide a system configured to measure concentrations of at least two target molecules from a single biological sample. Such system includes a porous pad impregnated with a solution that contains at least one chemical agent and a unfilled capillary matrix; and a gas sensor cooperated with the porous pad and configured to detect at least two different gases that are indicative of the at least two different target molecules. The system may additionally include a housing in which the porous pad is disposed and a hydrophobic and gas-permeable membrane placed to cover the porous pad in the housing. In substantially any implementation, the used chemical agent may include urease and/or the gas sensor maybe configured to detect a first one of the at least two different gases at a first time point and a second one of the at least two different gases at a second time point, the first and second time points being different from one another. Alternatively or in addition, the gas sensor may be configured to detect ammonia or an ammonia-containing gas and a non-ammonia material or non-ammonia-containing gas. In substantially any embodiment, the solution may be chosen to include at least one of polyhydroxy organic compounds selected from the group consisting of glycerol, sucrose, polysorbate, ethylene glycol, and propylene glycol. (Here, the chosen solution has a viscosity level higher than that of the biological sample to cause viscous fingering instabilities during mixing of the sample with the solution.) A particular embodiment of the system of invention may be additionally complemented a device configured to collect a biological sample, such device containing a funnel component (dimensioned to receive the biological sample), a container in fluid communication with the funnel and configured to store the biological sample; and a tray in fluid communication with the container and in fluid communication with the porous pad (here, the tray is dimensioned to deliver the biological sample to the porous pad to be absorbed therein). Substantially any embodiment of the system is configured to utilize and measure biological samples that include any of blood, serum, plasma, urine, saliva, spinal fluid, sweat, tears, vaginal fluid, mucous, and semen.

[0007] Embodiments additionally provide a method for measuring concentrations of at least two target molecules from a single biological sample with the use of an apparatus containing a porous pad. The method includes the steps of a) bringing such porous pad, impregnated with a solution containing at least one chemical agent and an unfilled capillary matrix, in contact with said single biological sample; and b) detecting at least two different gases, respectively indicative of the at least two target molecules, with a gas sensor. In at least one implementation of the method, the utilized apparatus includes a housing

in which the porous pad is disposed; and a hydrophobic and gas-permeable membrane placed to be carried by the porous pad; and such implementation may be configured to utilize urease as a chemical agent. In substantially any implementation, the step of detecting may include a process of detecting the at least two different gases with the gas sensor that is configured to detect one of the at least two different gases at a first moment of time and to detect another of the at least two different gases at a second moment of time, the first and second moments of time being different from one another; and /or a process of detecting the at least two different gases with the gas sensor configured to detect a first material that is not ammonia or a first-material-containing gas at the first moment of time and a second material that includes ammonia or the second-material-containing gas at the second moment of time that occurs after the first moment of time. Here, the first moment of time may be chosen to lag a moment when the porous pad came in contact with the biological sample by a time-period from about 1 minute to about 2 minutes, and a delay between the first moment of time and the second moment of time may be chosen to be between about 5 minutes and about 10 minutes. Alternatively or in addition, and in substantially any implementation of the method, the use can be made of the porous pad impregnated with the solution that includes a polyhydroxy organic compound selected from the group consisting of glycerol, sucrose, polysorbate, ethylene glycol, propylene glycol, and a combination thereof; and that has a viscosity level higher than that of the biological sample to create viscous fingering instabilities during the process of mixing of the sample with the solution. Substantially any implementation of the method may be configured to utilize the single biological sample that includes any of blood, serum, plasma, urine, saliva, spinal fluid, sweat, tears, vaginal fluid, mucous, and semen.

[0008] Embodiments further provide a method for measuring concentrations of at least two target molecules from a single biological sample. Such method includes bringing a single porous pad, impregnated with a solution that contains urease and a unfilled capillary matrix, with said biological sample; and detecting at least two different gases, respectively indicative of the at least two target molecules, with a gas sensor. In a specific case, the step of detecting may include detecting a first one of the at least two different gases and a second one of the at least two different gases at a different times.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The technology disclosed in this application will be better understood from the following description in conjunction with the in which:

[0010] FIGURE 1 is an exploded view illustrating one embodiment of the test apparatus of the invention.

[0011] FIGURE 2 is an exploded view illustrating another embodiment of the test apparatus.

[0012] FIGURE 3 shows an embodiment of a saliva-collecting apparatus in cooperation with an embodiment of the test apparatus.

[0013] FIGURE 4 is an exploded view illustrating another embodiment of the test apparatus.

[0014] FIGURE 5A is a plot illustrating a substantially linear dependence of the salivary urea nitrogen concentration generated from a gas sensor when an enzyme solution with density and viscosity greater than those of saliva is used.

[0015] FIGURE 5B presents data illustrating that insufficient mixing of the testing sample and urease (when using an enzyme solution with the density and viscosity that match the density and viscosity of saliva) produces non-linear results.

[0016] FIGURE 6 illustrates an embodiment of a method for measuring hydration levels configured according to an idea of the invention.

[0017] FIGURE 7 shows empirical data representing readouts of different urea concentrations as a function of time.

[0018] FIGURE 8 schematically illustrates an embodiment of a system featuring a porous pad and a gas sensor.

[0019] FIGURE 9 depicts data representing a comparison of Salivary Urea Nitrogen (SUN) using a DIUR-100 Assay and Prediction of SUN based on Sensor Calibration. Horizontal error bars show the uncertainty of 10% in the assay and potential errors introduced when measuring very small volume(s) while spiking a high concentration urea standard into the sample in order to simulate patients with moderate-to-high levels of salivary urea nitrogen.

[0020] FIGURE 10 depicts data collected based on seventeen saliva samples and four 20% polyethylene glycol solutions (blue filled circles). The dashed curve represents the predicted value based on the algorithm designed to assess the ammonia concentration in the sample that is also analyzed simultaneously for salivary urea nitrogen.

[0021] In the Drawings, generally, like elements and/or components may be referred to by like numerals and/or other identifiers; not all elements and/or components shown in one drawing may be necessarily depicted in another for simplicity of illustrations.

DETAILED DESCRIPTION

[0022] Embodiments of the invention address rapid and low-cost test and apparatus configured to determine the concentration of clinically- relevant molecules (such as urea) in biological fluids. For example, the concentration of urea in saliva of a person correlates with the state of hydration of such person. The embodiments of the test are configured to be easily administered and do not require instrumentation or expert knowledge.

[0023] The implementations of methodologies disclosed herein are described in one or more examples of embodiments. References throughout this specification to “one embodiment,” “an embodiment,” or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present technology disclosed herein. Thus, appearances of the phrases “in one embodiment,” “in an embodiment,” and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

[0024] The described features, structures, or characteristics of the disclosed technology may be combined in any suitable manner in one or more embodiments. In the following description, numerous specific details are recited to provide a thorough understanding of embodiments of the technology disclosed herein. One skilled in the relevant art will recognize, however, that the technology disclosed herein may be practiced without one or more of the specific details, or with other methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the technology disclosed herein.

[0025] Referring now to Figures 1, 2, and 4, several embodiments of an apparatus configured to measure a concentration of a target molecule in a biological sample are presented schematically. Referring specifically to Figures 1, an embodiment of the apparatus 100 is shown to include, among others, a bottom half housing 101, a top half housing 105, and a porous pad 103 disposed in between. The bottom half housing 101 and the top half housing 105 are structured to be attached together to form a cavity dimensioned to accommodate the porous pad 103. In this embodiment, a membrane 104 is placed on top of the porous pad 103 and couples to the top half housing 105. In one non-limiting example, the membrane 104 may be made of Teflon[®], while in other cases the membrane 104 may be made of polypropylene or polyethylene. Generally, to configure the membrane 104 any material that is substantially hydrophobic and gas permeable can be used. Further, a second water-proof membrane 102 is disposed underneath the porous pad 103 (such as to sandwich the pat 103 between the waterproof

membrane 102 and membrane 104) and couples to the bottom half housing 101. (Components 106 and 107 represent, respectively, a gas sensor and a housing and/or mount for the gas sensor, configured for use with the embodiment 100 as discussed below.) The top half housing 105 includes an opening 105A dimensioned for a gas sensor 106 to be brought in contact with the membrane 104.

[0026] Figure 2 illustrates another embodiment 200 of the apparatus of the invention with a strip 209, configured to indicate a target molecule level of presence. Similarly to the embodiment 100, apparatus 200 also includes the bottom half housing 101, the top half housing 108, and the porous pad 103. The bottom half housing 101 and the top half housing 108 are appropriately structured to be attached together to form a cavity dimensioned to receive and accept the porous pad 103. In this embodiment, the membrane 104 is disposed over the porous pad 103 and the target molecule level indicating strip 209 is disposed on top of (that is, is carried by) the membrane 104. (In some specific implementations, the target molecule level indicating strip 209 may include a multiplicity of areas or portions - such as those indicated as 205, 206, 207 in this example - to show low, medium, and high levels of concentrations of the target molecule, when detected.) Another (auxiliary) waterproof and transparent membrane 102' is placed over the target molecule level indicating strip 209 to provide coupling with the top half housing 105. The top half housing 105 contains the opening 105A to allow observation of the target molecule level indicating strip 209 through the membrane 102.

[0027] Further, Figure 4 illustrates yet another related embodiment 400 of the apparatus of the invention containing first and second porous pads 406A, 406B disposed between the bottom half housing 101 and the top half housing 105. The two porous pads 406A and 406B are separated by the waterproof membrane 102 when they are disposed in a cavity formed between the attached to one another the top half housing 105 and the bottom half housing 101.

[0028] In some embodiments - where different target molecules have to be detected - the apparatus may include a first target molecule indicating strip 412 and a second target molecule indicating strip 413. The first target molecule indicating strip 412 is shown to be disposed on top of (carried by) the first porous pad 406A with a waterproof membrane 104 placed in between. Similarly, the second target molecule indicating strip 413 is disposed underneath the second porous pad 406B. (In some specific implementations, at least one of the target molecule level indicating strips may include a multiplicity of areas or portions - as show schematically in Figure 4 - to show low, medium, and high levels of concentrations of the target molecule, when detected.) Yet some biomarker can be detected without the need for a membrane such membrane 104. For example, to detect hydrogen peroxide (as a reaction

product), colorimetric paper that is specific for hydrogen peroxide detection can be used without such a membrane.

[0029] Alternatively or in addition, waterproof membranes 416 laying over the first target molecule indicating strip 412 and/or the second target molecule indicating strip 413, respectively. All plastic components used for construction of the embodiments 100, 200, 400 of the apparatus of the invention would be of oral grade and sterile.

[0030] In one example, the embodiment 400 can be used to detect two different target molecules at the same time. To this end, in one case, the first porous pad 406A is impregnated with a first solution containing urease while the second porous pad 406B is impregnated with a second solution containing a different enzyme, (This different from the urease enzyme is judiciously chosen to react with creatinine to generate hydrogen peroxide. As result, the presence of hydrogen peroxide can be measured to determine the level of creatinine. The combined detection of urea and creatinine levels can be used to detect dehydration and assess kidney function of the patient.)

[0031] Figure 3 provides additional description by illustrating an assembled apparatus 301 (structured according to either embodiment 100 of FIG 1, or embodiment 200 of Figure 2, or embodiment 400 of Figure 4) in operable cooperation with an embodiment 300 of a biological sample collecting device. The sample-collecting device 300 a funnel 303, a container 302 cooperated with the funnel 303 at one end and a tray 304 at the other end of the container 302. As can be seen in Figure 3, the assembled porous pads and membranes protrude, 310, out the attached to one another together top and bottom housings of the apparatus 201. The tray 304 contains an opening (shown as 314 on the side of the tray 304) dimensioned to receive these protruded portion 310, which can be inserted into the opening 314 can be inserted into the tray 304. After a biological sample is collected at the funnel 303 (see arrow 320), the collected sample flows or otherwise propagates down the funnel 303 into the container 302, through the container 302, and into the tray 304, to be absorbed or otherwise brought in contact with the portion(s) 310 of the porous pads of the apparatus 301 that are received at the tray 304 through the opening 310.

[0032] In order to quickly absorb and distribute the collected biological fluid (sample) substantially evenly through the porous pad(s), the porous pads 103, 406A, and 406B are pre-treated - for example, impregnated with an appropriately chosen water-miscible solution. In certain embodiments, the water-miscible solution comprises polyhydroxy organic compounds and an agent that is designed to release/extract a target molecule from the biological fluid. Depending on the specifics of a particular implementation, such agent is enzyme urease (which can cause ammonia to release from urea nitrogen contained in a biological fluid such as saliva). Two other examples are provided by: (1) the detection /

measurement of high levels of creatinine in saliva with the use of the enzyme creatinine deaminase (which produces ammonia); and (2) the detection of total cholesterol with the use of cholesterolase enzyme via detection of hydrogen peroxide.

[0033] The polyhydroxy organic compounds for use in embodiments of the invention are selected from the group consisting of glycerol, sucrose, polysorbate, ethylene glycol, propylene glycol, and a combination thereof. In certain embodiments, the water-miscible solution comprises a liquid that has a higher density and a higher viscosity than those of the biological fluids. In one embodiment, for example, the water-miscible solution contains 50% by volume of glycerol and 50% by volume of water. Such solution may also include components that facilitate increase of shelf life of urease. Typically, the biological fluids of interest include some of blood, serum, plasma, urine, saliva, spinal fluid, sweat, tears, vaginal fluid, mucous, and/or semen.

[0034] In addition, the porous pads used in embodiments of the invention could utilize gauze or other suitable materials that have capacity to contain an unfilled capillary matrix after being impregnated with the water-miscible solution. The unfilled capillary matrix allows the porous pad to be filled quickly with a biological fluid by capillary action. When the biological fluid contacts the water-miscible solution in a confined space (such as a capillary inside the porous pad), Korteweg stresses occur within the porous pad due to the differences in density of the biological fluid and that of the water-miscible solution. Alternatively or in addition, as a skilled artisan will readily appreciate, viscous fingering occurs when a less viscous liquid (such as the biological fluid) is introduced into a pore or capillary that contains a more viscous liquid (such as the water-miscible solution). Both phenomena occur spontaneously and have been observed to increase the contact area between the two miscible liquids and speed their combination into one liquid characterized by substantially uniform density and viscosity at time-scales much shorter than the time needed for Fick's law diffusion to create/produce the same level of mixing in a liquid. When the porous pad contacts a biological fluid, the biological fluid initially pushes (through capillary pressure) the water-miscible solution further into the porous pad while also filling smaller pores that were not filled by the water-miscible solution. This initial process can occur within 30 or so seconds. Then Korteweg stresses and viscous fingering process can occur over a lengthier period of time of about 10 minutes. Since the capillary filling process is self-limiting (due to reaching the limited capacity of the porous test pad), precise operator control of sample contact time is not required.

[0035] A skilled artisan will readily appreciate that the amount of the biological fluid absorbed by the porous pad of an embodiment of the invention can be adjusted over a wide range by employing porous pads of different sizes. In some embodiments, for example, a porous pad that is about 2 mm in thickness

and about 15 mm in length and width can absorb about 300 microliters of saliva quickly. Sometimes, a larger amount of biological fluid is needed in order to accumulate a target molecule for accurate measuring. To scale up or down the amount of absorbed biological samples, the width and length of the porous pads can be increased or decreased while maintaining the thickness of the porous pad (in this example - at 2 mm). Although specific dimensions of the porous pad are described herein, these dimensions are use as but an example and are not meant to be limiting.

[0036] Some embodiments of the current technology additionally contemplate a salivary urea nitrogen level testing kit. The kit includes a saliva collecting device, a porous pad, an ammonia level indicating strip, and a hydrophobic and gas permeable membrane disposed between the porous pad and the ammonia level indicating strip. Further, in some embodiments, the ammonia level indicating strip may be sectioned / partitioned to include a multiplicity - for example, three - portions configured to detect three different levels of the target molecule and indicate these different levels by displaying correspondingly-different visually-perceivable indicia - in one example, different colors (see portions 205, 206, 207 of the strip 209 in FIGURE 2) to show corresponding low, medium, and high levels of salivary urea nitrogen (SUN) concentrations. In one example, one portion of the strip is configured to display green color to indicate a normal level of salivary urea nitrogen the color yellow displayed at another portion of the strip could be used to indicates a borderline high level of SUN; and the color red could be used to represent a high level of SUN thereby indicating that a subject / patient is dehydrated. In other embodiments, the ammonia level indicating strip may be additionally operably cooperated with a liquid crystal display (LCD) readout panel configured to display the ammonia concentration in numeric format, for example.

[0037] A related embodiment of the salivary urea nitrogen level testing kit may include a saliva collecting device, a porous pad, a gas sensor, and a hydrophobic and gas-permeable membrane disposed between the porous pad and the gas sensor. In some embodiments, the ammonia gas sensor includes an ammonia gas sensor Arduino MQ137 and is configured to detect and measure urea concentrations in ammonia gas. In certain embodiments, this kit may further contain an ammonia level indicating element, which includes or is cooperated with an LCD readout panel. The LCD readout panel may be configured to provide a numeric measure of the urea concentration after the ammonia gas sensor acquires this urea concentration. Alternatively, the LCD readout panel may be configured to provide a continuous display of the increased urea concentration until a maximum readout of the corresponding urea concentration is reached at the ammonia gas sensor. When the maximum readout of the urea concentration is reached, the ammonia level indicating element may provide a prompt or indication to testing personnel by generating a

visually-perceivable or audibly-perceivable indicia or signal (for example, by sounding a beep). The maximum readout of the urea concentration may be reached after 1 minute, 3 minutes, 5 minutes, 10 minutes, or 15 minutes of operation, depending on the particular circumstances.

[0038] Now, referring to Figure 6, a method of utilizing either of the above-described embodiment of kits for measuring the salivary urea nitrogen level in a saliva sample is illustrated schematically. At step 630, a saliva sample is acquired. If the saliva sample needs to be diluted to reduce the ammonia level in the sample, such decision is made at step 632 and the dilution process is effectuated at step 634 by transitioning the sample through an appropriate unit/cartridge in which dilution of the ammonia in the sample is achieved. A person of skill in the art will recognize that, in some embodiments, diluting ammonia would be useful when a high level of urea-determination accuracy is needed under rapid dehydration cause by exertion. In other situations, however, the ammonia dilution in a saliva sample is not performed under gradual dehydration that occurs over a longer period of time due to environmental or other conditions. In this case - that is, if the ammonia level in the saliva sample does not need to be diluted - step 630 is transitioned to step 636, where the salivary urea is hydrolyzed to produce ammonia in the vapor phase. At the following step 638, the amount of ammonia in the vapor phase is measured either quantitatively or semi- quantitatively. In some embodiments, the ammonia gas reaches the indicator chemicals contained in the ammonia level indicating strip and causes a change of the color of the ammonia level indicating strip. Different colors of the ammonia level indicating strip indicate and/or represent different levels of ammonia and, therefore, different hydration levels of a subject and can be determined semi-quantitatively. In other embodiments, the level of the ammonia gas produced at step 636 can be measured with a gas sensor as described above.

[0039] The apparatus structured according to any of the embodiments 100, 200, and 400 can also be used to detect other biomarker molecules through enzymatic action that generates target molecules that are present in the vapor phase at room temperature or produce vapor pressure sufficient to be readily detected in the vapor above an aqueous solution. Non-limiting examples of the target molecules are those of carbon monoxide, carbon dioxide, hydrocarbons, or other gases readily detectable in air. Several enzymatic reactions can be employed to generate hydrogen peroxide: (1) in one, by adding the enzyme catalase, hydrogen peroxide can be converted to oxygen; (2) in heme oxygenase, carbon monoxide can be produced; and (3) isoprene may be detected from various metabolic reactions in other biological fluids and human breath.

Non-Limiting Example 1 – Detection of salivary urea nitrogen using a gas sensor

[0040] The porous gauze pad was loaded with 300 microliters of urease in 50% glycerol by volume with an enzyme activity of 6.4 units/ml. Buffer, saliva samples, and saliva samples containing added urea were analyzed twice by measuring the voltage output from the gas sensor over a total time of 15 minutes from the moment when the sample was brought in contact with the porous gauze pad. Proper procedure for saliva collection included drinking 5 ounces of water and refraining from food and drink for 30 minutes prior to sample collection. The collected saliva samples were not pretreated or diluted, and at least 300 microliters of sample is needed, although exact measurement is unnecessary due to the fixed liquid capacity of the gauze pad. All that was done once sufficient saliva had been collected was to allow contact of the sample with the pad for 20 seconds in order for capillary action to take place and be completed. The maximum average reading from 10 through 15 minutes after contact with the sample were referenced with buffer containing no urea and are graphed in Figure. 5A. Based on the technical literature, it is anticipated that normal salivary urea nitrogen level would mirror the levels in blood which is between 7 and 20 mg/dl, correspond to a gas sensor reading of below 0.5 V, while a reading considered to be of concern would be above 50 mg/dl corresponding to a gas sensor reading above 1 V. The results illustrate that saliva can be measured within the required range to distinguish between normal salivary urea levels and up to very high levels of salivary urea nitrogen within 15 minutes. In comparison with the results of Figure 5A, Figure 5B illustrates that insufficient mixing of the testing sample and urease (when using an enzyme solution with the density and viscosity that match the density and viscosity of saliva) produces non-linear results.

Non-Limiting Example 2 - Detection of salivary urea nitrogen using color indicators

[0041] Empirical evidence showed that a very reliable, common, and simple way to measure urea in saliva is to use the enzyme urease to convert urea into ammonia and suggested. Another important aspect of working with saliva is that it has pH buffered such that measuring ammonia by pH alone is prone to low sensitivity since ammonia at low levels will not raise the pH of a buffered solution and since the pH scale is logarithmic with respect to ammonia concentration. These two facts suggested to us that a key way of measuring urea in saliva is to rely on a very accurate method of detecting ammonia and employing urease in a simple and inexpensive way in a disposable kit format. The current test apparatus can use the equilibrium ammonia concentration evolved at saliva pH and does not employ pH raising agents. According to the idea of the invention, the ammonia gas is measured by adding to the apparatus a

nanoporous water-repelling membrane to cover the porous pad and placing color changing test pads or a gas sensor over the membrane.

[0042] A color changing test strip that is specific for ammonia regardless of pH is used in order to keep the initial clinical testing simple. Particularly, Pad 1 as listed in Table 1 is the color indicator from Hach ammonia indicator test strip, and Pads 2 and 3 as listed in Table 1 are from Whatman, Panpeha Plus test strips that provide color variations in the range from about 5.5 to about 9.0 of pH. In the color indicator pads, ammonia permeating through the membrane is generating the color change, so the color does not reflect the pH of the liquid sample.

[0043] The porous gauze pad was loaded with 200 microliters of urease in 50% by volume of glycerol with an enzyme activity of 6.4 units/ml. Buffer, saliva samples, and saliva samples containing added urea were analyzed twice by measuring the gas sensor voltage output over a total elapsed time of 15 minutes from when the sample was contacted with the porous gauze pad. Proper procedure for saliva collection included drinking 5 ounces of water and refraining from food and drink for 30 minutes prior to sample collection. The collected saliva samples were not pretreated or diluted, and at least 200 microliters of sample were needed, although exact measurement was unnecessary due to the fixed liquid capacity of the porous gauze pad. All that was done once sufficient saliva is collected. The saliva sample was allowed to contact the porous gauze pad for about 20 seconds in order for capillary action to be completed.

[0044] Table 1. Average values of Color Scale from lowest (1) to highest (7).

Salivary Urea Nitrogen, mg/dl	Average Pad 1 Color Scale	Average Pad 2 Color Level	Average Pad 3 Color Level	Indicated Reading Using a Chart
0	1	1	1	No Urea/No sample
23	2	2	2	Normal
30	3	5	5	High
41	3	5/6	5/6	High
58	5	6/7	6/7	Mid-Range High
85	7	7	7	Highest Level

[0045] As noted in Table 1, the color pads are used in combination with reference to the chart developed based on observations of how the indicator paper responds to different levels of ammonia

vapor above a pH 7 solution with a hydrophobic membrane barrier. The color variations as a group are useful for detecting a wider range since Pad 1 is mostly used to indicate that the saliva sample was properly detected. If a saliva sample did not register above the original color denoted as color scale level 1, then there was a problem with the sample or it was a negative control. However, Pad 1 proved to be not very sensitive to changes in some of the lower parts of the salivary urea nitrogen scale and Pad 2 was best used for matching the color in the normal to borderline high level. In turn, Pad 3 became most useful in the high range. Above very high levels of salivary urea nitrogen, all of the color indicators achieved the maximum shade (level 7) within their respective scales. This methodology determines the concentration of urea in saliva by generating a color change that can be observed visually and compared to a color chart. The concentration of urea in saliva correlates with hydration state of a person. The test is rapid, low cost, and does not require instrumentation or expert operation.

Non-Limiting Example 3

[0046] A very reliable, common, and simple way to measure urea in saliva is to use the enzyme Urease to convert urea into ammonia. Another important aspect of working with saliva is that it is pH buffered so that measuring ammonia by pH alone is prone to low sensitivity since ammonia at low levels will not raise the pH of a buffered solution and since the pH scale is logarithmic with respect to ammonia concentration. These two facts suggested to us that a key way of measuring urea in saliva is to rely on a very accurate method of detecting ammonia and employing Urease in a simple and inexpensive way in a disposable kit format.

[0047] Therefore, a color changing test strip was chosen that was specific for ammonia regardless of pH and - in order to keep the initial clinical testing simple - it was decided to use a commercial test strip that is calibrated for the low end of ammonia concentration (0 – 6 ppm). The test strip relies on measuring ammonia by raising the pH of the test solution to force ammonia to permeate a membrane by converting it from the ionic to its dissolved gas form. Due to the low range of the test strip, we employ a rapid means of removing high levels of ammonia in the saliva sample prior to the Urease reaction step. This step currently adds 5 minutes to the overall test, but very little cost due to the use of a small amount of ammonia cation exchange beads in a 3-D printed cartridge. An added advantage of scaling back the ammonia in saliva to low levels is that if a patient has high SUN for a period of time, there will be more ammonia left in the saliva sample and the combined test result will be an accurate, commensurately higher reading.

[0048] The information below describes the empirical results procured with a 10 Minute SUN kit with healthy volunteers of ages 19-22 and 57. Here, a commercial skin impedance measuring device (New Spa SK-02 Skin Analyzer) was employed as a reference when testing the “10 Minute SUN” disposable kit. This skin impedance measuring device is one of many devices that provide dermatological information as the percent hydration of the skin, and it is not designed for medical purposes. Average readings of the face above 37% indicate what is considered to be normal level of hydration of skin, while readings from 32%- 36% are considered to indicate dry skin, and reading below 31% are considered to represent very dry skin.

[0049] 10 Minute SUN Beta Test Kit Results.

[0050] After calibrating the components of the test kit using laboratory instrumentation and simulated saliva solutions, two days of testing for all components of the kit were completed. The data are summarized in the following table. Individual volunteers are referenced in the table using a 4-digit code. The results in Table 1 were useful to help illustrate that the beta 10 Minute SUN test kit can be performed easily outside of the laboratory (testing was done in an office), and that the information provided is within the expected range of values. These data are not referenced with specific SUN or BUN (blood urea nitrogen) values, due to the limited capabilities of our ASU laboratory for human testing. Qualitative information gathered during these two days of testing that is not reflected in the table is that the mucin in saliva made operating the steps easier by lubricating the 3-D printed devices used to remove excess ammonia from the acquired saliva sample as well as lubricating the 3-D printed slider with Urease enzyme immobilized on the slider pad.

[0051] Table 2. Summary of Beta 10 Minute SUN test kit results with skin impedance measurements for 3 volunteers.

ID	Date	SKIN IMPEDANCE INFORMATION						SUN TEST RESULTS		
		Forehead	Face 1	Face 2	Neck	Wrist	Average	Test Strip Reading	Urea, mg/dl	Conclusion
2108	2/21/17	36	33	33	33	34	34	3	10	Normal
2108	2/21/17	35	33	30	34	33	33	3	10	Normal
6113	2/22/17	37	32	34	36	34	35	6	30	Normal
6113	2/22/17	34	35	35	38	34	35	6	30	Normal
2210	2/23/17	30	34	34	35	32	33	6	30	Normal
2210	2/23/17	29	33	34	35	30	32	8	45	Normal-High
1234	2/21/17	39	39	37	42	38	39	6	30	Normal
1234	2/21/17	35	39	36	38	38	37	2	8	Normal
1234	2/22/17	40	39	37	44	40	40	7	40	Normal
1234	2/22/17	40	37	36	40	38	38	4	15	Normal
1234	2/23/17	38	8	36	38	34	31	7	40	Normal

[0052] Prior to and post testing, Urease results and ammonia levels found in saliva were corroborated with the use of a spectrometer to track the change in phenolphthalein with pH as ammonia is introduced or when Urease produces ammonia. A laboratory in the Biodesign Institute working to produce urease from waste seed has been working with our laboratory to compare the standard method of measuring Urease activity (e.g., Nessler Method) with our measurements, and there is a reasonably good agreement between the two. Our spectroscopic method does not produce any hazardous waste and is a relatively simple method that can be accomplished with a portable spectrometer as well, if additional field-lab testing is needed.

[0053] 10 Minute SUN Beta Test Kit Details

[0054] The beta test kit used plastic disposable components, a timer, and a color reference card. No power or measurement instrumentation was required. The consumables of the test kit were provided in a sealed clear plastic container and should be stored in a refrigerator at approximately 4 °C, and the enzyme, beads, and test strip were expected to have a shelf life at 4 °C of up to one year. (This was due to the way the Urease was stabilized through formulation and entrapment onto a solid support.) The shelf life of the formulation has been tested in our refrigerator since July 2016, and we have thus far found that the enzyme appears to maintain its reactivity for 7 months. The entrapment method should extend the shelf life even more, based on the extensive literature of how immobilizing antibodies and enzymes increase shelf life of these proteins.

Non-Limiting Example 4

[0055] Using simulated saliva, experiments were conducted with 40 mg/dl and 10 mg/dl of Urea. The simulated saliva has buffering capacity to maintain the pH at 7.4, but with high ammonia production it is expected that buffering is not maintained. For 40 mg/dl Urea, complete conversion would result in approximately 227 ppm Ammonia, while for 10 mg/dl complete conversion would product 57 ppm Ammonia in solution.

[0056] A 12 mm x 12 mm x 3 mm thick porous gauze pad was soaked in 200 microL solution of 50 v.% glycerol/ 50 v.% water with urease and dried overnight in the refrigerator. The porous gauze pad was able to quickly wick up about 1 ml of simulated saliva. The porous gauze pad performed in the same manner when placed in a 3-D printed cartridge assembled based on the line drawings. Measurements with several color indicator paper preparations with a gas permeable membrane showed that ammonia in the gas phase can be detected while the enzymatic reaction is taking place. Room temperature experiments indicate that after 5 minutes readings can be taken. The result of universal indicator paper color change indicated that 40 mg/dl Urea simulated saliva can be distinguished from 10 mg/dl simulated saliva. The calibration of the paper with ammonia standards in simulated saliva provided values of between 100 – 1,000 ppm ammonia for 40 mg/dl and 50 ppm ammonia for 10 mg/dl Urea. These values were within the expected range.

Non-Limiting Example 5

[0057] An ammonia gas sensor (MQ 137) programmed to display voltage using an Arduino microcomputer was integrated into the SUN Device. Using 500 microliter pre-imbibed Urease solution onto a gauze pad, 1 ml of simulated saliva at pH 7 containing either 10, 20, and 40 mg/dl of Urea respectively was applied to the pad and recording commenced immediately. The final design would used a "razor and blades" model, whereby the gauze pad and saliva contacting portion would be detachable from the small sensor so that the electronics would be retained for more measurements while disposing of that portion of the device.

[0058] The data and a mathematical curve fitting are presented in Figures 7. While the signal from the sensor is non-linear, the signal behavior is predictable due to the way that measurement is taken. First, at the very beginning of the reaction of Urease with urea the rate of signal generation is slow to register since ammonia generated is at low concentration and at pH 7, which means that most of the ammonia is not in the dissolved gas form. As the reaction continues and ammonia reaches threshold value, the rate is at its highest since the pH is at the optimum value for Urease activity of about pH = 7-8. Once the amount of urea begins to be depleted and the concentration of ammonia increases enough to increase the pH of the solution, the rate of reaction slows but the signal continues to increase.

[0059] The mathematics of the signal generation can be modeled as a logistic curve series due to the different limitations in urea and enzyme activity as well as aqueous ammonia equilibria shift. In Figure 7, it appears that reading the signal at 5 minutes (300 seconds) is a useful way to clearly distinguish between 10 mg/dl (75 ppm) and 20 mg/dl (84 ppm) of Urea, as well as a strong difference as compared to 40 mg/dl urea (92 ppm). The logistic series used to fit the data is of the form:

$$[0060] \ C(t) = \frac{K_1}{1+e^{-r_1(t-t_{1,0})}} + \frac{K_2}{1+e^{-r_2(t-t_{2,0})}} + \frac{K_3}{1+e^{-r_3(t-t_{3,0})}}$$

[0061] where the maximum concentration, rates, and initial times used for curve fitting are given in the Table 3 below. The third term was only significant for curve fitting the highest urea concentration, which is understandable since the ammonia increase that shifts pH causes a more complex overall signal.

[0062] Table 3.

Urea Concentration, mg/dl	K ₁	r ₁	t _{1,0}	K ₂	r ₂	t _{2,0}	K ₃	r ₃	t _{3,0}
10	81	.0087	119	7	0.21	23	NA	NA	NA
20	74	.0835	120	18	0.0011	24	NA	NA	NA
40	62	0.013	200	24	0.0047	115	26	.096	4

Non-Limiting Example 6

[0063] In some embodiments, a system configured to measure concentrations of at least two target molecules from a single biological sample is disclosed. Preferably, the single sample is analyzed in one area (e.g., a single porous pad) rather than on multiple areas/pads. In this example, the system includes a porous pad impregnated with a solution containing at least one chemical agent (e.g., urease) and a unfilled capillary matrix, and a gas sensor configured to detect at least two different gases, wherein the two different gases are respectively indicative of the at least two different target molecules. For example, the gas sensor is configured to detect an ammonia or ammonia-containing gas and a non-ammonia or non-ammonia-containing gas.

[0064] Thus, the gas sensor (e.g., those available through SainSmart) may be a gas sensor 800 programmed to display (output) voltage using a programmable processor (in one specific case - an Arduino microcomputer) and placed in close proximity to porous pad 103 (see Figure 8; as shown in embodiments 100- with several elements interjecting in between the gas sensor 106 and the pad 103). Using 500 microliter pre-imbibed urease solution onto a gauze pad, 1 ml of simulated saliva at pH 7 containing either 10, 20, and 40 mg/dl of Urea respectively was applied to the pad and recording commenced immediately. Since the gas sensor is configured/programmed to detect a non-ammonia or non-ammonia-containing gas (e.g., carbon monoxide) at a first moment of time and an ammonia or ammonia-containing gas at a subsequent moment of time, the detection reading commenced at the first time point delayed by about 1 to 2 minutes from a moment when the porous pad has been brought in contact with the biological sample, with the subsequent moment of time being delayed by about 5 to 10 minutes after commencement of recording, to detect the ammonia or ammonia-containing gas. The data and a mathematical curve fitting thus form two separate peaks (with the first peak representing the

detected concentration of the non-ammonia gas and the second peak representing the ammonia gas concentration that, as explained above, is correlated to urea concentration in the biological sample.

Non-Limiting Example 7:

[0065] In this example, salivary urea nitrogen detection was achieved by using saliva samples, saliva spiked with urea, and a viscous solution spiked with urea. Quantitative analysis of fresh saliva required a device capable of managing its viscoelastic properties while accurately assessing target biomarkers in a sample that can scatter light due to proteins and microscopic debris. Moreover, saliva can contain dissolved gases due to underlying liver or kidney disease as well as digestive or oral health conditions. Viscoelasticity of the saliva sample facilitates accurate transfer of the sample to a test strip or assay kit challenging, thereby compromising accurate quantitation. Saliva light scattering properties create the need for some assay kits to rely on centrifugation in order to improve optical clarity. Urea detection relying on the enzyme Urease reaction to form ammonia that changes pH is subject to interference from dissolved ammonia.

[0066] Using some of the embodiments described above - and, specifically, the ones utilizing Korteweg stresses and viscous fingering (or viscous fingering instabilities) to mix the saliva sample directly after collection - salivary urea nitrogen and dissolved ammonia could be detected on the same sample in about 10 minutes. A gas sensor sensitive to ammonia vapor, records ammonia evolved from the test strip over time. Based on repeat studies of saliva and liquids with varying viscoelastic properties containing urea and ammonia, an algorithm was developed in order to predict salivary urea nitrogen and the level of dissolved ammonia.

[0067] Prepared test strips (containing urease enzyme) were connected to a sensor operated by a microprocessor and display. An adaptor connected to the test strip was used to automatically transfer a funnel with a breakable seal at the bottom. Saliva was collected by allowing it to pool in the mouth and with the following exculpation, at about 15 minutes after drinking 500 mls of water. The saliva sample was collected in the amount of at least 650 microL is collected, with the approximate average time of the collection of 2-4 minutes.

[0068] Once sufficient saliva was collected, the sample was flowed through the adaptor and the start button of the sensor was depressed since the saliva sample was now exposed to the test strip. Sensor data was collected for 10 minutes and software used the data in a proprietary algorithm in order to determine salivary urea nitrogen. In this example, ammonia gas dissolved in saliva and calibration samples is lower

than 15 ppm, which is within the range of normal (e.g., no underlying conditions generating high levels of ammonia or high bacterial production of ammonia due to poor oral health).

[0069] The algorithm prediction for 20 saliva samples and 4 high viscosity samples (20% weight percent polyethylene glycol in phosphate buffered saline at pH = 7.4) are shown in Figure 9. The assay used to determine the salivary urea nitrogen (DIUR-100, QuantiChrom Urea Assay Kit, BioAssay Systems) did not require pretreatment of biological samples, was based on the Jung method, and measured urea directly. In order to obtain saliva samples at higher salivary urea concentrations, a 100 mg/ml Urea aqueous solution was added to 1 ml of saliva in the range of 1-20 microliters in order to achieve desired SUN values. Several key features of the test strip can be noted in Figure 9. First, saliva samples over a wide range of SUN could be measured without dilution or sample pretreatment. Other quantitative assay systems have narrower ranges of detection, requiring dilution or some other sample pretreatment.

[0070] Secondly, the test was quantitative yet did not require careful measurement of sample volume. The sample specification was based on a minimum volume needed in order to wet the sample test strip pores, and continuous mixing due to viscous fingering (or viscous fingering instabilities) and Korteweg stresses controlled the mixing of the sample with urease enzyme in order to generate the ammonia gas being analyzed. Thirdly, all samples above normal salivary urea nitrogen were correctly identified. Fourth, saliva's complex viscous behavior was simulated using a high concentration of polyethylene glycol. The predicted SUN values agreed well with independent measured values regardless whether saliva, spiked saliva, or polyethylene glycol solutions were used. Finally, the prediction algorithm is based on calibration of the sensor with aqueous ammonia standards and sensor performance reported by the manufacturer. Accuracy, sensitivity, and response were based on the properties of the gas sensor used, and improvements or changes in gas sensor technology could be readily adapted using the algorithm and test strip designed for this application.

Non-Limiting Example 8

[0071] In this example, the detection of ammonia gas dissolved in saliva was demonstrated. Using the same procedure as described in Example 7, ammonia gas dissolved in the saliva sample could be detected along with salivary urea nitrogen. Output values produced by the algorithm based on analyzing the sensor signal over the 10 minutes used to detect salivary urea nitrogen are shown with a dashed curve in Figure 10. The data from 17 saliva samples and 4 polyethylene glycol solutions are also expressed in Figure 10 with closed circles. Higher concentrations of ammonia were obtained by spiking 1-50

microliters of a concentrated ammonia solution (10,000 ppm ammonium hydroxide) into 1 ml of saliva or polyethylene glycol samples. Ammonia in saliva was measured using a Hach colorimetric ammonia test strip for water, which increased the pH of the sample generating ammonia gas that was measured after passing through a membrane and changing the color of the test strip depending upon the range of ammonia measured (between 0-6 ppm). Saliva samples higher than 6 ppm ammonia required dilution in order to stay within the range of detection of the Hach test strip. The error bars of 25% in Figure 10 are based on uncertainty of accurately identifying the color of the test strip and in spiked ammonia sample preparation.

[0072] Several key features of the technology are illustrated in this example. First, dissolved ammonia gas present in the sample and urea can be measured simultaneously and independently. This is useful in order to distinguish between salivary urea and dissolved ammonia gas. Under certain chronic medical conditions, ammonia can be elevated at levels inconsistent with conversion of urea into ammonia due to bacteria. By rapidly detecting fresh passively collected saliva and simultaneous measurement of both biomarkers independently, there is an improved ability to screen for conditions such as chronic kidney or liver disorders. Also, in order to better screen for mild to moderate dehydration, measuring both biomarkers can better identify whether salivary urea nitrogen is high rather than the sample containing high dissolved ammonia.

[0073] Urease as the measuring reagent is advantageous due to its low cost and non-toxic characteristics since it is an enzyme vital to the ecological nitrogen cycle and is present in foods. Secondly, the range of detection is much greater than the range of ammonia that can be present in saliva while having sensitivity to distinguish between normal (about 6 ppm ammonia) and elevated ammonia within the expected elevated dissolved ammonia range of 30-50 ppm. Thirdly, measurement of two biomarkers within a time span of several minutes with the use of technology that has inherently lower cost (due to inexpensive disposable test strip reagent and reusable microelectronics based on low-cost gas sensors) provides a means for tracking on a regular basis (daily or weekly) to determine longer term trends or major changes in baseline values. This can be useful for monitoring patients out of hospital or doctor's offices or for evaluating physiological response for wellness purposes based on activity, diet, or training regimens. Fourthly, detecting ammonia via a gas sensor avoids potential error and inaccuracy in measurement as compared to colorimetric or potentiometric measurement of pH, which are frequently used and are indirect measurements of the weak base ammonia hydroxide that is formed in aqueous solution.

[0074] The data shown in Figure 10 illustrate that the proposed methodology is configured to consistently distinguish between normal and high dissolved ammonia, thereby making it practical to be used as a screening test to determine whether further testing to determine the underlying cause of high salivary ammonia gas is needed.

[0075] Any given embodiment of the apparatus of the invention may incorporate a programmable computer processor configured to perform the determination of the sought-after characteristic parameter(s) of the sample under test, or be operably coupled to a computer with program code implemented to perform such determination.

[0076] Reference throughout this specification to “one embodiment,” “an embodiment,” or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment,” “in an embodiment,” and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

[0077] The described features, structures, or characteristics of the invention may be combined in any suitable manner in one or more embodiments. In the following description, numerous specific details are recited to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that the invention may be practiced without one or more of the specific details, or with other methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the invention.

[0078] For the purposes of this disclosure and the appended claims, the use of the terms "substantially", "approximately", "about" and similar terms in reference to a descriptor of a value, element, property or characteristic at hand is intended to emphasize that the value, element, property, or characteristic referred to, while not necessarily being exactly as stated, would nevertheless be considered, for practical purposes, as stated by a person of skill in the art. These terms, as applied to a specified characteristic or quality descriptor means "mostly", "mainly", "considerably", "by and large", "essentially", "to great or significant extent", "largely but not necessarily wholly the same" such as to reasonably denote language of approximation and describe the specified characteristic or descriptor so that its scope would be understood by a person of ordinary skill in the art. In one specific case, the terms "approximately", "substantially", and "about", when used in reference to a numerical value, represent a range of plus or minus 20% with respect to the specified value, more preferably plus or minus 10%, even more preferably plus or minus 5%, most preferably plus or minus 2% with respect to the specified value. As a non-limiting example, two values being "substantially

equal" to one another implies that the difference between the two values may be within the range of $\pm 20\%$ of the value itself, preferably within the $\pm 10\%$ range of the value itself, more preferably within the range of $\pm 5\%$ of the value itself, and even more preferably within the range of $\pm 2\%$ or less of the value itself.

[0079] The use of these terms in describing a chosen characteristic or concept neither implies nor provides any basis for indefiniteness and for adding a numerical limitation to the specified characteristic or descriptor. As understood by a skilled artisan, the practical deviation of the exact value or characteristic of such value, element, or property from that stated falls and may vary within a numerical range defined by an experimental measurement error that is typical when using a measurement method accepted in the art for such purposes.

[0080] While the preferred embodiments of the present technology have been illustrated in detail, it should be apparent that modifications and adaptations to those embodiments may occur to one skilled in the art without departing from the scope of the present technology.

CLAIMS

What is claimed is:

1. A system configured to measure concentrations of at least two target molecules from a single biological sample, the system comprising:
 - a porous pad impregnated with a solution that contains at least one chemical agent and a unfilled capillary matrix; and
 - a gas sensor cooperated with the porous pad and configured to detect at least two different gases that are indicative of the at least two different target molecules.
2. The system of claim 1, further comprising:
 - a housing containing said porous pad; and
 - a hydrophobic and gas-permeable membrane placed to cover the porous pad in the housing.
3. The system of claim 1, wherein the at least one chemical agent comprises urease.
4. The system of claim 1, wherein the gas sensor is configured to detect a first one of the at least two different gases at a first time point and a second one of the at least two different gases at a second time point, the first and second time points being different from one another.
5. The system of claim 3, wherein the gas sensor is configured to detect ammonia or an ammonia-containing gas and a non-ammonia material or non-ammonia-containing gas.
6. The system of claim 1, wherein the solution comprises polyhydroxy organic compounds selected from the group consisting of glycerol, sucrose, polysorbate, ethylene glycol, propylene glycol, and a combination thereof; and the solution has a viscosity level higher than that of the biological sample to cause viscous fingering instabilities during mixing of the sample with the solution .
7. The system of claim 1, further comprising a biological sample collecting device that includes:
 - a funnel dimensioned to receive the biological sample;
 - a container in fluid communication with the funnel and configured to store the biological sample;and

a tray in fluid communication with the container and in fluid communication with the porous pad, the tray configured to deliver the biological sample to the porous pad to be absorbed therein.

8. The system of claim 1, wherein the biological sample includes blood, serum, plasma, urine, saliva, spinal fluid, sweat, tears, vaginal fluid, mucous, or semen.

9. The system of claim 1, wherein the biological sample is saliva.

10. **A method** for measuring concentrations of at least two target molecules from a single biological sample with the use of an apparatus containing a porous pad impregnated with a solution containing at least one chemical agent and an unfilled capillary matrix, the method comprising:

bringing a porous pad, impregnated with a solution containing at least one chemical agent and an unfilled capillary matrix, in contact with said single biological sample; and

detecting at least two different gases, respectively indicative of the at least two target molecules, with a gas sensor.

11. The method of claim 10, comprising utilizing the apparatus that contains
a housing upon which the porous pad is disposed; and
a hydrophobic and gas-permeable membrane placed to be carried by the porous pad.

12. The method of claim 10, wherein the at least one chemical agent is urease.

13. The method of claim 10, wherein the detecting includes detecting the at least two different gases with the gas sensor configured to detect one of the at least two different gases at a first moment of time and to detect another of the at least two different gases at a second moment of time, the first and second moments of time being different from one another.

14. The method of claim 13, wherein the detecting includes detecting the at least two different gases with the gas sensor configured to detect a first material that is not ammonia or a first-material-containing gas at the first moment of time and a second material that includes ammonia or the second-material-containing gas at the second moment of time that occurs after the first moment of time

15. The method of claim 14, wherein the first moment of time lags a moment when the porous pad came in contact with the biological sample by a time-period from about 1 minute to about 2 minutes, and a delay between the first moment of time and the second moment of time is between about 5 minutes and about 10 minutes.

16. The method of claim 10, comprising using the porous pad impregnated with the solution that includes a polyhydroxy organic compound selected from the group consisting of glycerol, sucrose, polysorbate, ethylene glycol, propylene glycol, and a combination thereof; and that has a viscosity level higher than that of the biological sample to create viscous fingering instabilities.

17. The method of claim 10, configured to utilize the single biological sample including any of blood, serum, plasma, urine, saliva, spinal fluid, sweat, tears, vaginal fluid, mucous, and semen.

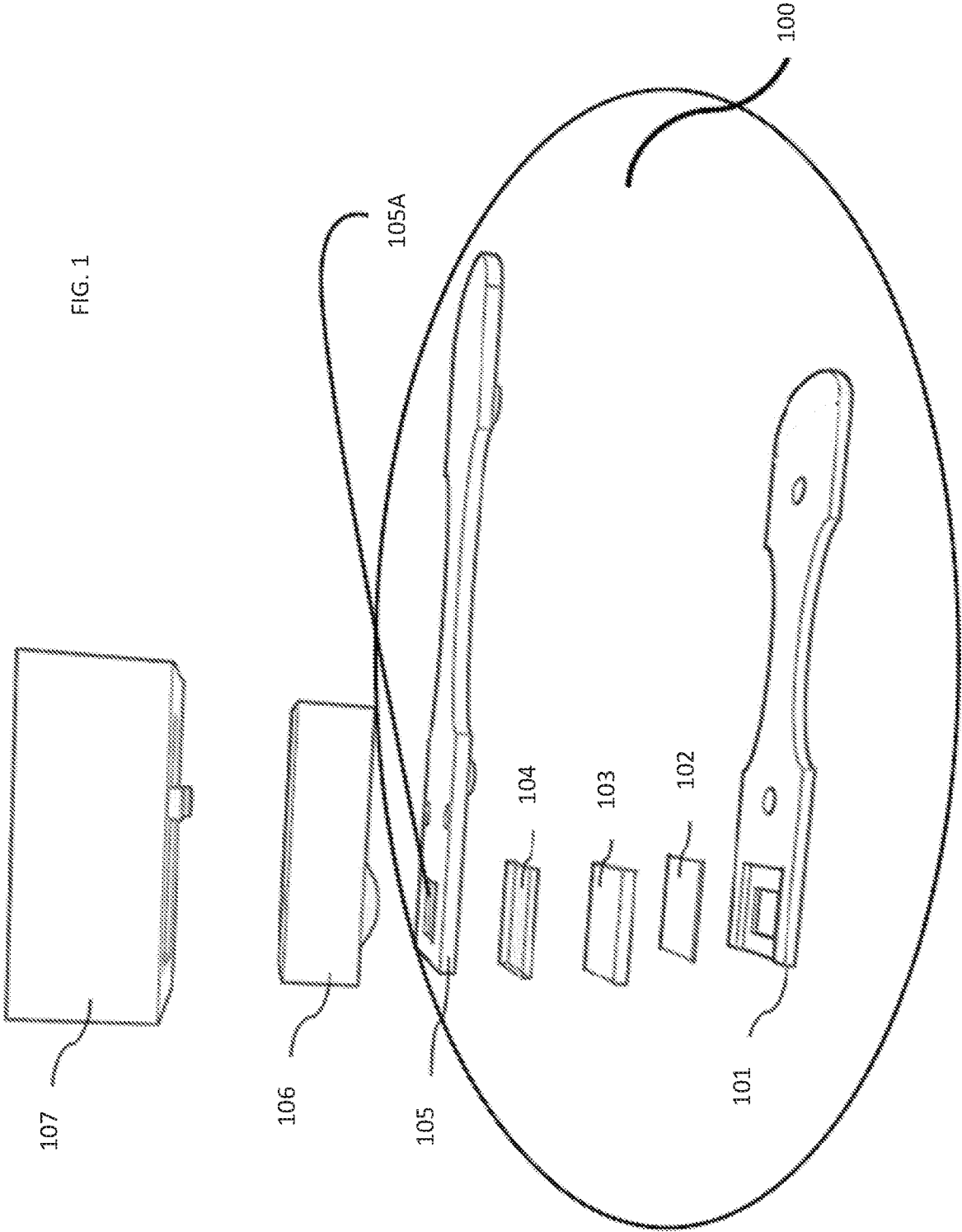
18. The method of claim 10, configured to utilize saliva as the single biological sample.

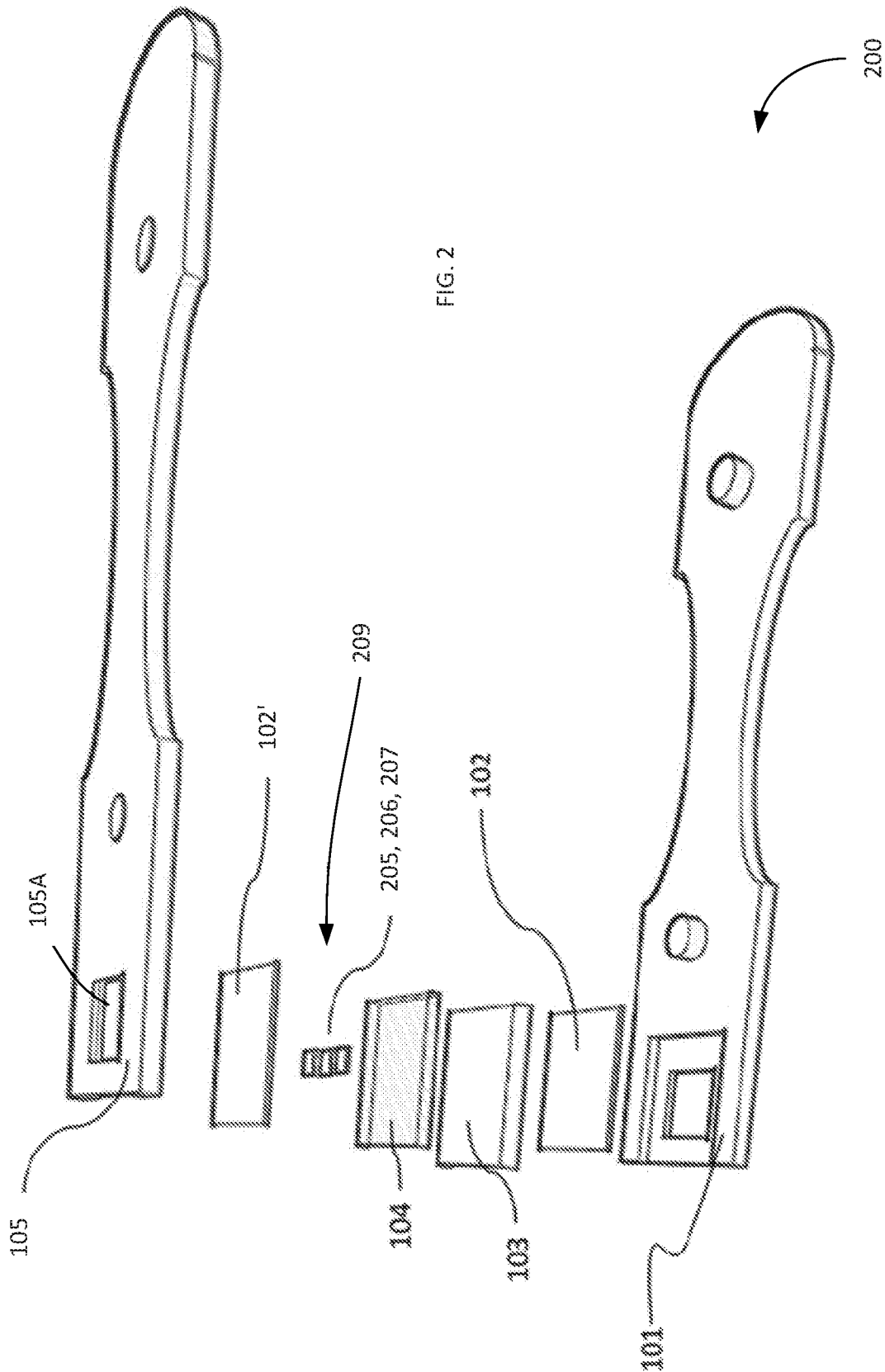
19. **A method** for measuring concentrations of at least two target molecules from a single biological sample, the method comprising:

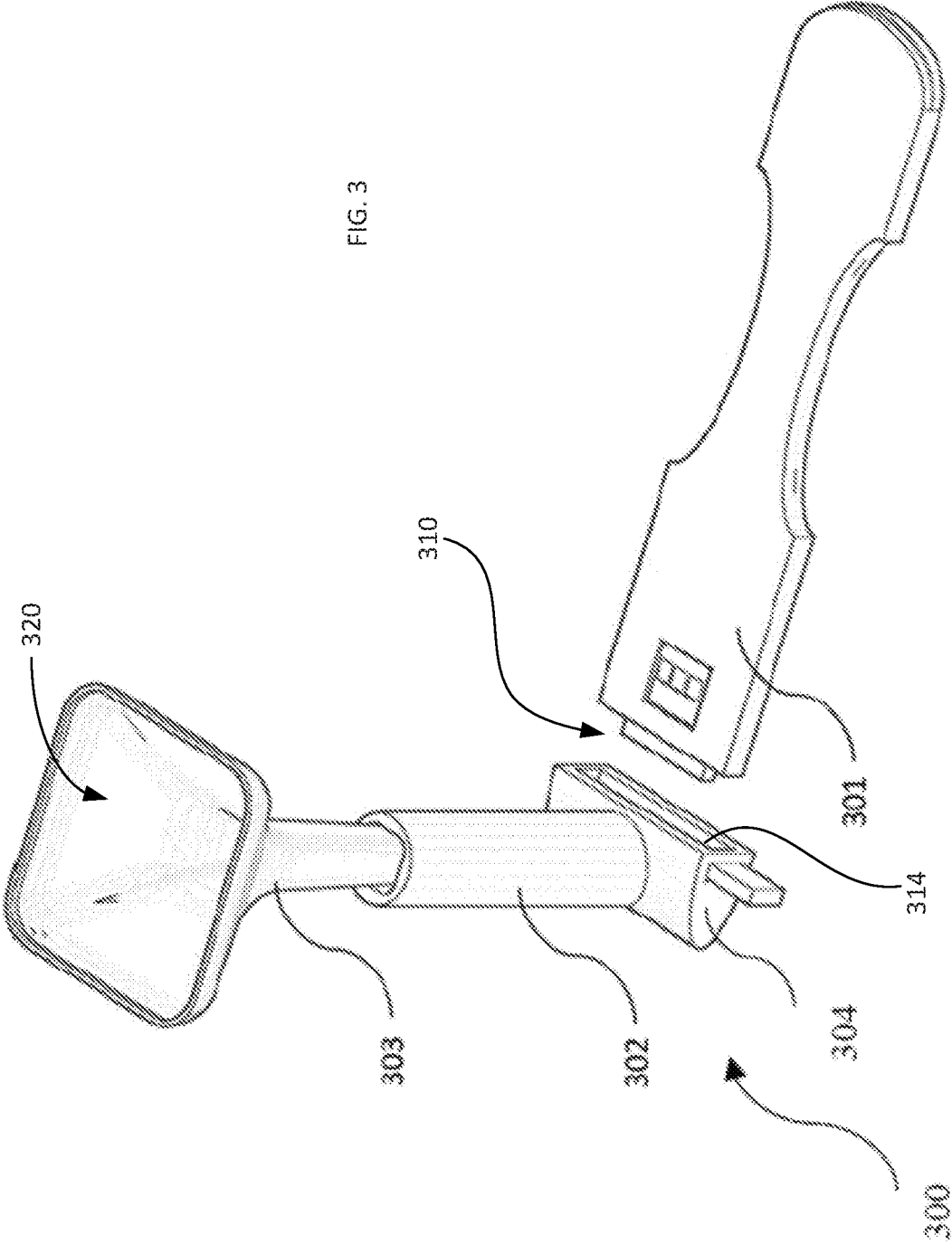
bringing a single porous pad, impregnated with a solution that contains urease and a unfilled capillary matrix, in contact with said biological sample; and

detecting at least two different gases, respectively indicative of the at least two target molecules, with a gas sensor operably juxtaposed with the single porous pad.

20. The method of claim 19, wherein said detecting including detecting a first one of the at least two different gases and a second one of the at least two different gases at a different times.







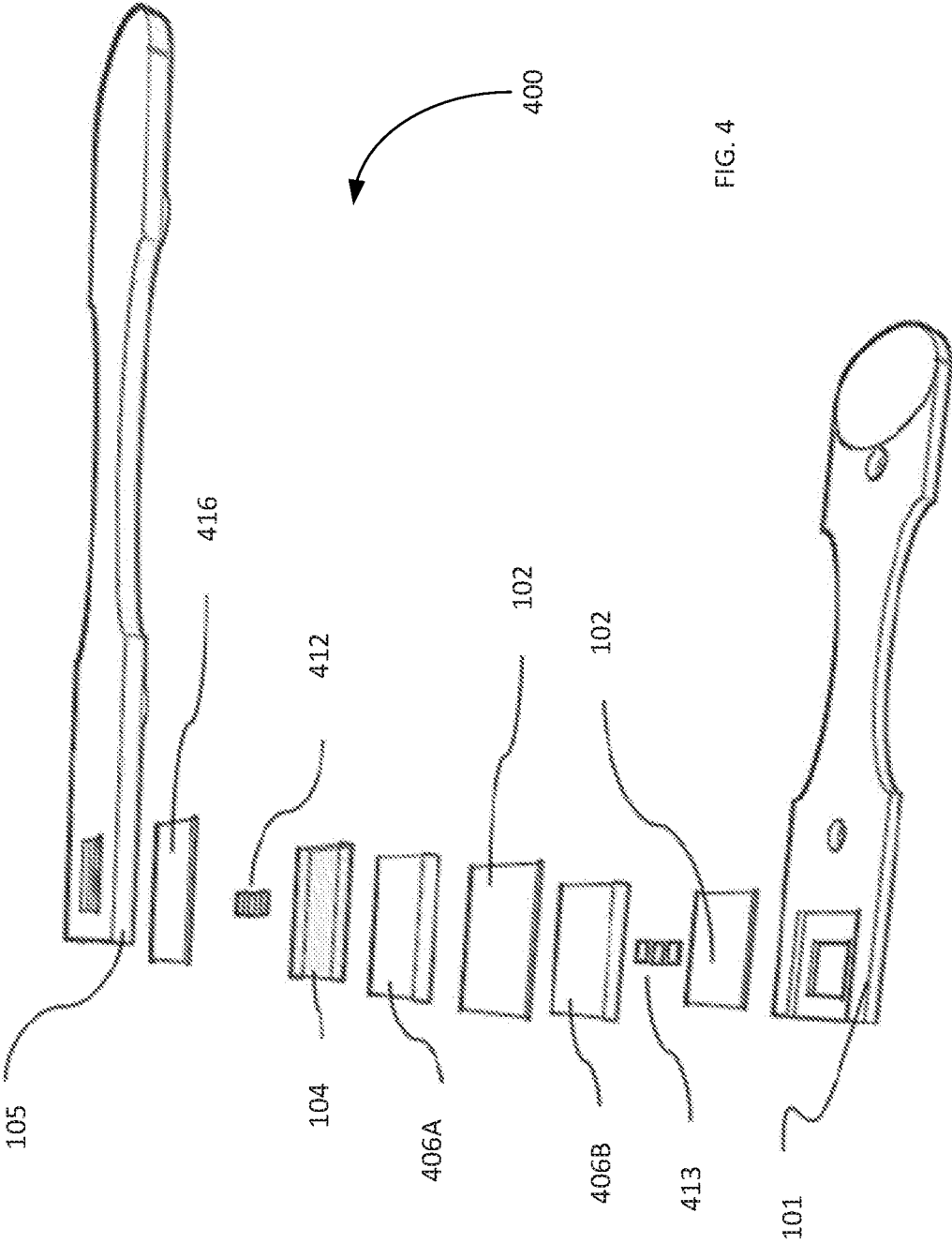


FIG. 4

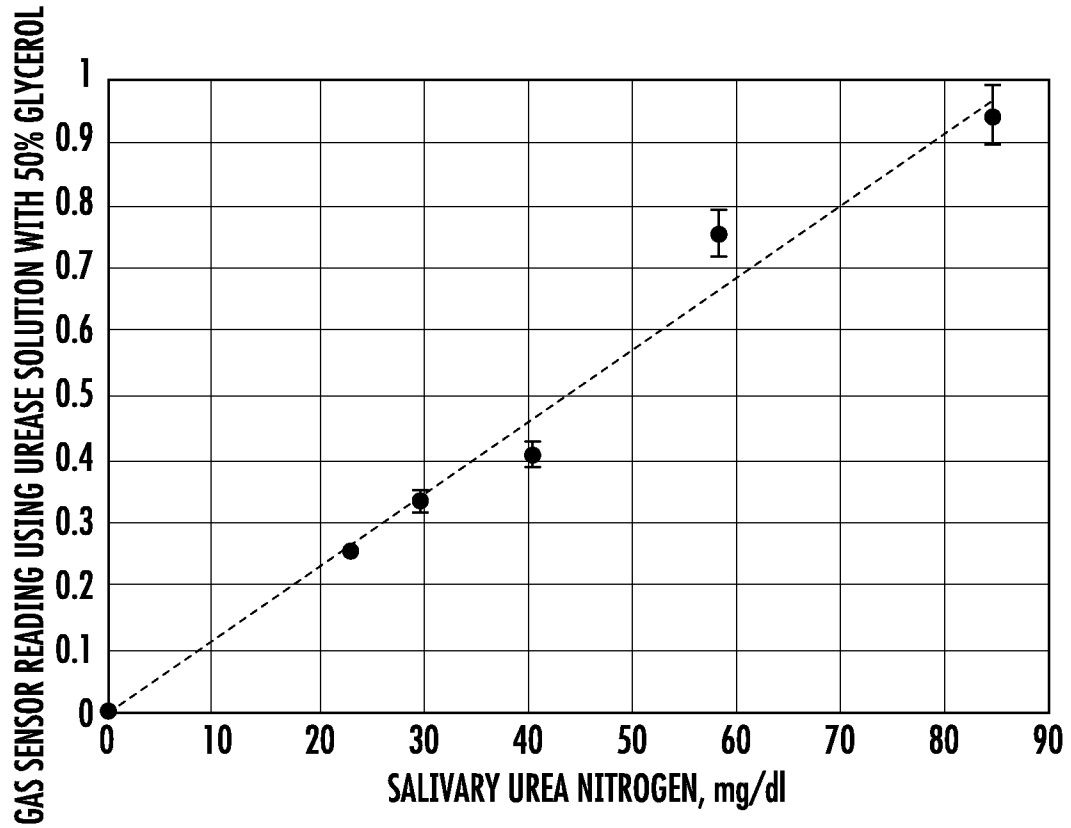


FIG. 5A

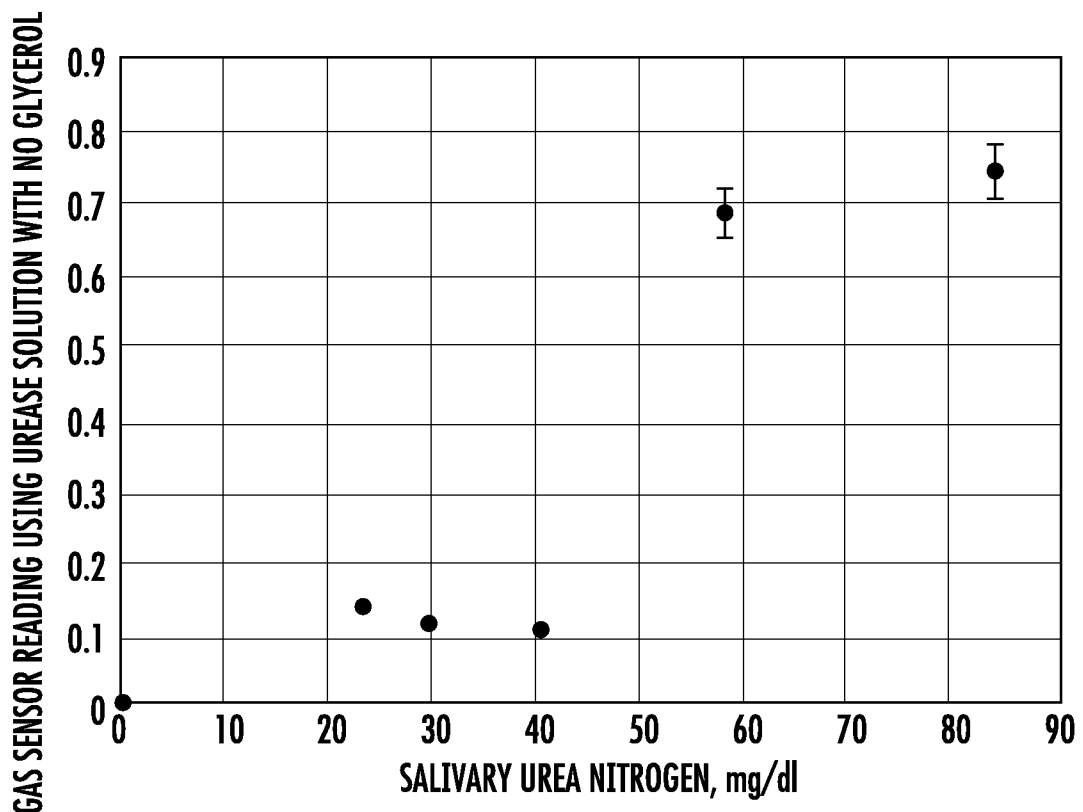


FIG. 5B

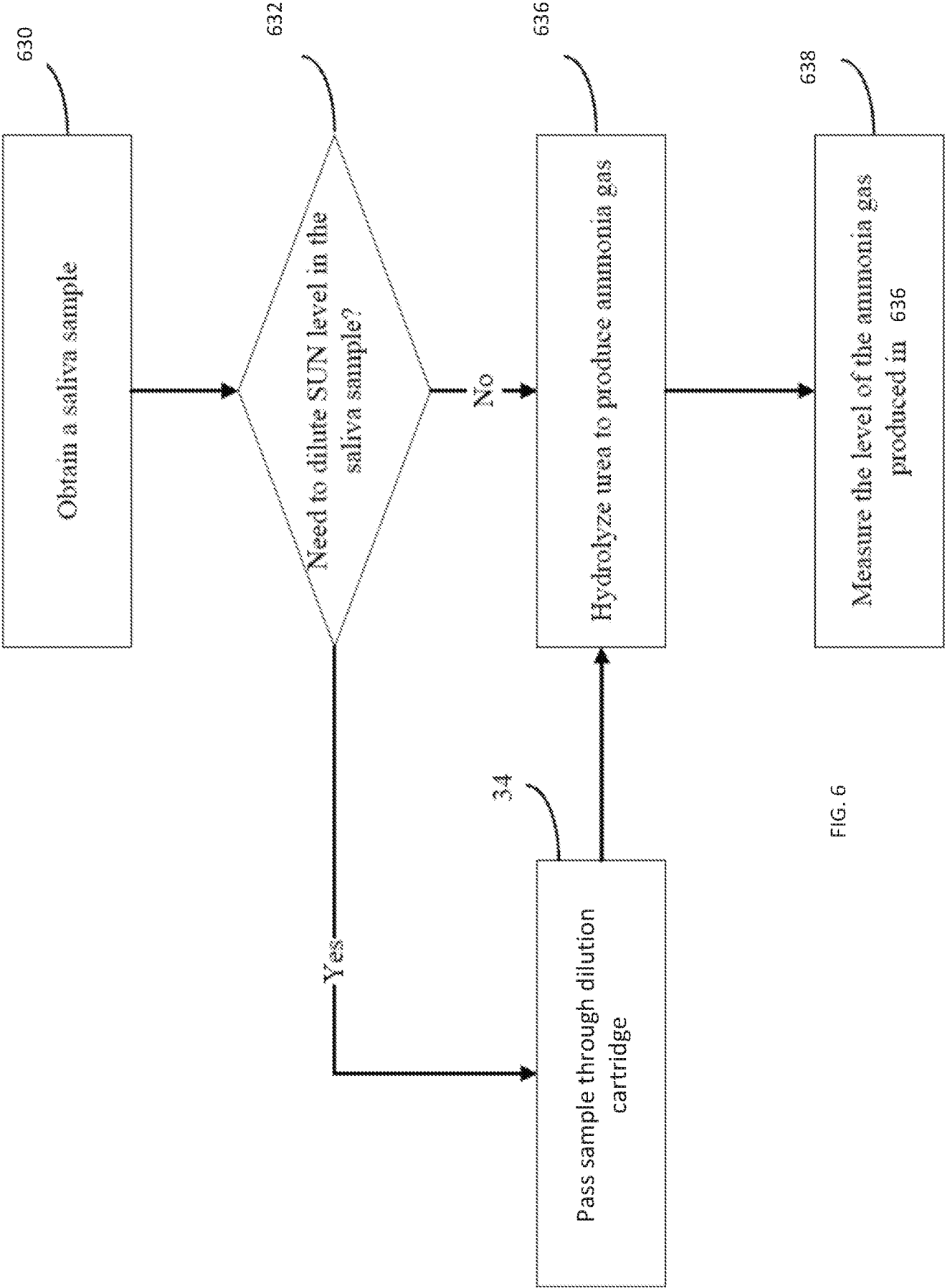


FIG. 6

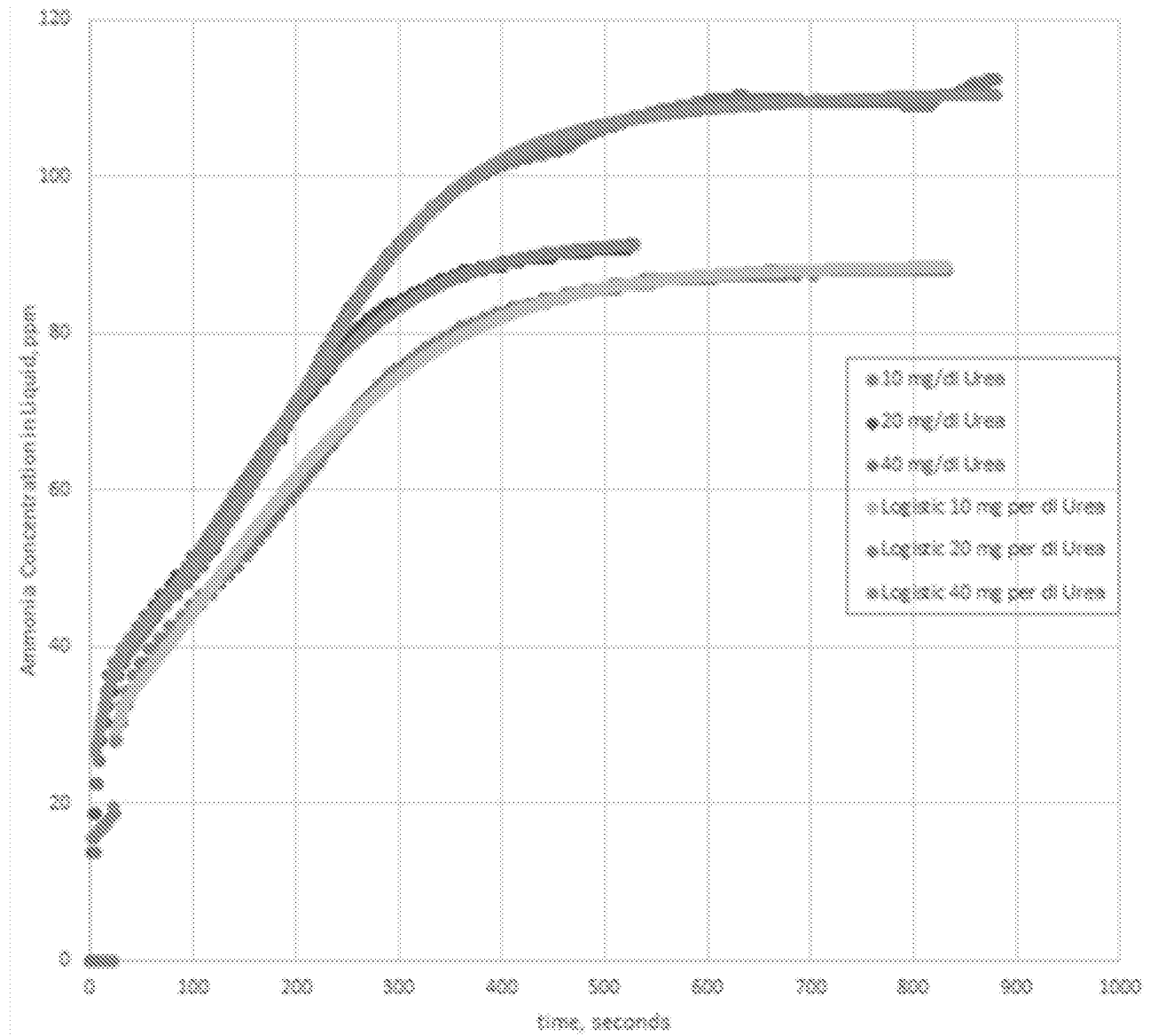


FIG. 7

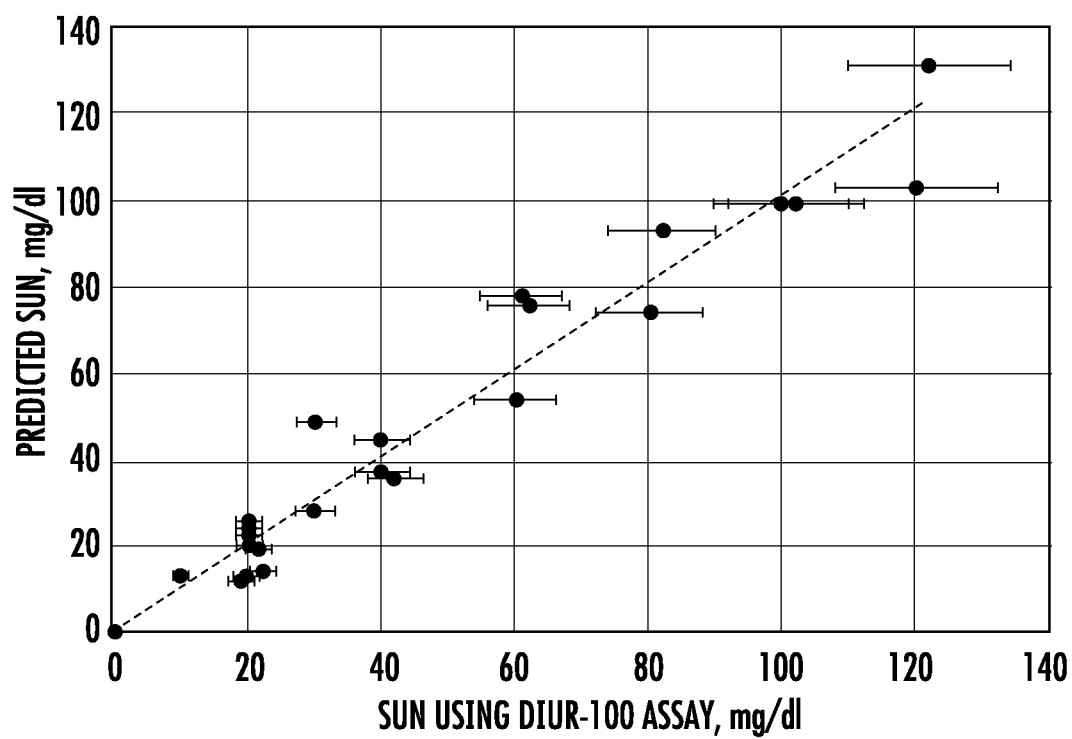
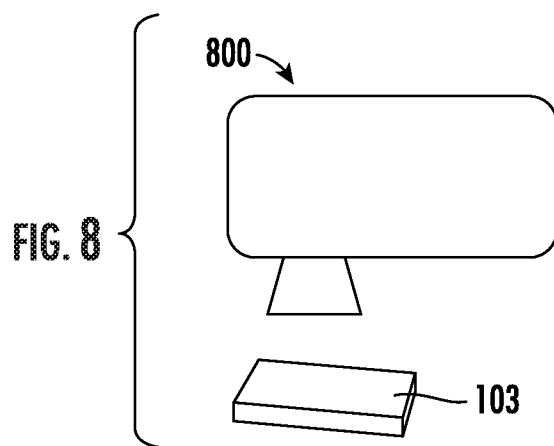


FIG. 9

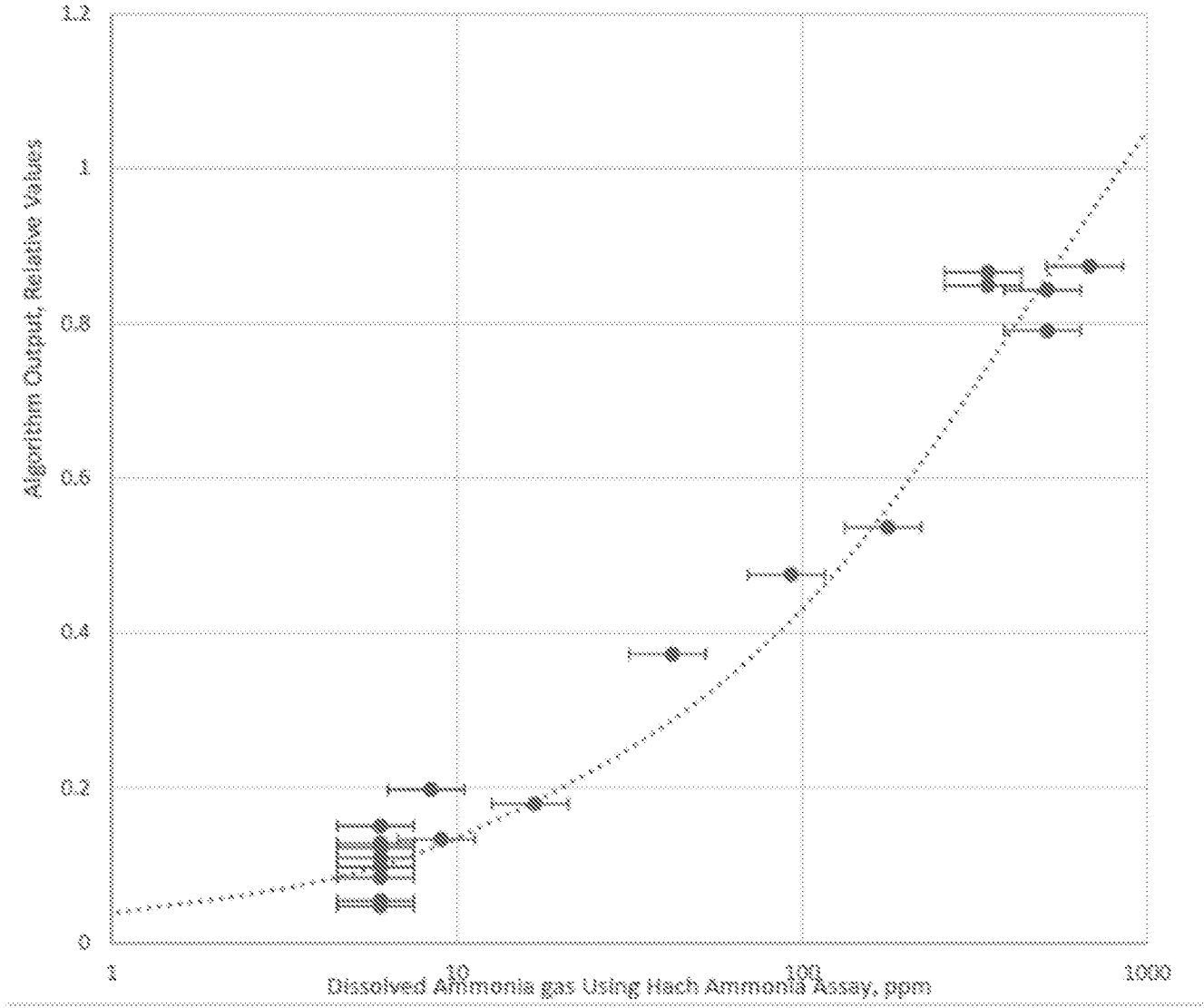


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/036000

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48; G01N 33/50; G01N 33/62 (2020.01)

CPC - G01N 33/48; G01N 33/50; G01N 33/5005 (2020.08)

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.
X	WO 2018/195043 A1 (DIGNITY HEALTH et al) 25 October 2018 (25.10.2018) entire document	1-20

☐

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

05 August 2020

Date of mailing of the international search report

20 AUG 2020

Name and mailing address of the ISA/US

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

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

Telephone No. PCT Helpdesk: 571-272-4300