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(21) International Application Number: PCT/US99/04785 (22) International Filing Date: 4 March 1999 (04.03.99) (30) Priority Data: 60/076,701 4 March 1998 (04.03.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/076,701 (CIP) Filed on 4 March 1998 (04.03.98) (71) Applicant (for all designated States except US): UNIVERSAL HEALTHWATCH, INC. [US/US]; 8990-E Oakland Center, Route 108, Columbia, MD 21045 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HE, Yongsheng [US/US]; 3450 Plumtree Drive, Ellicott City, MD 21042 (US). McCLINTOCK, Joseph, A. [US/US]; 2604 Manhattan Avenue, Baltimore, MD 21205 (US). CHOWDHURY, Mohammed, A. [BD/US]; 8881 Goose Landing Circle, Columbia, MD 21045 (US).		(74) Agents: SANDERCOCK, Colin, G. et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., Washington, DC 20007-5109 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: CHEMILUMINESCENCE DETECTION DEVICES (57) Abstract A rapid yet sensitive test is made possible by combining chemiluminescence detection with high porosity reagent pad and a movable absorbent pad to allow control of reaction timing. The test device, method and kits are suited for antibody based immunometric binding assays as well as for those based on nucleic acid hybridization reactions.		

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Chemiluminescence Detection Devices

Field of the Invention

The present invention relates to diagnostic test devices for rapid detection of analytes
5 using chemiluminescence in a strip format.

Background of the Invention

Clinical diagnostics procedures for the detection of pathogens such as *E. coli* 0157
and for the detection of aberrations such as the establishment and progression of tumors in
10 the body have been developed. However, sensitive assays for the most part, are time
consuming and difficult to use, yet convenient assays, although quick, are not very sensitive.
Thus, a need exists for rapid detection without compromising assay speed.

The combination of rapid speed and sensitivity is crucial in some clinical
circumstances. For example, the rapid and accurate detection of *B. coli* 0157 is very
15 important, both in clinical and in environmental settings. Because food contamination is the
main source of *B. coli* 0157 infections, there is a tremendous need in the food industry for
tests that can be performed outside of the clinical environment and that can give a result at
the site of suspected contamination. The development of a rapid and reliable method for *E.*
coli 0157 detection has been the highlight of recent research. While many methods have
20 been developed to detect and isolate *E. coli* 0157 there is no simple, rapid, sensitive and
specific detection method for convenient use in the medical diagnostic field or food industry,
as reviewed by Padhye et al., *J. Food Prot.* 55:555-65 (1992). The same limitations to *B.*
coli 0157 detection and treatment also limit the detection and treatment of other diseases.
Accordingly, a diagnostic system that successfully combines high sensitivity with a
25 convenient and rapid procedure would provide general benefits for a wide range of medical
conditions.

Summary of the Invention

The inventors have discovered a strip test device, test system and methods that combine fast chemiluminescence readout with a manually operable absorbent on the same strip with a reaction layer and sensitive light detection to provide quick testing of analytes at very low concentrations. The speed of this chemistry device, system that includes light detection from the device, and methodology allow measurements to be taken at clinical sites where they are most needed, thus allowing expedient medical intervention as needed. In the case of suspected infections by a pathogen such as botulism or E. coli 0157, where diagnosis and correction must be taken as soon as possible, the invention allows earlier life preserving treatment than previous art methods. Furthermore, the device and methods of the invention are convenient to use by relatively untrained individuals. This expands the usefulness of the diagnostic tests.

In one embodiment, the invention concerns a detection device for detecting an analyte from a liquid sample, comprising: (a) a test strip having a fixed portion with an opening and a movable portion having a surface with an area at least the size of said opening; (b) a reagent layer connected to said fixed portion for occluding said opening, said reagent layer comprising immobilized antibody or antigen, having at least 5uM porosity; and (c) an absorbent pad connected to said movable portion; wherein said absorbent pad has a volume that exceeds the volume of said reagent layer by more than ten-fold, wherein said movable portion is manually movable to allow said absorbent pad to receive said liquid sample from said reagent layer by contacting said reagent layer with said absorbent pad to dry said reagent layer sufficiently to allow chemiluminescence detection fluid to enter said reagent layer.

In another embodiment the invention comprises a kit for conducting an assay, comprising in packaged combination: (a) the device described above, and (b) reagents for conducting an assay.

In yet another embodiment, the invention is a system for detecting an analyte from a liquid sample, comprising: (a) a test strip having a fixed portion with an opening and a movable portion having a surface with an area at least the size of said opening, a reagent layer connected to said fixed portion for occluding said opening, said reagent layer comprising immobilized antibody or antigen, having at least 5uM porosity and an absorbent

pad connected to said movable portion wherein said absorbent pad has a volume that exceeds the volume of said reagent layer by more than ten-fold and (b) an electronic chemiluminescence monitoring device that accepts said test strip and which reports a signal in response to light emission from said test strip.

5 Other embodiments, objects and advantages will become apparent from the following detailed description taken in conjunction with the Figures.

Description of the Figures

Figure 1 shows chemiluminescence detection of a target gene that becomes immobilized on a solid phase. The overall procedure is pictorially represented as cross
10 linking of the gene to the membrane, hybridization with a biotinylated probe, binding with added streptavidin, binding with added biotinylated alkaline phosphatase and emission of light.

Figure 2 shows a top-side view of device 20 in accordance with the invention. The reagent layer covers opening 22. Absorbent pad 24 is positioned below this opening such
15 that strip portion 25 onto which pad 24 is mounted can move as shown by curved vector 26.

Detailed Description of the Preferred Embodiments

The inventors have examined the overall problem of speed in diagnostic tests. In doing so, the inventors were able to create a sensitive diagnostic test that requires only about
20 5 minutes to obtain a result, whereas previous tests are much slower. The inventive test was made possible by using the following features in combination for a single test: (1) a chemiluminescence reaction to carry out the detection step; (2) an automated detector having a sensitive light detection element selected from the group consisting of a photon multiplier tube, photo diode, photo field effect transistor, charge coupled device, and charge injection
25 device, to detect the test results; (3) multiple fluid movements with the same absorbent pad; (4) a solid phase reaction site having rapid flow-through characteristics due to porosity of at least 5 microns; (5) carrying out all reactions in this same reaction site (no transfer or chromatographic movement) ; and (6) detecting a product of the reaction from this same reaction site.

In some embodiments, the automated detector further comprises a peltier device to cool the light detection element and in some embodiments the porosity of the solid phase reaction site is greater than 10, 15, 20, 25 or 50 microns.

Chemiluminescence, noted as the first feature above, is particularly important to the practice of the invention because this sensitive method allows shorter reaction times and shorter detection times. Particularly advantageous in this regard is the use of horseradish peroxidase catalyzed chemiluminescence because, unlike other reactions, such as dioxetane chemiluminescence, horseradish peroxidase chemiluminescence provided a stable light signal only a few seconds after initiation of the reaction. Furthermore, the high light output of horseradish peroxidase, when matched with a small solid state photodetector such as a photo diode amplifier, CCD or CID, allows the detector to be used without expensive cooling.

Another particularly important feature of the invention is the use of a solid phase material having a porosity of at least five microns. This minimum size ensures that liquids and suspensions are quickly absorbed and pass through the reagent layer into the absorbent pad. The inventors emphasize that by combining this feature with the use of the chemiluminescence feature and the use of a movable absorbent pad, their diagnostic test is very rapid, yet very sensitive. Accordingly, this particular combination is most advantageous in using the invention. In other embodiments a minimum porosity of 10, 15, 20, 25, 40 or 50 microns is preferred as the larger porosity provides greater flow rates, especially when used with liquid suspensions of particles.

An important mechanical feature of the invention is the use of manual placement of absorbent pad in contact with the reagent layer to control reaction time. By waiting, for example, a minute after applying the sample to the reagent layer before removing fluid with this contact, reactions can proceed for a longer time, giving better sensitivity, compared to other devices in which the absorbent pad contacts the reagent layer at all times.

One embodiment of the invention is a device that contains both an immobilized binding partner, such as an antibody, antibody fragment or antigen, and a second binding pair chemilumigenic conjugate, such as an antibody-horseradish peroxidase conjugate in the reagent layer. Thus, various chemistries that are linked to a chemiluminescence detection step are contemplated. An exemplary test strip in accordance with the invention is a device for detecting E. coli 0157, given in Example 1. Further examples of chemistry and

instrumentation contemplated by the inventors are found in U.S. utility application entitled COMBINED SAMPLING-ASSAY DEVICE AND HOLDER filed March 4, 1999 (Attorney docket No. 073294/0195), and PCT publication WO 98/49544 from PCT/US98/08551 entitled HAND HELD LUMINOMETER (Attorney docket No. 073294/0174).

5 Test strips in accordance with the invention have a flat base comprised of a water impermeable material onto which a reaction layer and an absorbent pad are affixed in separate locations. The portion containing the absorbent pad can move with respect to the portion that contains the reaction layer such that the layer and the pad can come into contact with each other, allowing fluid transfer from the layer to the pad.

10 A simplified version of this arrangement is shown in Figure 2, although other physical arrangements are possible and readily conceived by a skilled artisan after reading this disclosure. The reagent layer covers an aperture in the flat base such that a liquid sample can be added to one side of the reagent layer and the absorbent pad can contact the other side of the reagent layer after, for example, bending the respective portion of the flat
15 base over to move the absorbent pad in position.

The strip may be constructed from well-known materials in accordance with well-known methods of the prior art. Material suitable for the invention should not interfere with the production of a detectable signal and should have a reasonable inherent strength, or strength can be provided by means of a supplemental support. Natural, synthetic, or
20 naturally occurring materials that are synthetically modified, can be used including, but not limited to: cellulose materials such as paper, cellulose, and cellulose derivatives such as cellulose acetate and nitrocellulose; fiberglass; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; porous fibrous matrixes; starch based materials, such as Sephadex(TM) brand cross-linked
25 dextran chains; ceramic materials; films of polyvinyl chloride and combinations of polyvinyl chloride-silica; and the like. See, for example, U.S. Patent Nos. 5,075,078, 5,552,276, 4,912,034 and 5,212,065 which are incorporated herein by reference in their entireties. Constructions other than those described in these patents readily will be apparent to the skilled artisan.

In one advantageous embodiment devices of the invention are particularly suited for detecting gram negative bacteria such as *E. coli* 0157. This embodiment advantageously detects the bacteria via reactivity with lipopolysaccharide of the bacterial outer membrane. In this case, a preferred material for attaching the lipopolysaccharide is polymyxin-coated resins, such as polymyxin-coated polyester cloth, as described by Blais and Yamazaki in *Inter. J. Food Micro.* 11: 195-204 (1990)

The physical assembly of components from known materials within the housing generally will be understood to a skilled artisan but for clarity, some details are provided here in the form of definitions of some terms used in the claims.

An "absorbent pad" imbibes water generally and serves as a final repository for much or most of the liquid that contacts the reagent layer. Examples of an absorbent pad include paper, ethyl cellulose, porous plastic, sintered glass, and other polymers that accept water such as polyvinyl pyrrolidone and acrylamide copolymers. An absorbent pad may consist of more than one piece. A most acceptable absorbent pad is comprised of ethyl cellulose and is held in place on the strip surface by an adhesive.

An "antibody" is IgG, IgE, IgM, IgA, or a fragment thereof, and the like and may be obtained from blood, a blood product or saliva. Although the use of saliva samples is not discussed in great detail, it is understood that the invention is used for the detection of an analyte from saliva as well as from blood and fluid from wiping suspected environmental surfaces. Advantages of the invention, such as the operation of the filter extend to saliva samples as well as to blood samples.

An "opening in the reagent layer" may be a hole, aperture or simply a region that accepts a fluid sample from one side and allows movement of the fluid to the other side to contact the absorbent pad. In some cases, the strip itself may be partly water permeable and partly water impermeable. In these instances the water permeable portion(s) act as an "opening" in context of the invention. More than one opening may be used in the test device. Multiple opening, and their associated reagent layers are advantageous when simultaneous testing of two analytes is desired. In this case, the instrument separately can detect light emitted from the different reagent layers. This last design feature is particularly advantageous for a confirmatory test. An example of two antigens, the co-presence of which

gives a diagnostic measurement of greater reliability, is LPS with H7 flagellin from *E. coli* 0157.

The phrase "passes fluid" has its regular meaning that is well known to the skilled artisan. The relative rate of passing fluid can be determined by a number of techniques known to the artisan, including for example, measuring how much time is required for the material to become completely wetted upon contact with water.

A "reagent layer" is an absorbent or group of absorbents that contains at least one reagent, can contact the absorbent and transfers fluid to the absorbent by virtue of this contact. An advantageous reagent layer is a membrane that comprises an immobilized test reactant such as antibody or antigen.

Typically, the absorbent pad contacts the reagent layer to accept and draw in fluid from the reagent layer after a period of time determined by the user. Although the reagent layer preferably comprises an immobilized test reactant, the test reactant may become immobilized during the test itself by for example, precipitation or by binding to the reagent layer during the test procedure. The reagent layer may assume any of a variety of shapes. An advantageous shape is a thin layer lying over the opening although another shape such as a plug may be used, depending on the features of the strip. Fluid preferably moves traverse to the major surface of the reagent layer during transfer to the absorbent pad, although some device configurations may utilize lateral flow along the longest axis of a reagent layer.

In an advantageous embodiment for testing *E. coli* 0157 in a stool sample, the reagent layer is a nitrocellulose membrane with antibody bound to it. Preferred in this context is an antibody that reacts with lipopolysaccharide or H7 flagellar antigen from this bacterium. The reagent layer, however, need not be a "layer" per se, but may consist of a pad, rod or other shaped absorbent.

An advantageous device configuration comprises an absorbent pad and a reagent layer attached to a single plastic base having a hinge region between the two attachment sites as shown in Figure 2. Acceptable materials for the plastic base include water impermeable plastics such as polystyrene, polypropylene, polyvinyl chloride and the like. Acceptable materials for the optional filtering portion of the filter, the reagent layer and the absorbent pad are glass fiber, nitrocellulose and ethyl cellulose respectively. When nitrocellulose is used for the reagent layer, however, the material of the filter should be chosen for its ability

to premix the test sample and any test reagent that may be present in the filter. Two or more materials can be physically combined to make up the optional filter, reagent layer or absorbent pad. Two materials are acceptable for the filter, an upper filtering material and a lower dispersal material. Most acceptable is a dispersal layer that passes fluid at a slower flow rate compared to passage of fluid through an upper filtering material as described in co-
5 pending U.S. No. 09/069,935 filed 4/30/98, which is herein incorporated by reference in its entirety. The use of a dispersal layer is superior over the use of a single part filter.

A skilled artisan will readily appreciate that one or more portions of the device summarized above can be replaced by a single piece of a suitable material. In this context,
10 many absorbent, porous or capillary possessing materials through which a solution containing the analyte can be transported by a wicking action are suitable for construction of the test device and are known to the skilled artisan.

Also acceptable is to place a colloidal gold detection reagent in the filter in a dried form so that the detection reagent may become re-suspended during application of a test
15 sample.

During use, a single test device is obtained and sample and wash fluids are added directly to the reagent layer of the device. After adding the fluid sample, an aqueous wash solution may be applied. The wash solution preferably contains a non-ionic detergent such as Tween 20 (TM) and may also contain a protein such as bovine serum albumin.

20 Reagents needed for operation of the test device may be placed in the optional filter, reagent layer, and/or in an aqueous fluid that is applied to the device. For convenience in manufacturing, a reagent such as hydrogen peroxidase antibody conjugate or hydrogen peroxidases substrate solution can be prepared and used as a liquid phase reagent and added during operation of the device.

25 Adding sample and wash solution starts sequential and automatic reactions in the test device. These reactions include two or more binding reaction(s), an optional separation step and an optional enzymatic reaction step according to the test format employed. Many types of ligand antigens are useful for the test device and are contemplated for the invention.

One advantageous format is a binding assay in which at least one, but more
30 preferably two or more binding pair members such as antibodies or antigens are immobilized. In this format the test substance in the test sample, such as, for example, E.

coli, has at least one epitope, or binding site that participates in a binding reaction with a specific binding member immobilized in the reaction layer. In such a binding assay two molecules that bind specifically (i.e. with an association constant of at least 100,000 under typical assay conditions) form the basis of concentrating a signal producing substance to form an optical signal. An advantageous signal producing substance is horseradish peroxidase conjugated to an antibody that binds to the test analyte. Molecules that act as "binding partners" with an analyte in a test sample typically are antibodies specific to the analyte, but, as the skilled artisan will readily appreciate, other molecules that bind specifically to the analyte molecule also can be used.

The assay typically uses multiple immobilized antibodies targeted against antigens that are diagnostic of the analyte. These antibodies can be selected from lists of antibodies that are known to react with and indicate, for example, the presence of antibodies against a particular microbe that displays or sheds such antigens.

After adding one or more fluids to the test device and then allowing one or more reactions to proceed, emission of chemiluminescence light is determined by an instrument that uses a light detection element to generate an electrical signal. Preferred chemiluminescence reactions are the alkaline phosphatase-dioxetane reaction and horseradish peroxidase-luminol reaction and others as described in the publications and applications referenced herein. Enhanced horshradish peroxidase chemiluminescence is most preferred because it is faster, taking less than 10 seconds to reach a plateau of light production and generates much light.

Light detection advantageously is carried out with a photon-multiplier in an integrative (voltage measurement across a capacitance) mode, although photon counting also is acceptable and the use of semiconductor devices is acceptable, particularly in combination with catalyzed light output from an enzyme. The light detector used should be in a light tight box having an opening for receiving the test strip after its development. A chemiluminescence light measurement advantageously is carried out within one minute of inserting the strip.

The above-summarized procedure can be modified by the use of a fibrous pre-filter positioned on top of the reagent layer, as described in U.S. patent application No. 08/577623

filed 12/22/95 and entitled FECAL TEST METHOD, which is herein incorporated in its entirety by reference.

In a particularly acceptable embodiment, a horseradish peroxidase antibody conjugate is included in the optional filter or in the reagent layer itself.

5 The detection devices of the invention are particularly advantageous when used in combination with a small battery operated hand held luminometer. One example of a hand held luminometer in this context is the BioLIGHT (TM) instrument obtainable from Universal HealthWatch, Inc., Columbia Maryland, U.S. The BioLIGHT detects light with a photon multiplier tube. In other advantageous embodiments, the detection devices are used
10 with a portable instrument reader that employs a sensitive semiconductor device as a light detection element. Most preferred in this context are a photodiode (generally incorporated within a high gain low noise amplifier package), a photo field effect transistor (photo fet), charge coupled device (CID) and a charge coupled device (CCD) as described for example in U.S. No. 4,020,463. Such light detection elements are known and can be adapted by a
15 skilled artisan to detect light in a chemiluminescence monitor as for, example, described by Jansen *et al.*, *J. Biolumin. Chemilumin.* 3: 53-7 (1989). Another example of a useful light detecting element is a static induction transistor based device as described in U.S. No. 4,502,203. An adaptation of a CID that is particularly useful for chemiluminescence detection is described by U.S. No. 4,681,440. In the latter example of a sensitive CID
20 semiconductor sensor, the sensor combines pixels to form a super pixel for simultaneous readout, and achieves sensitivity on the order of several photons per second by cooling. Such cooling can be achieved in a hand-held device with a peltier device thermally connected to the sensor.

For the present invention, the inventors realized that by choosing a chemilumigenic
25 enzyme, such as horseradish peroxidase to generate light inside the detection device, less sensitivity is needed for the detector. This allows simpler detection (photomultiplier operation in an integration mode instead of photon counting mode) or substitution of a semiconductor for a photon multiplier and, where appropriate, less cooling to achieve satisfactory sensitivity. In one embodiment of using a semiconductor in this context, only a
30 single peltier device is needed to cool the detector to less than 0 degrees centigrade. In another embodiment, no peltier cooler is needed and the semiconductor is used at room

temperature. Thus, the inventors discovered that although semiconductor sensors generally are less sensitive than photo multipliers, they could combine the high light output of an enzyme-based chemiluminescence reaction with a semiconductor sensor to take advantage of the sensor, which may have lower power consumption, greater stability and smaller size.

5 A skilled artisan will readily appreciate that a device of the present invention can be packaged with other components such as an instruction pamphlet, a wash reagent that may be either a dried material that is redissolved in water or buffer, or already present as a fluid, and optionally one or more reagents to add to the device such as gold particles treated with a binding partner for a binding reaction. Accordingly, the invention allows the production and
10 use of kits for infectious disease testing from blood. Preferred procedures according to embodiments of the invention are used in combination with kits.

The present invention will now be illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE ONE

15 Chemiluminescence assays were carried out using a simple plastic cassette as indicated in Figure 2. Two cassettes, one for 0157 and another one for H7 antigens was tested in parallel. The barrier for capturing the bacterial antigens is mounted on a hole 8mm in diameter. The samples were directly added onto the barrier for specific (spotted with specific antibodies) or non-specific binding. Unbound or non-specific antigens and others
20 were washed away by adding a wash buffer to the barrier and the solution was absorbed with the absorption pad adhered to the cassette tongue. An enzyme-labeled antibody solution specific for 0157 or H7 antigen was placed on the bacterial antigen captured barrier at an appropriate concentration and allowed to bind for a defined period of time. Extensive washing of the barrier with the wash buffer was carried out to eliminate the unbound or
25 excess enzyme-labeled antibody. A chemiluminescence substrate solution was added onto the barrier and the intensity of the emitted light was measured by a photomultiplier tube in a commercial instrument BioLIGHT (TM) luminometer, from Universal HealthWatch, Columbia, Maryland.

More specifically, the assay was carried out by adding two drops of sample and
30 allowing the sample to contact the membrane for about 1 minute. The absorption pad was

closed and two to five drops of wash buffer were added. The absorption pad was opened and one drop of peroxidase labeled antibody solution was added to the membrane. The antibody solution remained in contact with the membrane for about 1 minute. The absorption pad was closed and 5 to 10 drops of wash buffer were added to wash away the unbound antibody. Then one drop of chemiluminescence solution was added and the cassette was inserted into the BioLIGHT (TM), followed by recording of the light emission by the machine.

The preparation of the reagents used is generally known to the skilled artisan. For example, the preparation of nitrocellulose membrane having antigen or antibody attached is described in co-pending US patent application nos. 08/577,108 (Childs et al., entitled PARTICLE ASSISTED IMMUNOASSAY), 08/577,630 (Childs et al., entitled STRIP TEST FOR HIV) and 08/912,580 (Childs et al., entitled DIAGNOSTIC TEST DEVICES WITH IMPROVED FLUID MOVEMENT AND RESISTANCE TO INTERFERENCES) which are herein incorporated in their entireties by reference. Monoclonal antibodies useful in the procedure are described, for example by Westerman *et al.*, J. Clin. Microbiol. 35: 679-684 (1997) and by He *et al.*, Appl. Environ. Micro. 62: 3325-3332 (1996) Microbiol. 57: 2693-2698 (1991).

EXAMPLE II

Antibodies against human immunodeficiency virus (HIV) antigens can be detected by the following procedure.

Immobilize antigens (virus lysate, recombinant protein, or synthetic peptide) on a capture membrane. Wash the membrane with BSA blocking buffer to block the remaining protein-binding sites of the membrane. Add 1-2 drops of sample (serum, plasma, urine, saliva, etc.) on the membrane and incubate for 1-2 minutes at room temperature. Wash the membrane with BSA containing buffer to eliminate the unbound antibodies and other proteins. Add 1 drop of anti-human immunoglobulins labelled with horseradish peroxidase or protein A (or protein G) labelled with horseradish peroxidase. Incubate for 1-2 minutes at room temperature. Wash the membrane with BSA containing buffer to eliminate the unbound enzyme-labelled antibodies. Add 1 drop of luminol substrate and read the cassette in the BioLIGHT(TM) luminometer immediately.

EXAMPLE III

Hepatitis can be detected from blood, using non-specifically immobilized DNA on the cassette membrane. A specific DNA probe which is complementary to hepatitis DNA is conjugated to alkaline phosphatase and used to detect hepatitis DNA. In the procedure, sample DNA is immobilized onto a membrane such as nylon or nitrocellulose by any of a number of procedures known to the skilled artisan, such as by spotting and ultraviolet cross linking. Then one drop of DNA probe-alkaline phosphatase conjugate is added in salmon DNA containing hybridization solution (2X SSPE and 0.2% SDS) and incubated at 42° C for 10-15. The strip membrane then is washed with 10 drops of salmon DNA containing buffer. One drop of dioxetane substrate solution is added and chemiluminescence is determined with a BioLIGHT (TM) chemiluminometer.

EXAMPLE IV

Chemiluminescent detection and quantitation of a target nucleic acid also is carried out by the following procedure. Specific details of how to carry out this procedure are known to the skilled artisan, as exemplified by Karamohamed et al., BioTechniques 24: 302-306 (1998), Bryant et al., BioTechniques 23: 476-483 (1997) and the J. of NIH Research, 9:23-25 (1997), which are herein incorporated in their entireties by reference. Fifty microliters of sample are added to a nylon membrane in the test cassette. This is crosslinked by exposure to ultraviolet light for 25 seconds. Then, 100 microliters of blocking buffer consisting of 5% SDS, 125 mM NaCl and 25 mM sodium phosphate, pH 7.2 are added. This is allowed to incubate for 30 seconds and then washed through by addition of wash fluid and contact of the membrane with the absorbent pad.

Fifty microliters of biotinylated target gene probe specific for a tumor marker, (biotinylated telomere-specific oligonucleotide TTAGGG) are added, allowed to react for one minute and washed through. Then 10 microliters of streptavidin (100 micrograms per ml) diluted in 40 microliters of blocking buffer are added and allowed to react for one minute. The membrane is washed twice with 50 microliters of 1:10 diluted blocking buffer. Chemilumigenic substrate is added and light emission is monitored.

The target gene is detected by the presence of a chemiluminescence light signal above the instrument noise background signal level. The level of the signal is proportional to the number of copies of the target nucleic acid in the sample.

Although the present invention has been described with reference to certain
5 acceptable embodiments, modifications or changes may be made therein by those skilled in
this art without departing from the scope or spirit of the present invention, as defined by the
appended claims. Further, although the inventors have described certain embodiments,
designs, materials, etc. as preferred, it is understood that such preferred embodiments merely
represent the preferred embodiments that have resulted from the inventors' work to date and
10 that many other acceptable embodiments can be discovered and employed through practice of
the invention and routine trial and error, and that some of such other embodiments ultimately
may be more preferred than those specifically described. All documents referenced herein
are specifically incorporated in their entireties by reference.

In the appended claims, the articles "a" and "an" shall mean "at least one" unless
15 otherwise indicated.

What is claimed is:

1. A detection device for detecting an analyte from a liquid sample, comprising:
- (a) a test strip having a fixed portion with an opening and a movable portion
5 having a surface with an area at least the size of said opening;
- (b) a reagent layer connected to said fixed portion for occluding said opening,
said reagent layer comprising immobilized antibody or antigen, and having at
least about 5uM porosity; and
- (c) an absorbent pad connected to said movable portion; wherein said absorbent
10 pad has a volume that exceeds the volume of said reagent layer by more than
about ten-fold,

wherein said movable portion is manually movable to allow said absorbent pad to
receive said liquid sample from said reagent layer by contacting said reagent layer with said
absorbent pad to dry said reagent layer sufficiently to allow chemiluminescence detection
15 fluid to enter said reagent layer.

2. The device of claim 1, wherein said analyte is E. coli 0157 and said
immobilized antibody is a monoclonal antibody having cross reactivity to a
lipopolysaccharide or H7 flagellar antigen.

3. The device of claim 1, wherein said chemiluminescence detection fluid
comprises a peroxide and a thiazole.

4. The device of claim 1, wherein said liquid sample comprises a nucleic acid
25 hybridization fluid.

5. The device of claim 4, wherein said antibody has a binding site for a nucleic
acid hybrid.

6. A kit for conducting an assay, comprising in packaged combination:
- (a) the device of claim 1 and

- (b) reagents for conducting an assay.

7. The kit of claim 6, comprising a peroxide and thiazole for reacting with horseradish peroxidase enzyme.

8. A system for detecting an analyte from a liquid sample, comprising:

- (a) a test strip having a fixed portion with an opening and a movable portion having a surface with an area at least the size of said opening, a reagent layer connected to said fixed portion for occluding said opening, said reagent layer comprising immobilized antibody or antigen, having at least about 5uM porosity and an absorbent pad connected to said movable portion wherein said absorbent pad has a volume that exceeds the volume of said reagent layer by more than about ten-fold and

- (b) an electronic chemiluminescence monitoring device that accepts said test strip and which reports a signal in response to light emission from said test strip.

9. A chemiluminescence strip method for detecting an analyte from a sample comprising:

- (a) applying said sample to a test strip having a fixed portion with an opening and a movable portion having a surface with an area at least the size of said opening, a reagent layer connected to said fixed portion for occluding said opening, said reagent layer comprising immobilized antibody or antigen, having about at least 5uM porosity and an absorbent pad connected to said movable portion wherein said absorbent pad has a volume that exceeds the volume of said reagent layer by more than ten-fold;
- (b) waiting for analyte in said sample to react with said immobilized antibody or antigen;
- (c) manually moving said absorbent pad to contact said reagent layer, thereby transferring said liquid sample from said reagent layer into said absorbent pad;
- (d) applying chemiluminescence detection fluid to said reagent layer of said strip;

- (e) inserting said strip into a chemiluminescence detector that uses a light detection element selected from the group consisting of a photon multiplier, CCD, CID, photo fet and photo diode; and
- (f) detecting light emitted from said reagent layer in response to the presence of said analyte.

10. The method of claim 9, wherein said sample is a nucleic acid hybridization solution and said sample is a nucleic acid.

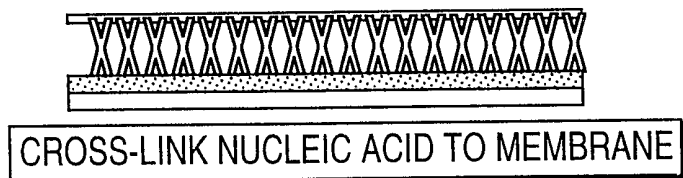
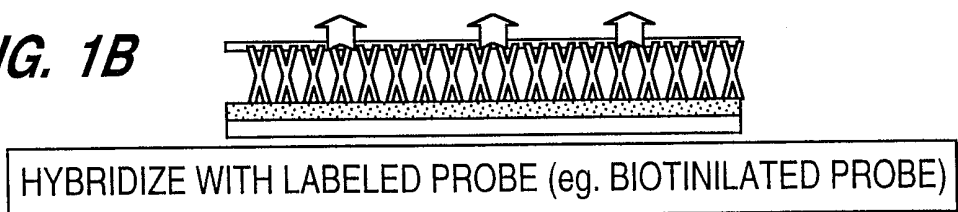
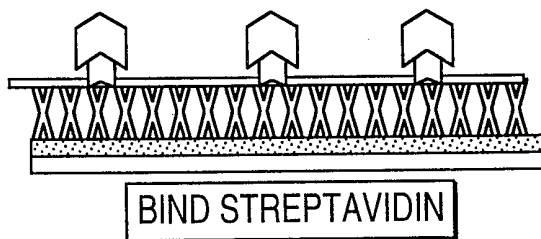
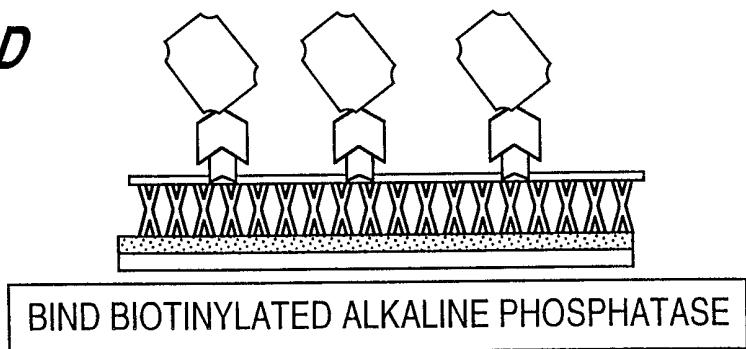
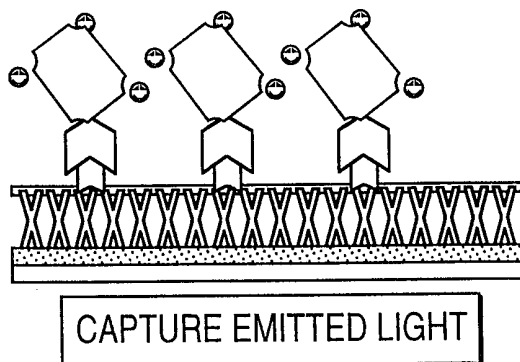
11. The method of claim 10, further comprising a sample pre-treatment step of hybridizing a sample with a hybridization solution.

12. The method of claim 10, wherein said chemiluminescence detection fluid comprises a peroxide substrate for reaction with horseradish peroxidase.

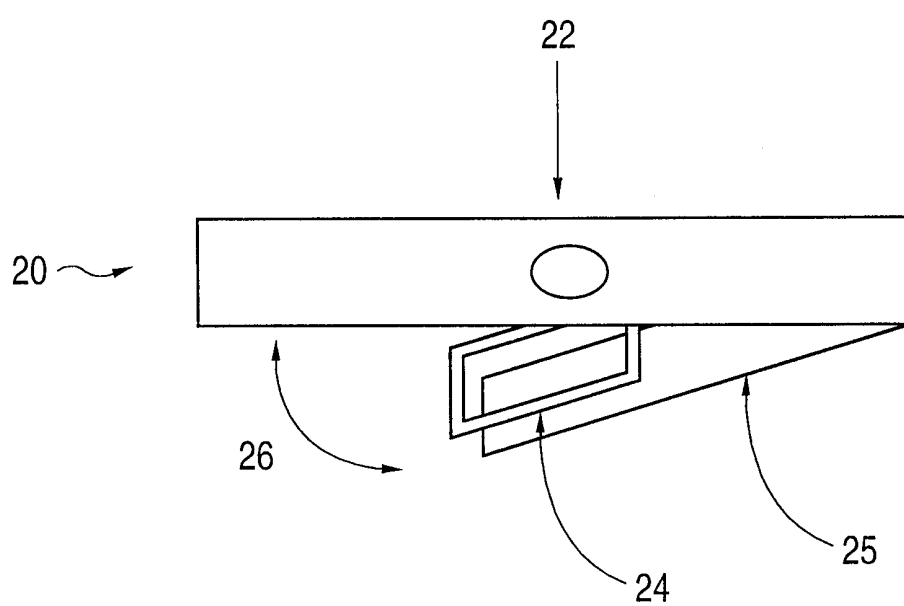
13. The method of claim 10, wherein the light detection element used for step (f) is a photon multiplier tube.

14. The method of claim 10, wherein the light detection element used for step (f) is a charge injection device.

1/2

FIG. 1A**FIG. 1B****FIG. 1C****FIG. 1D****FIG. 1E**

2/2

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/04785

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/543 G01N33/52 G01N33/569 G01N33/58 C12Q1/68

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)

IPC 6 G01N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

Special categories of cited documents:

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Date of the actual completion of the international search

27 July 1999

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 85 02466 A (VERTRIK BIOTEKNIK AB) 6 June 1985 (1985-06-06) the whole document	1-14

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