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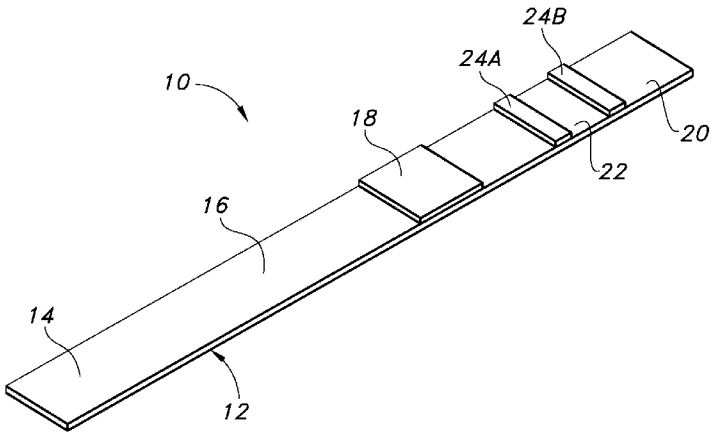

FIG. 1

(57) Abstract: Various modifications to a porous substrate, such as employed in lateral flow assay devices, to regulate or modify the flow rate and/or flow path pattern of a fluid through the porous substrate is described. The lateral flow assay device has a porous substrate matrix in fluid communication with a flow-rate control zone having a number of flow-rate control devices arranged as features in or on a substrate surface or laminates thereof in a body. The flow-rate control devices may include: a density gradient, porosity gradient, ion affinity gradient, micro-channels, and combinations thereof.

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LATERAL-FLOW POROUS MEMBRANE ASSAY WITH FLOW RATE CONTROL

FIELD OF INVENTION

The present invention relates to fluidic control devices and channels as employed in a porous membrane or substrate. In particular, the invention describes alterations of a lateral flow substrate or membranes to regulate and modify fluid flow rate and patterns for certain assay formats.

BACKGROUND

Dehydration is the depletion of fluids and associated electrolytes from the body. Normally, a person's daily, total fluid amount is regulated to be within about ± 0.02% of body weight, and water in the body may comprise approximately 63% of the entire body mass. A balance of bodily fluids is achieved and maintained by matching the input and excretion of liquid from the body, and an imbalance in fluids can be linked to either dehydration or hypohydration. Dehydration can be of particular concern for either the infirm, elderly, or infants, and can have serious consequences to a dehydrated person if not cared for properly. Loss of body fluids in amounts of less than about 2-5% body mass have been associated with reduced heat dissipation, loss of cardiovascular function, and decreased physical stamina.

Specific gravity of an individual's urine is a routinely measured means of evaluating the relative hydration status of the individual. Determination of urine volume and electrolyte concentrations can aid in monitoring whether the individual's body fluid amounts are in balance. Urine specific gravity (USG) refers to the ratio of the density of urine to the density of water. USG is affected mainly by the solids and ions in urine. USG correlates proportionally with the solid concentration and ion concentration of urine. USG normally ranges from 1.002 to 1.030. It is accepted that USG < 1.020 is considered to be well hydrated, USG between 1.020 and 1.025 is considered to be semi-dehydrated and USG > 1.025 is considered to be severely dehydrated. USG can be measured by an instrument such as either a urinometer or urine test dipsticks or strips. Modern dipsticks are commonly based on lateral flow assay technology. Three major methods, namely refractometry, hydrometry and reagent strips, are commonly used for USG measurements.

Although refractometry and hydrometry are very accurate, they require special instruments and trained persons to operate.

Over the years, various manufacturers have attempted different methods to improve the performance of the dipsticks for specific gravity, such as different formulations to increase sensitivity and specificity. Problems, however, persist for all the commercially available dipsticks. A major problem is that the user has to read a change in color within a few brief minutes after dipping in the sample because the color development is not stable under test conditions. The signals that one may observe outside of a limited time window time window are often inaccurate, hence normally invalid. For some analyte tests, such as ion concentration in urine (i.e., specific gravity for dehydration), a certain time period is needed before a signal is fully developed and a valid reading can be achieved. This situation may not be a problem for a test that a user can constantly monitor; however, it becomes a problem when constant monitoring of the test is not feasible and sample introduction time is uncertain. For instance, it is difficult, if not impossible, to predict accurately when a baby or incontinent adult will urinate to provide a sample for an assay device in a diaper or other personal care product. Therefore, the assay device requires a validation mechanism to make sure that a reading is within the valid reading time window.

In recent years, reagent strips have become more popular, particularly in the over-the-counter and point-of-care markets, mainly due to their low cost and ease of use. In general, conventional reagent strips change color in response to the ionic strength of a urine sample. The ionic strength of urine is a measure of the amount of ions present in the urine. The USG is proportional to the ionic strength of the urine. Therefore, by assaying the ionic strength of the test sample, the USG can be determined indirectly and semi-quantitatively by correlating the ionic strength of the urine to the USG.

Conventional reagent strips are usually made in such a way that all the relevant reagents are diffusively immobilized together on a small porous zone on the strip. A sample of urine is then applied to the zone or the entire strip is dipped in the urine sample and withdrawn quickly to allow color to develop. Examples of such conventional reagent strips are described in U.S. Patent No. 4,318,709 to Falb *et al.* and U.S. Patent No. 4,376,827 to Stiso *et al.*

U.S. Patent No. 4,318,709 to Falb *et al.* and U.S. Patent No. 4,376,827 to Stiso *et al.*, both of which are incorporated by reference herein, describe the polyelectrolyte-dye ion

exchange chemistry utilized in conventional test strips for measuring USG. In such conventional test strips, ions present in urine induce an ion-exchange with a polyelectrolyte, thereby introducing hydrogen ions into the urine. The change in hydrogen ion concentration is detected by a pH indicator.

However, conventional reagent strips for USG measurement suffer from major shortcomings, particularly for over-the-counter and point-of-care markets. For instance, conventional reagent strips have a limited reading window because the signal produced by such strips begins to change only a short period of time after sample application. Signal change can be caused by reagent leaching (the result of diffusively immobilized reagents) and sample evaporation. Unless the strips are analyzed shortly after application of the sample, the signal change can lead to erroneous test results. Furthermore, because the reagents in conventional strips are typically water soluble, the strips must also be pulled-out quickly in the urine sample to prevent the reagents from leaching into the sample. In addition, conventional reagent strips are often designed for only a single urine sample application. Multiple urine insults can lead to erroneous test results making such strips unsuitable for applications in absorbent articles where multiple urine insults cannot be controlled. Finally, conventional reagent strips do not provide a way for a user to know if the test has been performed correctly or if enough sample has been applied.

Thus, an unsatisfied need exists for an assay device that can provide such assurance to caregivers in a cost effective way.

SUMMARY OF THE INVENTION

The present invention describes various modifications to a porous substrate, such as employed in lateral flow assay devices, to regulate or modify the flow rate and flow path of a fluid through the porous substrate. The lateral flow assay device has a porous matrix in fluid communication with a flow-rate control zone having a number of flow-rate control devices or mechanisms as surface features or laminates thereof in a body. The flow-rate control zone regulates the flow rate from a buffer pad to a sample observation-feedback zone of a wicking pad. The flow-rate control zone may contain a separate, discrete substrate, such as a membrane or film, which can have a different porosity gradient or have a variety of flow-path features or micro-channels that help to regulate the progress of a sample volume from one section of the substrate to another. The flow-rate control devices

can take the form of: a density gradient, filter porosity gradient, ion affinity gradient, micro-channels, and combinations thereof. The micro-channels can be arranged in a pattern oriented either in parallel, orthogonal, on a diagonal to a primary direction of lateral flow, or in combination thereof. The micro-channels can have either a constant cross-sectional dimension or alternating regions with either enlarged or constricted channel cross-sectional dimensions.

In another aspect, the invention pertains also to a fluidic test device that includes the flow-rate control features described. The flow-rate control devices can be employed to regulate and modulate the manifestation of test results to reduce or eliminate errors. The lateral assay device has a first substrate with a porous matrix adapted for conducting lateral flow; and may further include a sample contact zone, a buffer pad, a wicking pad, and said flow-rate control zone is situated between said buffer pad and said wicking pad. On the buffer pad and wicking pad can be allocated a sample contact zone, a detection zone, an observation-feedback zone, and a flow-rate control zone situated between the detection zone and feedback zone. The sample observation-control zone can change color upon contact with a urine sample. A supporting member secures each of the zones together in an integrate device.

In another aspect, the present invention also describes a method of monitoring dehydration, the method comprises: providing a later flow strip with a porous matrix in fluid communication with a buffer pad, wicking pad, and a flow-rate control zone situated between said buffer pad and wicking pad; introducing a test sample to a sample zone on said buffer pad, allowing the sample to travel through a detection zone to the flow-rate control zone before developing a visual signal in an observation-feedback zone; controlling the flow rate by means of manipulating porosity, density, or ion affinity gradient in a matrix forming at least part of the flow-rate control zone.

The present invention also relates to a process or method for controlling flow rate and time intervals of fluids in a lateral flow assay device. The method involves: providing a substrate with a porous matrix in fluid communication with a flow-rate control zone; forming a number of flow-rate control devices as features in a substrate surface; and positioning at least one substrate or a plurality of laminated substrate layers thereof together in the flow-rate control zone. The flow-rate control devices can be created

according to at least one of the following processes: cutting with a die, laser etching, chemical etching, or printing reagents on said substrate surface.

In another aspect, the present invention relates to an assay apparatus for monitoring specific gravity of a urine sample, the apparatus comprising: a lateral flow assay format having a porous matrix in fluid communication with a buffer pad, wicking pad, and a flow-rate control zone situated between said buffer pad and wicking pad, said flow-rate control zone regulates an amount of time needed for development and appearance of a visual signal in a observation-feedback zone of said wicking pad until a color transition in a detection zone of said buffer pad attains color stability. The flow-rate control zone has a porosity gradient differential relative to the adjacent buffer pad or the wicking pad. The flow-rate control zone may have a combination of layers of laminated substrates, each with a particular physical or chemical property.

In another aspect, the invention relates to an absorbent article incorporating a lateral fluidic assay device as described above. Examples of absorbent articles may include, diapers, adult incontinence products, or personal or feminine hygiene products, or absorbent pads for medical or hospital uses.

Additional features and advantages of the present three-dimensional sensor or assay device and associated absorbent articles containing such a sensor will be described in the following detailed description. It is understood that the foregoing general description and the following details description and examples are merely representative of the invention, and are intended to provide an overview for understanding the invention as claimed.

BRIEF DESCRIPTION OF FIGURES

FIG. 1 is a three-quarter schematic representation of a lateral flow assay device according to the present invention.

FIGs. 2A-C are schematic representations of different flow-rate control mechanisms which may be incorporated into the flow-rate control zone of the present assay device.

FIGs. 3A-D are enlarged schematic representations of flow-rate control mechanisms having a micro-channel pattern (similar to a brick-work pattern) to direct the fluid sample through the sensor.

FIG. 3E is an enlarged schematic representation of a combination of density or porosity gradient and micro-channel pattern.

FIG. 4 is a representation of an alternate micro-channel pattern design.

FIGs. 5A and 5B are representations of another alternate micro-channel pattern design. FIG.5B shows an amount of fluid beginning to enter and pass through the micro-channel.

DETAILED DESCRIPTION OF THE INVENTION

The lateral flow assay (LFA) format is a well established technology that can be adapted for a variety of testing applications for sensors, diagnostics, and indicators. LFAs typically consist of a porous material or combination of materials, which transport a fluid sample of interest from the point of application (e.g. the sample collection zone) to the detection zone(s) via passive capillary action. Because the method of fluid transport is passive, the rate of flow as well as the specific flow path is largely fixed by the viscosity of the liquid sample, the substrate material, and the chemical nature of any coatings that may be applied (e.g., hydrophilic or hydrophobic). In terms of particular test applications, there are several situations in which it would be advantageous to alter the flow rate or flow path without unnecessarily adding extra components or complicating the existing testing procedure. The present invention discloses a approach to modify and regulate the flow rate and flow path of the fluid sample within the test, while maintaining the traditional LFA format. Additionally, this technology may have potential applications outside the field of diagnostic testing.

Conventional urine testing devices, such as dipsticks or test strips, use a lateral flow format that operates by addressing a sample at one end of the strip, which wicks to another area of the strip where the sample reacts with chemical agents, and then to a location where a signal can be detected. Typically these test strips have a short reading window, typically about or less than two minutes, and do not have any user feedback mechanism. Recently, an improved dehydration monitoring and test format was developed, as described in U.S. Patent Application No. 11/956428, the contents of which are incorporated herein by reference. Unlike previously developed lateral flow hydration test formats, the improved dehydration monitoring and assay device has a reading window with a much longer duration of at least about 2 hours, typically about 4-6 hours or greater, with stable color signal and a user feedback zone to indicate a sample volume and sample contact with the test zone. The long reading window and long term stability of the color signal and user

feedback mechanism are important features for an over-the-counter (OTC) test format; in particular, for a test in a personal care product, where constant monitoring is not practical.

I. Lateral Flow Format

The present invention pertains, in part, an assay apparatus that monitors specific gravity of an urine sample, the apparatus includes: a lateral flow strip having a porous matrix in fluid communication with a buffer pad, wicking pad, and a flow-rate control zone situated between said buffer pad and wicking pad, said flow-rate control zone regulates development and appearance of a visual signal in a control-feedback zone of said wicking pad for a predetermined time period, until a color transition in a detection zone of said buffer pad attains color stability.

The present invention builds upon the successes of prior lateral flow test formats and retains all the advantages of a lateral flow device for dehydration monitoring, but addresses some of their shortcomings. The flow-rate control zone has a porosity gradient differential relative to the adjacent buffer pad or the wicking pad. The flow-rate control zone is made from a nitrocellulose membrane, fiberglass pad, nylon membrane, cellulose pad, filter paper, nonwoven material, or polymeric film.

The porous matrix should not interfere significantly with the association and dissociation constant of the buffer. The matrix is preferred to be porous and urine friendly to allow rapid penetration of urine. The dehydration test device has a wicking pad that is porous and water friendly. The wicking pad is preferred to be porous and have a significant capacity of holding water or urine. The dehydration test device has a sample control pad (zone) that change color upon contact with urine.

The dehydration test device has a buffer pad where a buffer is loaded in a porous matrix. The buffer non-diffusively immobilizes with a pH indicator. The buffer's pH experiences change with different ion concentrations or ion strengths or specific gravity of a sample. The pH indicator is preferred to have a color transition around neutral pH, from about 5.5 to about 10.5. Examples of the pH indicator include bromothymol blue, thymol blue, m-cresol purple, brilliant yellow and neutral red. The detection zone can use a pH indicator that exhibits a color transition at a pH from about 5.5 to about 10.5. The sample observation-feedback zone has a non-diffusively immobilized pH indicator and pH adjuster, said pH indicator exhibits a color transition at a pH of either less than 5.5 or

greater than 10.5. The buffer may have partially neutralized weak polymeric acid or base. Examples of weak polymeric acids or bases include poly(acrylic acid), poly(maleic acid), poly(vinylamine) and poly(4-vinylpyridine). The buffer may consist of non-polymeric weak acids such as 2-(N-morpholino)-ethanesulfonic acid and bis-(aminoethyl)- glycol ether N,N,N',N'-tetraacetic acid. The buffer components may or may not permanently be immobilized on the porous matrix. Examples of porous matrices include cellulose pads, filter papers, non-woven materials and glass fibers pads.

The sample observation-control pad can have a non-diffusively immobilized pH indicator and a pH adjuster on a porous and water/urine friendly matrix. The pH indicator is preferred to have a color transition at a pH either <5.5 to >10.5. Examples of the pH indicator include bromophenol blue, bromochlorophenol blue, phloxine B, Bromocresol green and Congo red. Examples of the pH adjuster include citric acid, oxalic acid and tartaric acid. The matrix is preferred to be porous and urine friendly to allow rapid penetration of urine. The dehydration test device has a flow-rate control zone that can regulate the flow rate of a sample from the buffer pad to the wicking pad.

According to the present invention, several methods exist to regulate the flow rate. One example is a piece of porous membrane to be bridged between the buffer pad and wicking pad. The membrane's pore size, width and length of the zone, geometry of the zone, wettability of the pore surface and their combination can be used to tailor the liquid flow rate from the buffer pad and wicking pad. Examples of the porous membranes include Nylon membrane, papers and tissues. The dehydration test device may also have a supporting substrate that helps secure those components together to make an integrated device.

II. Flow-rate Control

A schematic illustration of an example of a lateral flow assay device according to the present invention is shown in accompanying Figure 1. The device 10 has a supporting substrate 12 upon which are located a sample deposition zone 14, a buffer zone or pad 16, a detection zone 18, and a sample reading or observation-control zone 20. Situated between the detection zone 18 and sample reading-observation zone 20 is a flow-rate control zone 22, each respectively with a pad or membrane. According to an embodiment, overlapping areas 24a, 24b are located before and after the flow-rate control zone in reference to the

direction of general fluid flow, from a first end A near the sample deposition zone to a second end B near the control zone.

In particular, the buffer pad 16 is laminated on one side of the supporting substrate 12 and a wicking pad is laminated on the other side of the substrate. Situated in between the two is a porous membrane with an overlap 24a with the buffer pad at one end and an overlap 24b with the other end. The signal indicator in the sample observation zone is situated in the example on the top of the wicking pad. A sample test pad is situated over a portion of the buffer pad. These components are laminated together, for instance with a liquid-insoluble adhesive or tape. The buffer pad, the test pad, the flow-rate control membrane, the wicking and control pad are in fluid communication, either directly or indirectly through other components. A portion of the buffer pad can also act as a sample zone where sample is introduced. As an alternative, a separate sample pad can be laminated with the buffer pad with fluid communication between them. The whole device is enclosed in a sealed casing to prevent sample evaporation except in the sample deposition zone. In an alternate embodiment, the dehydration test device can be integrated as part of a personal care product such as a diaper or used as an exchangeable insert.

In traditional lateral flow devices, the flow rate is constant from one end of the device to the other. Typically in many lateral flow-based assay, for the tests to perform properly and to their full capabilities, it may require about 5-10 minutes to reach reaction equilibrium and to stabilize their signal. The consumer/user may not be aware of this time delay, and so may perceive an erroneous result. An object of the present invention is to minimize errors that arise from premature reading. In the present invention, one can modify and regulate the flow rate of flow of a urine sample through the lateral flow device to serve as a timing mechanism. A function of the flow-rate control zone is to retard or delay progress of a liquid sample across the lateral flow substrate by about 3-5 minutes up to about 10 or 15 to about 20 minutes, depending on the desired application. This can help the consumer compare a stabilized signal in the detection zone to one that manifests in the sample observation zone. In other words, the flow-rate control zone permits the detection zone to perform to its optimal stability.

In the middle of the lateral flow is located an extra or separate zone, which has a discrete membrane, in fluidic communication with the sample flow. The flow-rate control zone can be configured as an interchangeable, separate assembly or component that either

the manufacturer or ultimate consumer can tailor for particular applications. Alternatively, the flow-rate control zone can integrated as a permanent inset part of the lateral flow assay substrate. To adjust the speed of liquid permeation, one may employ a difference in substrate porosity. The materials in the flow-control zone can be calibrated to provide a predetermined rate so as to enable one to know beforehand or have a preset timer to know when to read a signal. Such as wait until at least a second control line is developed for signal validation in the dehydration sensing lateral flow substrate. Depending on the nature of the substrate material, one can tailor the reactivity of the materials with sample fluids or mechanisms of the reactions at the detection zone.

In some embodiments, the flow-rate control zone can be part of the same substrate as the wicking pad; but in other embodiments, the flow-rate control zone is at least part of a second substrate separate from the first substrate. The flow-rate control zone can have a porous membrane that bridges a gap between the buffer pad and the wicking pad. A variety of flow-rate control devices or mechanism may be employed in the flow-rate control zone 22 and arranged along the substrate between the detection zone and the sample observation-control zone, with regions that overlapping with the flow-rate control zone. The devices can be arrayed as surface features of the flow-rate control zone or arranged in laminated layers to provide some thickness and to either assist or hinder the progress of capillary transport of a sample liquid. The devices can either increase or decrease the time that it may take for a sample volume to traverse from the buffer and wicking pads through the flow-rate control zone 22 to the sample observation or control zone 20. For example, such as represented in Figures 2-4, flow-rate control devices can be take the form of 1) a density gradient, filter gradient of varying degrees of porosity, or combined elements thereof, and 2) designs of micro-channel patterns either in parallel, orthogonal, on a diagonal to a primary direction of lateral flow, or in combination thereof, and 3) a combination of all of the aforementioned features. These devices can be incorporated along a single layer or surface of the flow-control zone or layered in the body of the flow-rate control zone of each assay unit to help regulate the flow rate of the liquid from one side of the lateral flow assay to the other, along the primary flow direction. The micro-channels can have a cross-sectional dimension from about 0.01 microns to about 50 or 60 microns. Typically, the cross-sectional dimension may range from about 0.5 micron to about 35 or 40 microns, or from about 1-3 or 5 microns to about 18-20 or 25 microns.

Parallel oriented flow-rate control devices can be placed to follow the general direction of liquid flow, such that the liquid can travel largely along one plane from the detection zone to sample observation control zone, such as depicted in Figures 3-5. In contrast, orthogonally oriented flow-rate control devices can be situated largely perpendicular to the liquid flow direction such that the liquid passes through the plane of each horizontal layer, such as depicted in Figures 1. Laminations can include various types of tapes and polymer films. The present device can include a large number or combination of layers of laminated substrates, each with a particular physical or chemical property, as long as the layers are in fluid contact with each other. The lamination can involve forming a number of flow-rate control devices as features in a substrate surface and joining a plurality of substrate layers together in laminate structure.

Figures 2A-C are schematic representations of different flow-rate control mechanisms which may be incorporated into the flow-rate control zone of the present assay device. The mechanisms may take, for example, the form of micro-channels arranged in predetermined patterns and/or one or a number of differentiated substrate densities. These mechanisms may be oriented either parallel or orthogonal to the flow path of fluid. The flow-rate control delays development and appearance of a visual signal in the observation-feedback zone for a predetermined time interval, until said color transition in said detection zone reaches color stability. The membranes in the flow-rate control zone can have a variety of porosity. In particular, the figures represent configurations with relative density or porosity of the filter medium in the flow-rate control zone. Figure 2A represents a relatively high density, about 2,100 or 2300 to about 2,500 or 2,700 per square inch; Figure 2B represents a medium density, about 1,100 or 1,200 to about 1,900 or 2,000 per square inch; and Figure 2C represents a relatively low density of about 1,000 or 1,100 per square inch or less. Each dot represents either a concave pocket or convex nodule.

The physical dimensions of the flow-rate control and associated overlapping regions on the lateral flow assay device can vary depending on the desired application and absorbent needs. Physical dimensions along x-y directions, defining a plane, can be any size that is area large enough to satisfy the dictates or needs of a specific use. Physical dimensions along the thickness or z –direction should be sufficient to accommodate the volume of liquid in a sample; which typically is a faction or the whole thickness of a substrate body of a testing article. For instance, possible practical dimensions along the x-y

direction can range from as short as a few millimeters (e.g., ~1-7 mm) or up to a few centimeters (e.g., about 1-4-5-7 or 10 cm). Typically, the length dimensions are between about 2 or 3 cm for each side as desired. The overall flow-rate control zone can have an area of about 1-4 cm^2 up to about 100 cm^2 (e.g., about 2-3-5-8-10-12-16-20-25 cm^2) as desired. The thickness of the flow-rate control zone may range from about 0.01 or 0.04 cm up to about 1.0 or 2.0 cm thick (e.g., about 0.1-0.25-0.50-0.8-1.5 cm).

According to certain embodiments, the flow-rate control mechanisms of the present invention involve physically altering the lateral flow membrane so that particular fluid flow rates and flow patterns. One of the major advantages of the present flow-rate control mechanism is that it does not need to introduce special or additional materials into current lateral flow assay products. All of the physical alterations are made to the existing testing membrane, such as mentioned above, appropriate nitrocellulose membranes, glass fiber pads, cellulose pads, non-woven of thermoplastic polymers, or filter papers. Another advantage of the present invention is that it could be implemented into existing manufacturing processes without requiring large recapitalization of equipment. A third advantage of this invention is that it can be seamlessly included into the test without negatively affecting the performance or the accuracy of the test. It can be applied to numerous testing formats, including immunoassays, chemical assays, small molecule detection, nucleic acid testing, and others.

One embodiment of this invention involves using a laser to remove specific sections of one or more of the lateral flow membranes. To maintain test integrity, the laser intensity can be tuned so that only the membrane is removed and not the supporting backing card. The membrane can be removed in sections or patterns of laser cuts can be created. Examples of patterns include, but are not limited to dots, dashes, circles, triangles, other geometric shapes. Additionally, the laser can be dynamically tuned such that a three dimensional relief of a picture or complex pattern is achieved. The density and cross-sectional depth of these patterns can effectively control the rate of the flow of the fluid sample. In addition, the pattern and shape of the laser cuts can control the direction of fluid flow on the test strip.

Figure 2 shows examples of patterns created on a laminated nitrocellulose test strip with a laser. Other methods of physically altering the membrane through mechanical means include punch dyes, vinyl cutters and others. Another embodiment of this invention utilizes

printing techniques to apply hydrophobic materials to the lateral flow membrane material. The hydrophobic materials can be applied in sections or patterns much like the laser cut embodiment. Similarly, the pattern, the density, and the shape can control and alter flow rate and flow direction of the fluid. Additionally, the penetration depth of the hydrophobic ink into the membrane can change the rate of fluid flow. Examples of the hydrophobic materials include but are not limited to commercial Sharpie® ink and hydrophobic polymers (e.g., polystyrene and polyvinyl chloride). An example of a permanent ink (e.g., Sharpie™ Ink) on nitrocellulose is depicted in Figure 3. Yet another embodiment includes removing sections of the lateral flow membrane through chemical etching. An example of this methodology was previously described in two U.S. Patent Publications US 2006/0246597 A1, and US 2006/0246600 A1, the content of which are incorporated herein by reference, describing flow control and metering techniques, respectively.

In particular, one or more recessed regions are formed in the substrate by applying a solvent treatment. The solvent treatment is selected based on its particular dissolving capacity for the material used to form the membrane. For example, an alcohol-based solvent, such as methanol, may be used for nitrocellulose membranes. Upon contact with the solvent treatment, a recessed region is formed that may serve a variety of different functions relating to flow-rate control. The solvent treatment may be applied to the membrane using any of a variety of well-known application techniques. Suitable application techniques include, for example, standard lithography and photo resist technology, spraying, printing (e.g., inkjet, pad, etc.), pipetting, air brushing, metering with a dispensing pump, and so forth. In one particular embodiment, for example, the solvent treatment is applied using a dispensing and optional drying process commonly employed to form detection lines on lateral flow strips. Such a system could involve placing a sheet of the porous membrane on a dispensing machine and threading it through a rewind spindle. This may be accomplished using either a batch or continuous process. The dispensing machine delivers a precise volume of the solvent treatment in a straight line as the membrane passes beneath. The sheet then passes through a drier and is wound back on a spool for further processing. For instance, a lab-scale dispensing pump system for batch processes is available from Kinematic Automation, Inc. of Twain Harte, Calif. under the name "Matrix® 1600."

The solvent treatment may also be applied in any amount effective to form a recessed region having the desired size and shape. The ultimate amount employed may depend on a variety of factors, including the dissolving capacity of the solvent for the membrane material, the speed of application, etc. Regardless of the manner in which it is formed, the recessed region generally acts as a flow-rate control mechanism for the lateral flow device. For example, the recessed region may block the flow of the fluid through the membrane until such time that the assay is initiated, such as by placing the membrane in fluid communication with another membrane. Alternatively, the recessed region is simply used to slow down or otherwise control the flow of fluid through the membrane. For example, a plurality of discrete recessed regions (e.g., dots) may be formed to reduce the continuity of the membrane structure. Thus, a fluid flowing through the membrane structure is forced to follow a tortuous pathway, which increases the amount of time for the fluid to reach the detection zone. Such an increased flow time may provide a variety of benefits, such as to promote uniform mixing and ensure that any analyte within a test sample has sufficient time to react with the desired reagents. For example, the time for the test sample to reach the detection zone may be at least about 1 minute, in some embodiments at least about 2 minutes, in some embodiments from about 3 or 5 minutes to about 8 or 10 minutes, and in some embodiments, from about 10 or 12 minutes to about 25-30 minutes.

Particular uses for the present invention, it is envisioned, may include any lateral flow assays in which timing is critical, such as ensuring a minimum time has elapsed before reading and interpreting the results. One such example is a dehydration test designed for inclusion in a personal care garment, such as a diaper, where precise monitoring of the test is not practical. For a dehydration indicator developed internally, the detection zone requires 5-10 minutes to stabilize and reach equilibrium after coming in contact with the urine sample. If the test is read prior to equilibrium, inaccurate results may be given. Thus, it would be useful to include one of these flow-rate control zones in between the detection zone and the sample observation-control zone such that the sample fluid would not react with the sample observation-control zone until 10 minutes after reaching the detection zone. In such an embodiment, the user would be assured that the test is ready to read once the observation-control zone color has formed. The flow-rate control zones could also be used in between detection zones of a multi-analyte test in which the signal from the zones

forms at different rates. In such situation, it would be advantageous that most or all of the signals develop at the same time, so as not to confuse the user. Otherwise, the user may assume the test is complete once one signal is formed and therefore miss the other signals that develop later.

Although the present inventive concept is described in terms of solving issues associated with reading time for a dehydration test to be incorporated into an absorbent article (e.g., diaper, or adult incontinence product), the concept has potential for broader applications. Additionally, applications for this technology are envisioned to reach beyond lateral flow technology and diagnostic applications. Potentially, this technology could be used in any type of situation where filtering is required or where fluid flow rates need to be controlled.

In another aspect, the invention also relates to a method for testing specific gravity of a urine sample, the method comprises: introducing a urine sample to a sample zone, passing said urine through a buffer pad in a detection zone, causing a color change in a pH indicator in said detection zone, passing the urine through a flow-rate control zone to regulate the appearance of a visual signal in an observation-feedback zone of the wicking pad (for a predetermined interval), until a color transition in the detection zone attains color stability.

The testing is normally performed according to the following: A urine sample is introduced into the sample zone and flows through the buffer zone through capillary action. The ions in the urine cause the change of the buffer's pH in the buffer pad. Some of the samples flows into the detection zone where the pH indicator will show different colors depending upon the pH of the buffer, which is determined by the ion concentration of the urine sample. It is the color of the detection zone that correlates with the urine ion strength, or specific gravity of the urine, which reflects a person's hydration status. It was found that the color signals in the detection zone normally take some time (normally 10 to 30 minutes depending upon the device dimension and configuration) to be fully developed. Some of the sample further flows to the flow-rate control zone, then to the wicking zone, and then to the sample observation-control zone to finally trigger a color change in the reading zone. The time it takes for the sample to fully reach the observation-feedback zone to develop the feedback signal can be easily regulated through many parameters of the flow-rate control zone, including the selection of the material, width and length of the zone

and pore size. The color change in the feedback pad can be used to provide not only assurance that the test is properly done, but also to ensure a minimal time that the sample has contacted with the detection zone before reading the signal. For instance, the test is not valid if the feedback pad has not experience a color change, indicating that one either did not have sufficient amount of sample introduced or had not allowed sufficient time for the signal to develop in the detection zone.

III. Examples

The flow-rate control devices can be generated in or on the porous substrate by means of a variety of methods or processes, such as die cuts, laser etching, chemical etching, or printing reagents on the substrate surface. The particular method of creating the flow-rate control devices may depend on the chemical or physical nature of the substrate material (i.e., porosity, hardness, chemical reactivity/composition) and the desired geometries, shapes, patterns, ablation depths. The following examples are illustrative of two such processes.

Example 1.

Prepartion of components:

An 81mm x 300mm Mylar backing card is laminated with 50mm wide nitrocellulose membrane from Millipore. The distal end of the card is laminated with a 20mm wide porous membrane from Millipore with a high liquid capacity that serves as an absorptive sink. The absorptive sink is positioned in fluid contact with the nitrocellulose membrane, with a total overlap zone of 3mm. A 25mm wide glass fiber pad from Millipore is laminated to the proximal end of the card and is in fluid contact with the nitrocellulose membrane with a total overlap zone of about 3mm. The glass fiber serves as both the sample deposition zone and the conjugate pad. The conjugate is dispensed into three discrete bands on the conjugate pad with a liquid handling system from BioDot prior to assembly of this device. The conjugate consists of a C-reactive protein (CRP) monoclonal antibody conjugated to a gold label, with optical density 3.3. The detection zone contains a CRP antibody and is dispensed with a BioDot machine in a 1mm wide line on the nitrocellulose membrane.

A pattern of alternating diagonal lines (back/forward slashes) is cut in the nitrocellulose membrane with a CO_2 laser to control the flow rate of a sample or conjugate, as well as to promote mixing of the two. The position of this pattern is between the glass fiber-nitrocellulose overlap and the detection zone and spans the entire 300mm width of the card. The card is then slit into 4mm wide strips with a guillotine cutter from Kinematic Automation, Inc.

The laser-etched pattern in the nitrocellulose enabled the rate of fluid flow to be controlled and thus the time of signal development after sample addition could be controlled. Additionally, the color development in the detection zone formed from the center of the test strip to edges, thus concentrating it in the center. This is advantageous for a quantitative system that uses an apertured optics configuration that excludes the edges of the test strip.

Example 2.

Preparation of components:

A 2 cm x 30 cm piece of cellulose pad from Millipore Co. is soaked with 5 ml of polyacrylic sodium salt that is titrated to pH of 8.1 with 1N HCl. The pad is air-dried overnight to make a buffer pad. A 10 cm x 10 cm piece of Biodyne Plus Nylon membrane from Pall Co. is soaked in a 30ml of bromothymol blue aqueous solution (0.1 mg/ml) for 10 minutes and air-dried overnight to make a test pad. A 10 cm x 10 cm piece of Biodyne Plus membrane is soaked with an aqueous solution containing bromocresol green (0.2 mg/ml) and citric acid (2 mg/ml) for 10 minutes and air-dried overnight to make a control test pad.

Assemble the device with a 3mm wide flow-rate control zone:

On an 8cm x 30cm supporting plastic card was laminated with a 5mm wide strip of Biodyne B membrane, 2cm from the edge of the card to make a flow-rate control zone. A 6mm wide strip of a cellulose wicking pad was laminated on the card with a 1mm overlap with the Biodyne B membrane on one side. A 2cm wide buffer pad was laminated on the other side of the Biodyne B membrane with 1mm overlap with the Biodyne B membrane to make a buffer zone. A 2cm wide cellulose sample pad was laminated with 5mm overlap with the buffer pad to make a sample zone. A 5mm wide strip of the test pad was laid on

the top of the buffer pad, 2.2mm from the edge and secured by a Scotch tape, to make a test zone. A 5mm wide strip of the sample control pad is laid on the top of the wicking pad and secured by a tape. The card was cut into 5mm wide devices. The devices were sealed by tape except the sample zone. The particular embodiment creates a multi-layered structure with sections of the lateral flow device adjacent to the flow-rate control zone overlapping the flow-rate control substrate material.

Assemble the device with an 8mm wide flow-rate control zone:

All the steps are the same as above except using a 10 mm wide Biodyne B membrane to replace the 5 mm wide Biodyne B membrane to make a flow-rate control zone.

Test the devices with 3mm wide flow-rate control zone:

To each of six wells in a microtiter plate was added 200µl of synthetic urine with a specific gravity of 1.002, 1.008, 1.014, 1.020, 1.025 and 1.035, respectively. A dehydration test device was inserted into each sample well. About two minutes later, the color in the detection zone started to develop. Five minutes later, the sample observation-control zone shows no color change. About ten minutes later, a small portion of the observation-control zone started to show color change from yellow to blue. At this time, the color signal in the detection zone reaches stability. About 15 minutes later, the whole control zone became blue.

Test the devices with 8mm wide flow-rate control zone:

The results were similar except that the sample observation-control zone started to show color change at about 15 minutes after sample application and at about 20 minutes the whole sample observation-control zone showed color change.

In summation, the present inventive concept describes a fluidic testing device, an absorbent article incorporating the test device and a process for controlling flow rate and flow path of a fluid sample. The testing format and process that involves: providing porous substrate material which transports a fluid sample along a pathway from a point of deposition to at least one detection zone by means of capillary action; modifying a section of said pathway to a) create a physical pattern of channels either on or in said porous

substrate material, b) provide varying density and cross-sectional depth of said porous substrate, or c) a combination of a) and b). The substrate material includes at least a portion having a porous membrane with a flow-rate and flow-path control mechanism, either as part of the substrate or as a separate laminate layer in fluid communication with adjacent components.

The present invention has been described both generally and in detail by way of examples and the figures. Persons skilled in the art, however, can appreciate that the invention is not limited necessarily to the embodiments specifically disclosed, but that substitutions, modifications, and variations may be made to the present invention and its uses without departing from the spirit and scope of the invention. Therefore, changes should be construed as included herein unless the modifications otherwise depart from the scope of the present invention as defined in the following claims.

CLAIMS

We Claim:

1. A lateral flow assay device comprising: a porous matrix in fluid communication with a flow-rate control zone having a number of flow-rate control devices as surface features or laminated substrate layers thereof in a body.
2. The device according to claim 1, wherein said device further comprises a sample contact zone, a buffer pad, a wicking pad, and said flow-rate control zone is situated between said buffer pad and said wicking pad.
3. The device according to claim 1, wherein said flow-rate control devices are selected from the group consisting of: a density gradient, filter porosity gradient, ion affinity gradient, micro-channels, and combinations thereof.
4. The device according to any one of claims 1-3, wherein said micro-channels are arranged in a pattern, oriented either in parallel, orthogonal, on a diagonal to a primary direction of lateral flow, or in combination thereof.
5. The device according to any one of claims 1-4, wherein said micro-channels have either a constant cross-sectional dimension or alternating regions with either an enlarged or constricted channel cross-sectional dimension.
6. The device according to any one of claims 1-5, wherein said micro-channels have a cross-sectional dimension of from about 0.01 micron to about 60 microns, desirably about 1 micron to about 40 microns.
7. The device according to claim 3, wherein said micro-channels encourage mixing of fluids.
8. The device according to any one of claims 1-7, wherein said flow-rate control zone has a combination of layers of laminated substrates, each with a particular physical or chemical property.
9. An assay apparatus to monitor specific gravity of a urine sample, the apparatus comprising: a lateral flow assay format having a porous matrix in fluid communication with a buffer pad, wicking pad, and a flow-rate control zone situated between said buffer pad and wicking pad, said flow-rate control zone regulates an amount of time needed for development and appearance of a visual signal in a observation-feedback zone of said wicking pad until a color transition in a detection zone of said buffer pad attains color stability.

10. The assay apparatus according to claim 9, wherein said flow-rate control zone has a porosity gradient differential relative to said adjacent buffer pad or said wicking pad.

11. The assay apparatus according to claim 9 or 10, wherein said flow-rate control zone has a number of flow-control devices selected from either: a micro-channel pattern design, density gradient, ion gradient, degree of filter porosity, or a combination thereof.

12. The assay apparatus according to claim 9, 10, or 11, wherein said flow-rate control zone comprises a combination of layers of laminated substrates, each with a particular physical or chemical property.

13. An absorbent article comprising: a first substrate with a porous matrix adapted for conducting lateral flow, said substrate having a sample contact zone, a detection zone, an observation-feedback zone, and a flow-rate control zone situated between said detection zone and said feedback zone, wherein each of said zones is in fluidic communication with each other with directly or indirectly by an adjacent component, said flow-rate control zone having a number of flow-rate control devices as surface features or laminated substrate layers thereof, such devices being at least one of the following: a density gradient, filter porosity gradient, ion affinity gradient, micro-channels, and combinations thereof.

14. The absorbent article according to claim 13, wherein said absorbent article is one of the following: diapers, adult incontinence products, feminine hygiene products, or absorbent pads.

15. A method for controlling flow rate and time intervals of fluids in a lateral flow assay device, the method comprising: providing a substrate with a porous matrix in fluid communication with a flow-rate control zone; forming a number of flow-rate control devices as features in a substrate surface; and positioning at least one substrate or a plurality of laminated substrate layers thereof together in said flow-rate control zone, said flow-rate control devices being selected from the group consisting of: a density gradient, porosity gradient, ion affinity gradient, micro-channels, and combinations thereof.

16. The method according to claim 15, wherein said micro-channels are arranged in a pattern, oriented either in parallel, orthogonal, on a diagonal to a primary direction of lateral flow, or in combination thereof.

17. The method according to claim 15, wherein said micro-channels have either a constant cross-sectional dimension or alternating regions with either an enlarged or constricted channel cross-sectional dimension.

18. The method according to claim 15, wherein said flow-rate control zone has a combination of layers of laminated substrates, each with a particular physical or chemical property.

19. The method according to claim 15, wherein said flow-rate control devices are formed according to at least one of the following processes: cutting with a die, laser etching, chemical etching, or printing reagents on said substrate surface.

20. A method of monitoring dehydration, the method comprises: providing a later flow strip with a porous matrix in fluid communication with a buffer pad, wicking pad, and a flow-rate control zone situated between said buffer pad and wicking pad; introducing a test sample to a sample zone on said buffer pad, allowing said sample to travel through a detection zone to said flow-rate control zone before developing a visual signal in an observation-feedback zone; controlling the flow rate by means of manipulating porosity, density, or ion affinity gradient in a matrix forming at least part of said flow-rate control zone.

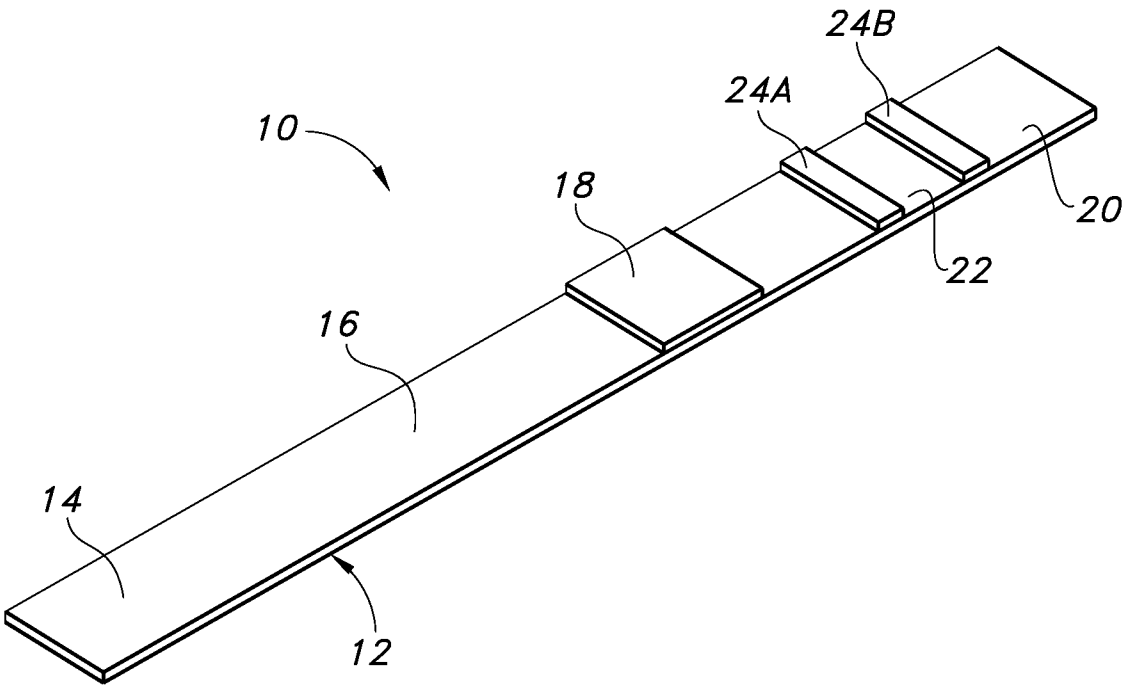
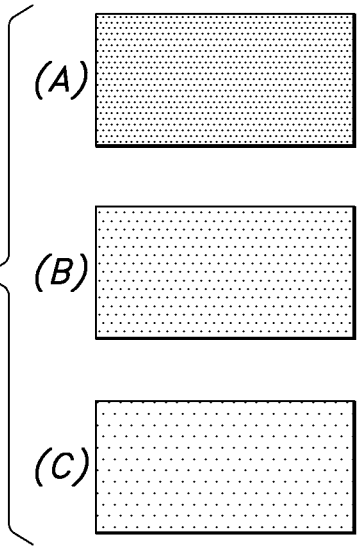


FIG. 1

FIG. 2



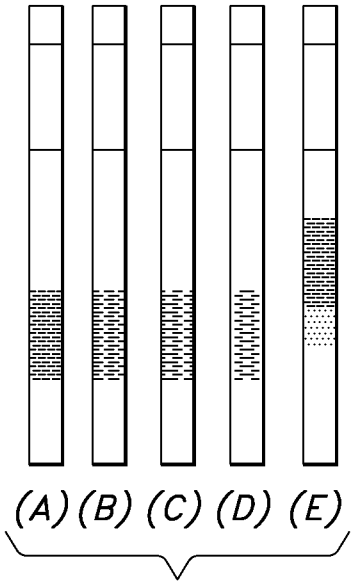


FIG. 3

FIG. 4

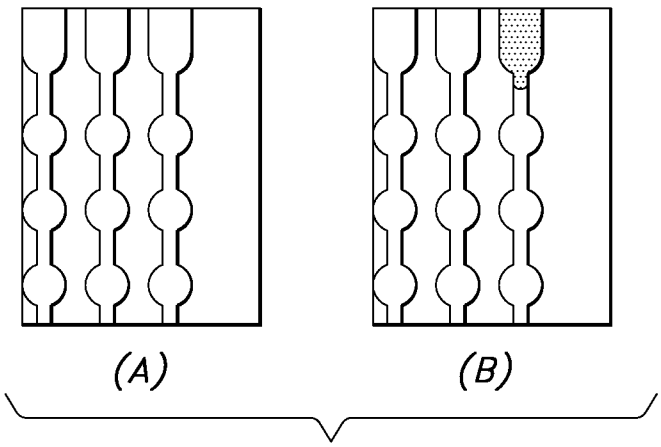


FIG. 5

Marking Key Numbers:
10 - entire device
12 - support card
14 - sample zone
16 - buffer pad
18 - detection zone
20 - control zone
22 - flow control zone
24 - overlap area (mark the two as 24a, 24b)

A - first end (near sample zone)
B - second end (near control zone)