

DOUBLE-CHAMBER BI-DIRECTIONAL REVERSE FLOW DEVICE

FIELD

[0001] The present invention relates generally to a solid phase chromatographic assay device, test kit and method for the detection of an analyte in a sample. More particularly, the present invention provides a Bi-directional chromatographic assay device with double diagnostic chambers to detect two or more analytes.

BACKGROUND

[0002] Bibliographic details of references provided in the subject specification are listed at the end of the specification.

[0003] Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

[0004] Chromatographic assay systems employed as rapid assay devices are one of several means for detecting the presence of a given analyte from a biological sample. One advantage of these systems is that they do not require specialized equipment or trained personnel. Another advantage is the wide variety of analytes that can be detected using this type of assay. The use of rapid chromatographic techniques for detecting the presence of an analyte in a biological sample has thus progressed beyond the bounds of the clinical laboratory, as assay devices employing these techniques have been found to be especially valuable in "point of care" situations such as in a medical practitioner's office or in a home setting.

[0005] Typical rapid chromatographic tests utilize either a "sandwich" assay or a "competition" assay to detect the presence of a desired analyte. In the sandwich assay, an analyte is bound, or "sandwiched," between an unlabeled first binding partner and a labeled second binding partner. For example, an analyte, such as an antibody to HCV, can be captured by a first binding partner, in this case, an HCV antigen, immobilized on a membrane. The antibody-antigen complex can then be detected by a second binding partner having a label, such as another HCV antigen/anti-human antibody tagged with a colored particle.

[0006] In contrast, in a competition assay, the analyte in the sample competes with a labeled analyte, or labeled analog to the analyte, for a binding partner immobilized on a solid support. A greater concentration of analyte in the sample results in a lower signal in the assay, as the labeled analytes are competed away from the binding partner on the solid support (i.e., the signal produced during a competition assay decreases as the concentration of analyte in the sample increases). Thus, the sandwich assay provides a qualitative assessment with great sensitivity, whilst the competition assay provides a quantitative measure of analyte concentration.

[0007] Regardless of the analyte-detection method used, the rapid assay devices currently available are often categorized into one of four basic formats: the "dipstick" format, the "flow through" format, the "lateral flow" and the reverse flow format.

[0008] The "dipstick" format (as exemplified in U.S. Patent Nos. 5,275,785; 5,504,013; 5,602,040; 5,622,871 and 5,656,503) typically consists of a strip of porous material having a sample receiving end, a reagent zone and a reaction zone. The sample is wicked along the assay device starting at the sample-receiving end and moving into the reagent zone. The analyte to be detected binds to a reagent incorporated into the reagent zone, preferably a labeled binding partner, to form a complex. Typically, these binding pairs are antibody:antigen complexes, or receptor:ligand complexes having a label such as a colloidal metal incorporated into the reagent portion of the complex. The labeled binding partner-antigen complex then migrates into the reaction zone, where the complex is captured by another specific binding partner firmly immobilized in the reaction zone. Retention of the labeled complex within the reaction zone thus results in a visible readout.

[0009] The "flow through" format (such as exemplified in U.S. Patent No. 4,020,046) also utilizes porous solid phase materials. This assay format usually has a porous membrane that contains an immobilized binding partner positioned above an absorptive layer. Once the sample has been added to the membrane surface, the analyte of interest reacts with the immobilized binding partner to form an analyte-binding partner complex. The complex is visualized by addition of a second binding partner having a label, such as an enzyme, dye particles or colloidal metals. The absorptive layer acts as a sink for excess assay reagents, and can be used to regulate the flow rate of the reactants to achieve optimal reaction between the analyte and the binding partner. In this format, the sensitivity of the readout can be improved by "washing" the membrane with additional solution to reduce any non-specific binding of the label, or remove any other materials which can interfere with the assay readout.

[0010] The "lateral flow" format (see for example U.S. Patent Nos. 5,075,078; 5,096,837; 5,354,692 and 5,229,073) utilizes a porous solid phase material and has a linear construction similar to that of the dipstick assay format: a sample application site, a reagent releasing site and a reaction site. However, instead of vertically wicking the samples up the "dipstick," the lateral flow format allows a sample to flow laterally across the porous solid phase material. The sample is applied directly to the application site and the analyte of interest flows laterally to the reagent-releasing site, and forms a complex with a labeled binding partner. The analyte:binding partner complex then migrates into the reaction site where it is captured by a second, immobilized binding partner and detected.

[0011] The "reverse flow" format (such as in U.S. Patent No. 6,316,205) utilizes two directional flows of analytes which allows first binding partner flows from one end and second binding partner from other end of the device and makes the format more sensitive with a clearer background.

[0012] Conventional rapid assays are useful for determining the presence of a given analyte in samples at all levels of the health care system ("point of care test") without need of specialized equipment or personnel, and produce results in a short period of time. For example, simple and rapid immunoassay devices for infectious diseases such as AIDS have been available for almost a decade. However, the existing rapid tests are not without their shortcomings. Most importantly, the sensitivity of such devices has often been questioned, due to various limitations with the currently available formats (Giles *et al*, *Journal of Medical Virology*, 59:104-109, 1999). In addition, there are several practical limitations to the use of these assay devices inherent in the design of the assay format, as exemplified below.

[0013] The dipstick format, which was originally designed for urine analysis, uses a relatively large volume of sample for analysis. This is a considerable limitation to the use of such a device for analysis of serum or blood samples. In contrast, assay devices based on the flow-through format reduce the volume requirement of samples significantly.

[0014] However, the flow-through format cannot be employed in a truly self-contained device. In devices based on the flow-through format, the detecting reagent (i.e. the labeled binding partner) is not directly incorporated into the porous solid matrix of the device and thus must be separately provided. This leads to additional limitations regarding reagent transportation and stability.

[0015] The lateral flow format overcomes both the sample volume problem of the dipstick format, as well as the detecting reagent issue of the flow-through format. However, the lateral-flow format does not allow for a washing step, as inherent in the flow-through format. Any interfering species, such as particulate or colored material introduced by the sample solution, or unbound label, can potentially interfere with the readout of the assay device. As a result, the lateral flow format often employs filtration (e.g., using specialized filters) during the assay procedure, e.g., using specially coated filters to remove potential interfering species prior to detection of the analyte. Moreover, the intensity of test line in lateral flow format is not stable which may change after 30 minutes because of the back flow of coloring agent from absorbent pad into the chromatographic and/or reaction zone.

[0016] The reverse flow format provides more facilities to the end-user because it only needs a small volume of sample, higher titration end-point, clearer background and stable test lines. However, this format cannot be used to detect both antigen and an antibody in the same chromatographic zone due to immunological interactions and is unable to meet the demands of early detection required for infection by agents such as HIV, HCV and HBV when sero-conversion is yet to develop.

[0017] There is a need to develop an improved assay device.

SUMMARY

[0018] The present invention provides an assay device, test kit and method for detecting the presence of an analyte or signature molecule and in particular multiple analytes or signature molecules in a sample. By controlling the release of the various reagents used in the assay device, the sensitivity of the assay is improved as compared to conventional assays, without compromising the specificity of the assay. Improved sensitivity without loss of specificity against multiple disease conditions by simultaneous detecting both antigen and antibody in a single platform is a highly desirable improvement in the field of rapid chromatographic assay. In addition, the assay can be performed by untrained personnel in a short period of time, and without the need for specialized equipment. The assay device or test kit may be referred to as a "double chamber multi-lateral reverse flow device". In a particular embodiment, the device is a "double chamber bi-directional reverse flow device".

[0019] Adverse immunological interactions which can interfere with conventional assays are avoided by using separate chambers where binding partners instructive or diagnostic of particular conditions are detected and thus the assay device of the present invention provides an approach to achieve optimal control of the assay reactions without requiring specially-developed specific binding partners, large volumes of sample, or complicated arrays of reagents or fluid pathways. The present invention further provides an assay device that is particularly suitable for rapid chromatographic assays using a controlled series of reactions. The assay device of the present invention uses a small volume and central addition of sample and achieves a much higher titration end-point activity with immunologically interacting signature molecules than conventional lateral flow assays. In addition, the assay device of the present invention provides greater assay sensitivity against multiple conditions without compromising specificity.

[0020] Accordingly, the subject invention provides an assay device or test kit for use in detecting the presence of analytes specific for particular conditions such as multiple disease conditions and/or multiple biomarkers against single disease condition like cardiac markers.

[0021] One aspect of the present invention provides an assay device or test kit comprising:

(a) a chromatographic element comprising a central sample receiving area and a multiplicity of reagent releasing ends and reaction zones;

- (b) an absorbent pad adjacent the central sample receiving area; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad.

[0022] Another aspect of the present invention is directed to an assay device or test kit of an assay device or kit comprising:

- (a) a chromatographic element comprising a central sample receiving end, two reagent releasing ends, and a double reaction chamber;
- (b) an absorbent pad adjacent to said reaction zone; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad.

[0023] Yet another aspect of the present invention relates to an assay device or test kit comprising:

- (a) a chromatographic element comprising a centrally located sample receiving area, a reagent releasing area at both ends of the device, and two reaction chamber or windows;
- (b) an absorbent pad positioned in between said reaction chambers; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier; and wherein the sample receiving end comprises a releasable first binding partner, the reaction chamber comprises an immobilized second binding partner, and the reagent releasing zones comprise a labeled releasable third binding partner.

[0024] Still another aspect of the present invention provides an assay device or test kit comprising:

- (a) a chromatographic element comprising a sample receiving area and a multiplicity of reagent releasing ends reaction zones;
- (b) an absorbent pad positioned in between said reaction zones; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier; and wherein the reaction zone comprise an immobilized first binding partner, and the reagent releasing end comprises a releasable second binding partner and a labeled third binding partner.

[0025] Another aspect of the present invention is directed to an assay device or test kit configured to detect multiple analytes in a sample comprising:

- (a) a chromatographic element comprising a central sample receiving area having a releasable first binding partner, a multiplicity of reaction zones having immobilized one or more second binding partners, and a multiplicity of reagent releasing ends having a releasable labeled third or more binding partners;
- (b) an absorbent pad; and
- (c) a separator positioned between the chromatographic element and the absorbent pad.

[0026] Even another aspect of the present invention contemplates a use of:

- (a) a chromatographic element comprising a central sample receiving area and a multiplicity of reagent releasing ends reaction zones;
- (b) an absorbent pad adjacent the central sample receiving area; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier, in the manufacture of an assay device or test kit for the detection of multiple signature molecules in a sample.

[0027] Another aspect of the present invention provides a method for detecting an analyte in a sample, the method comprising:

- (a) adding the sample to the central sample receiving area of the chromatographic element of the assay device or test kit as herein described;
- (b) allowing the sample to flow into two directions from the central sample receiving area and through at least a portion of the reaction zones in both sides of the chromatographic element;
- (c) reacting the analyte within the sample with a first binding partner immobilized within the reaction chambers to form a first complex;
- (d) adding an aqueous solution to the reagent releasing ends of the chromatographic elements and solubilizing a releasable second binding partner incorporated therein, wherein the releasable second binding partner comprises a label;
- (e) removing the separator from the assay device to bring the absorbent pad into contact with the part of the chromatographic element;
- (f) allowing the releasable second binding partner to flow from the releasing ends and through at least the portion of the reaction chambers of the chromatographic element;
- (g) forming a second complex between the releasable second binding partner and a substrate selected from the group consisting of the analyte, the first binding partner, and the first complex; and
- (h) detecting the second complex through color development.

[0028] In an alternative embodiment, the present invention relates to a method for detecting an analyte in a sample, the method comprising:

- (a) adding a sample to the central sample receiving area of the chromatographic element of the assay device or test kit as herein described;
- (b) allowing the analyte to flow from the sample receiving area into both and opposite directions through at least a portion of a reaction chamber of the chromatographic element;
- (c) reacting the first complex with an immobilized agent within the reaction chamber to form a first immuno-complex;
- (d) adding to the reagent releasing ends of the chromatographic element an aqueous solution and solubilizing a releasable second binding partner incorporated therein;
- (e) removing the separator from the assay device to bring the absorbent pad into contact with the part of the chromatographic element which allows absorbent pad to absorb fluid from the sample pad;
- (f) allowing the releasable second binding partner to flow in reverse direction of the sample flow through at least the portion of the reaction chamber;
- (g) forming a second complex between the releasable second binding partner and a substrate selected from the group consisting of the analyte, the releasable first binding partner, and the first complex; and
- (h) detecting the second complex through color development.

[0029] Yet another embodiment of the present invention contemplates a method for detecting an analyte in a sample, the method comprising:

- (a) adding the sample to the central sample receiving area of the chromatographic element of the assay device or test kit as herein described;
- (b) allowing the sample to flow from the central sample receiving area in both and opposite directions and through at least a portion of the reaction chamber of the chromatographic element;
- (c) reacting the analyte within the sample with a first binding partner immobilized within the reaction zone to form a first complex;
- (d) adding an aqueous solution to the reagent releasing ends of the chromatographic element and solubilizing a releasable second binding partner and a labeled releasable third binding partner incorporated therein;
- (e) removing the separator from the assay device to bring the absorbent into contact with the portion of the chromatographic element;
- (f) binding the releasable second binding partner to the releasable third

binding partner to form a second complex;

(g) allowing the second complex to flow from the reagent releasing end and through at least the portion of the reaction zone of the chromatographic element;

(h) forming a third complex between the first complex and the second complex; and

(i) detecting the third complex through color development.

[0030] Additional features of the present invention will become apparent from the description which follows or may be learned by practice of the instant invention.

[0031] The contents of U.S. Patent No. 6,316,205 are incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Figure 1 is a schematic representation of a general view of the multi-lateral reverse flow device of the present invention

[0033] Figure 2 is a schematic representation of lower case of the device showing different supporting elements to hold the chromatographic elements.

[0034] Figure 3 is a schematic representation depicting internal view of lower case and the positioning of different parts of chromatographic elements.

[0035] Figure 4 is a schematic diagram of MULTISCAN Blood Test

[0036] Figure 5 is a schematic diagram of simultaneous detection of ToRCH panels

[0037] Figure 6 is a schematic diagram of Cardiac Markers using Double Chamber Bi-directional Reverse Flow Technique.

DETAILED DESCRIPTION

[0038] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0039] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural aspects unless the content clearly dictates otherwise. Thus, for example, reference to "a releasable binding partner" includes a combination of two or more such binding partners; reference to "an analyte" includes mixtures of analytes; reference to "the invention" includes singular or multiple aspects of an invention; and the like.

[0040] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein.

[0041] One aspect of the present invention provides a multi-chamber reverse flow assay device or kit comprising: (a) a chromatographic element comprising a central sample receiving area and a multiplicity of reagent releasing ends and reaction zones; (b) an absorbent pad adjacent the sample receiving area; and (c) a removable separator positioned between the chromatographic element and the absorbent pad.

[0042] In a particular embodiment the assay device of the present invention comprises (a) a chromatographic element comprising a central sample receiving area, two reagent releasing ends, and two reaction zones; (b) an absorbent pad generally adjacent the central sample receiving area; and (c) a removable separator positioned between the chromatographic element and the absorbent pad. This particular embodiment describes a bi-directional reverse flow device.

[0043] The assay device or test kit is configured to simultaneous detection of multiple analytes or signature molecules in a sample.

[0044] Consequently, another aspect of the present invention is directed to an assay

device or test kit configured to detect multiple analytes in a sample comprising:

- (a) a chromatographic element comprising a central sample receiving area having a releasable first binding partner, a multiplicity of reaction zones having immobilized one or more second binding partners, and a multiplicity of reagent releasing ends having a releasable labeled third or more binding partners;
- (b) an absorbent pad; and
- (c) a separator positioned between portion of the chromatographic element and the absorbent pad.

[0045] The separator, in an embodiment, generally comprises a fluid-impermeable barrier. In operation, a sample is applied to the central sample receiving end of the chromatographic element and allowed to migrate laterally in opposite direction by capillary action towards the reagent-releasing ends. After the sample covers the reaction chamber and the analytes within the sample have interacted with one or more binding partners immobilized within each reaction chamber, an aqueous solution is added to the reagent releasing ends of the chromatographic element. The separator is pulled out from the device, allowing the absorbent pad to come into contact with the portion of the chromatographic element. The aqueous solution is then added prior to concurrently or immediately after the removal of the separator. The separator can be removed by pulling the separator entirely from the assay device, or it can be partially removed such that the sample receiving area of the chromatographic element and the absorbent pad come into contact. One or more reagents embedded at the reagent-releasing area, for example, a second binding partner labeled with a detectable label such as a naturally colored particle, is/are released by addition of the aqueous solution and moved toward the reaction chamber by the pulling force of the absorber pad.

[0046] Thus, the device according to this embodiment allows the analyte to form a complex with the first binding partner prior to the reaction between the labeled second binding partner and the bound analyte complex. In addition, the aqueous solution added to the reagent releasing end of the chromatographic element acts not only as a reagent releasing solvent but also as a wash liquid. As a result, a visual readout with a clear background is observed within each reaction chamber.

[0047] Another embodiment of the assay device of the present invention comprises (a) a chromatographic element comprising a central sample receiving area having a releasable first binding partner, a multiplicity of reaction chambers having immobilized one or more second binding partners, and a multiplicity of reagent releasing ends having a releasable labeled third or more binding partners; (b) an absorbent pad; and (c) a

separator positioned between the chromatographic element and the absorbent pad. In a particular embodiment, there are two reaction chambers and two reagent releasing ends. This embodiment of the assay device is particularly useful when a capture assay is desired. Using an assay device of this embodiment, the analyte (for example, an antibody or toxicant) reacts with at least one first binding partner (such as an antigen or a recombinant protein or other binding partner) impregnated at the sample receiving end of the chromatographic element. The analyte-binding partner complex then migrates to the reaction zone, where this first complex is captured by an immobilized second binding partner (the "capturing reagent," such as anti-human IgG or anti-human IgM antibodies) to form a second complex. When the aqueous solution is added and the separator is removed, one or more third binding partners labeled with a detectable label, such as a naturally colored particle, are released from the reagent releasing end of the chromatographic element, and allowed to laterally flow to the reaction zone. Detectable labels include moieties which can be detected by visual inspection (e.g., moieties which include or produce colored elements), or with the aid of artificial detection systems, including, e.g., optical systems, spectroscopic systems, radiographic systems, and the like. For ease of operation, visually detectable labels are particularly contemplated.

[0048] The third binding partner can interact with the second complex to form a third complex, which can be detected *via* the label incorporated in the third binding partner. Whilst the first binding partner can be single antigen or a mixture of antigens, a generic reagent can be used as the third labeled binding partner, for example, an anti-GST antibody which will react with all GST-constructed recombinant antigens.

[0049] Similarly, in another embodiment of the present invention, the use of two or more reagents interacting at the reagent releasing end of a chromatographic element is contemplated prior to migration across the reaction zone. In this embodiment, the assay device comprises: (a) a chromatographic element comprising a sample receiving end, a reaction zone having an immobilized first binding partner, and a reagent releasing end having two releasable binding partners, at least one of which carries a label; (b) an absorbent pad; and (c) a separator positioned between the chromatographic element and the absorbent pad. Using an assay device of this embodiment, the first complex is formed at the reaction zone between an analyte and a first binding partner bound to the reaction zone. The second reaction occurs at the reagent releasing end between the second and third binding partners once the aqueous solution has been added, to form a second complex bearing a label. The third reaction takes place in the reaction zone, when the analyte-binding partner first complex and the second binding partner- third binding partner second complex interact to form a third, labeled complex which can be detected.

As in the embodiment described above, the second (embedded) binding partner can be single or a mixture of antigens, while the third (labeled) binding partner acting as the detector can also be a generic reagent such as an anti-GST antibody.

[0050] These and other embodiments of the present invention address changes in the reagents used in the assay, and in the order in which the reactions take place. Yet another aspect of the present invention involves the composition of the separator component of the assay device. Rather than using a barrier that must be manually removed during the assay, the separator can be composed of a material that will provide a "time-controlled" barrier, such as a semi-permeable membrane or a material that dissolves over time. When the device is in use in accordance with this embodiment, by the time that the sample added to the sample receiving end has migrated laterally and covered the reaction zone, the separator is dissolved or permeabilized, and the absorbent pad is readied for operation. An aqueous solution can then be added and the assay completed.

[0051] Other aspects of the present invention include a method for detecting an analyte in a sample and a test kit for use in conducting the assays. Other permutations of the present invention are also possible, such as the simultaneous detection of multiple analytes using a single sample and a single device.

[0052] In relation to a test kit, multi-component kits are also contemplated herein comprising a chromatographic element having a central sample receiving area and a multiplicity of reagent releasing ends and reaction zones; an absorbent pad; and a removable separator capable of being positioned between the chromatographic element and the absorbent pad. The test kit may also be packaged for sale with instructions for assembly and use.

[0053] Regardless of the embodiment employed, the assay device of the present invention does not need to include any additional filtration techniques using filters with special coatings, as employed in conventional lateral flow devices. The assay device is versatile and can be used to assess a variety of biological fluids including, but not limited to saliva, serum, plasma whole blood, mucus, sputum, urine, fecal matter, tissue fluid, semen and sebum samples. It can also test environmental samples, industrial samples and waste samples such as for contaminants, toxicants and microorganisms. This versatility is achieved by controlling the order in which the reactions occur, and by the additional "washing" of the reactants as provided by passage of the aqueous solution through the chromatographic element and into the absorbent pad.

[0054] An additional benefit of the present invention is that the design of the assay device provides a generic platform versatile enough to accommodate the needs and requirements for different product lines. An assay device specific for detection of a particular analyte can be easily adapted to detect a different analyte with minimal modification to the overall design, such as replacing the binding partner immobilized within the reaction zone, but still using a "generic" labeled binding partner for detection purposes. There is no need for the development of additional specialized reaction reagents for the detection of each desired analyte. This not only reduces the time needed to design and produce new assay devices, but also significantly reduces the costs for product development. Furthermore, since the major components of the assay device are the same, manufacturing parameters can be maintained without major changes. Thus, a production facility for manufacture of a series of products based on the assay device of the present invention utilizes the same equipment and a minimal inventory of raw materials for the manufacture of all of the products, which in turn reduces the cost of operation significantly.

[0055] In addition, the assay device or test kit is capable of participating in existing pathology architectures to provide diagnostic information and point of care information to a clinician or subject.

[0056] The term "assay device" is used herein to describe a multi-component chromatographic apparatus used for the detection and/or measurement of one or more analytes of interest. Alternatively, terms such as kit, apparatus, configuration may also be used. An assay device, kit, apparatus and/or configuration may also be packaged for sale with instructions for assembly and/or use.

[0057] The term "chromatographic element" refers to a solid matrix upon which a sample can be applied and allowed to migrate during the assay procedure.

[0058] The term "sample receiving end" refers to the portion of the chromatographic element at which the sample is administered during the assay. The expression "sample receiving area" or "sample receiving region" may also be used. The sample receiving end, area or region refers to a single or multiplicity of sample receiving ends, areas and regions.

[0059] The term "reagent releasing end" refers to the portion of the chromatographic element distal to the sample receiving end, and at which one or more releasable assay

reagents are incorporated. The expression "reagent releasing area" or "reagent releasing region" may also be used. The reagent releasing end, area or region refers to a single or multiplicity of reagent releasing ends, areas and regions.

[0060] The term "reaction zone" refers to the region of the chromatographic element between the sample receiving end and the reagent releasing end, within which one or more binding partners specific to one or more analytes (or to a complex containing an analyte) has/have been immobilized.

[0061] The term "absorbent pad" refers to an absorbent or bibulous material usually positioned at the base of the assay device.

[0062] The term "separator" refers to a barrier structure positioned between the chromatographic element and the absorbent pad.

[0063] The term "casing" or "housing" as used herein refers to an optional component of the assay device, which surrounds at least a portion of the chromatographic element, absorbent pad and separator and provides some structural support.

[0064] The term "sample" refers to any desired material for sampling, usually of biological, environmental, industrial or synthetic origin.

[0065] The term "analyte" refers to a compound or composition to be detected or measured in a sample. Examples of analytes include antigens, antibodies, contaminants, toxicants, allergens, microorganisms and viruses.

[0066] The term "binding partner" is used herein to describe a member of a binding pair which interact either chemically or physically to form a complex. An "immobilized" binding partner refers to a binding partner that is adsorbed, embedded or affixed, either permanently or semi-permanently, to a solid substrate or matrix (for example, the reaction zone of the chromatographic element.) A "releasable" binding partner refers to a molecule which is not permanently immobilized or affixed to a solid substrate or matrix, and is capable of migration or movement for example, by diffusion.

[0067] The term "label" as used herein refers to any substance that is capable of producing a detectable signal. Various labels suitable for use in the present invention include, but are not limited to, chromatogens, fluorescent or chemiluminescent compounds, catalysts, enzymes, enzymatic substrates, dyes, colloidal metallic and

nonmetallic particles, and organic polymer latex particles. Particularly useful for the present invention are the visually-detectable colored particles, such as colloidal metals and nonmetals, and dye particles.

[0068] The term "bibulous" refers to materials that are absorbent.

[0069] The present invention is directed towards an assay device for detection of one or more analytes instructive of a particular condition such as a disease condition or contamination in a single platform. The assay device is constructed in a manner to allow for the controlled release and interaction of the assay reagents. Furthermore, included in this invention is a method for detecting the analyte, as well as test kits employing the assay device. The term "subject" includes human and non-human subjects. Hence, the assay device or test kit has application in human medicine and in veterinary or animal husbandry. Non-human subjects include sheep, cattle, horses, goats, pigs, poultry, non-human primates and captive wild animals. The sample may also be an environmental, waste, industrial or chemical or synthetic sample.

[0070] As will be understood by the ordinary skilled artisan upon reading the specification, the analyte can be any specific substance or component that one is desirous of detecting and/or measuring in a chemical, physical, enzymatic, or optical analysis. Analytes of interest include, for example, antigens (such as antigens specific to a microorganism, virus or protozoan organism); antibodies, particularly those induced in response to an infection, allergic reaction, or vaccine; hormones, proteins and other physiological substances (for example, human chorionic gonadotropin, estrogens, progestins, testosterone, corticosteroids, human growth factors, hemoglobin, and cholesterol); nucleic acids; a variety of enzymes; therapeutic compounds and illicit drugs; contaminants and environmental pollutants including toxicants; or any number of natural or synthetic substances including allergens. As is appreciated by one skilled in the art, the number of natural and synthetic substances which can be detected by the assay devices and methods of the present invention is extensive, and include, but is not limited to, the following groups of compounds: ACE inhibitors, adrenergics and anti-adrenergics, alcohol deterrents (for example, disulfiram), anti-allergics, anti-anginals, anti-arthritis, anti-infectives (including but not limited to antibacterials, antibiotics, antifungals, antihelminthics, antimalarials and antiviral agents), analgesics and analgesic combinations, local and systemic anesthetics, appetite suppressants, antioxidants, anxiolytics, anorexics, antiarthritis, anti-asthmatic agents, anticoagulants, anticonvulsants, antidiabetic agents, antidiarrheals, anti-emetics, anti-epileptics, antihistamines, anti-inflammatory agents, antihypertensives, antimigraines,

antinauseants, antineoplastics, antioxidants, antiparkinsonism drugs, antipruritics, antipyretics, antirheumatics, antispasmodics, antitussives, adrenergic receptor agonists and antagonists, anorexics, appetite suppressants, cardiovascular preparations (including anti-arrhythmic agents, cardiotonics, cardiac depressants, calcium channel blockers and beta blockers), cholinergics and anticholinergics, contraceptives, diuretics, decongestants, growth stimulants, herbal preparations, hypnotics, immunizing agents, immunomodulators, immunosuppressives, muscle relaxants, neurologically-active agents including anti-anxiety preparations, antidepressants, antipsychotics, psychostimulants, sedatives and tranquilizers, sore throat medicaments, sympathomimetics, vasodilators, vasoconstrictors, vitamins, xanthine derivatives, various combinations of these compounds, and the like.

[0071] The device according to the present invention is particularly useful for detection of analytes in samples of biological, environmental or industrial origin. As indicated above, such samples include, but are not limited to, whole blood, serum or plasma; saliva, sputum, tears, sweat, or other secreted fluids; urine or fecal matter; as well as biologically derived fluids such as cerebrospinal fluid, interstitial fluid, cellular extracts and the like. A minimal volume of sample is used for the assay device of the present invention, particularly as compared to sample volumes used in a flow-through assay format. Desired sample volumes range from about 1 μL to about 500 μL , preferably from about 1 μL to about 100 μL , more preferably from about 5 μL to about 50 μL , most preferably between about 10 μL and about 30 μL .

[0072] The assay device of the present invention is based on binding assays such as, but not limited to, immunoassays. The binding partners involved in such binding assays include, but are not limited to, the following binding pairs: antibody and antigen or haptens; hormone and receptor; biotin and avidin; carbohydrate and lectin; effector and receptor molecules; enzymes and cofactors, substrates, or inhibitors; and complementary nucleotide sequences. Thus, the descriptions and examples included below are for demonstration purposes and should not be considered limiting to the particular applications addressed.

[0073] The devices of the invention are particularly well adapted to detecting antibody-antigen binding. Thousands of antibody-antigen binding partners are known and can be detected using the devices herein. A number of basic texts describe antibody-antigen interactions, antibody production processes, and other related matters, including, e.g., Borrebaeck (ed.), *Antibody Engineering*, 2nd Edition Freeman and Company, NY, 1995; McCafferty *et al*, *Antibody Engineering*, A Practical Approach IRL at Oxford Press,

Oxford, England, 1996; Paul, *Antibody Engineering Protocols Humana Press*; Towata, N.J., 1995; Paul (ed.), *Fundamental Immunology*, Fourth Edition, Lippincott-Raven, N.Y., 1999; Coligan, *Current Protocols in Immunology Wiley/Greene*, NY; Harlow, 1991 and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, NY, 1989; Stites *et al*, (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, Calif., and references cited therein; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, N.Y., 1986; and Kohler and Milstein, *Nature* 256:495-497, 1975.

[0074] Figure 1 is an illustrative embodiment of the double chamber Bi-directional reverse flow device of the present invention. The assay device 1 in Figure 1 is composed of the view of upper face 2 which is made of plastic and has several windows or chambers: middle sample receiving window A, two reagent releasing windows C and C¹ and two reaction chambers B and B¹. The middle sample receiving window A is used to add sample (whole blood/plasma/serum/saliva/urine and the like) and allows the release of sample in two directions over reaction chambers B and B¹. The reagent windows C and C¹ are used to receive chase buffer which release labeled reagents over reaction chambers B and B¹ in opposite directions. The Reaction chambers B and B¹ expose the chromatographic membrane which contains immobilized capturing agents and indicator lines and allows the flow of test samples and reagent in reverse direction and show visible indication of test results. A portion of separator D protrudes from casing at the middle of device just opposite the sample receiving window A which facilitates the removal of separator D during the assay procedure.

[0075] Figure 2 is a view of the double chambers bi-directional reverse flow device showing the view of lower case of the device which is made of plastic and has several supporting areas to hold chromatographic elements, separator and absorbent pad. The central notch 1 is designed to hold an absorbent pad on top of which the separator is placed to separate the sample pad which is located of one of the ends of the chromatographic element 4 and 5. The each chromatographic element is located in chamber 2 & 3 and has three distinct areas: sample pad area (common for two membranes), reaction zone and reagent pad area. The membrane is held by a lower case of the device with appropriate supporting notches on the lower plastic case.

[0076] Figure 3 is illustrative of the different components of the assay device and how they adjust on the bottom part of the plastic case. Each side of the device has three major components: chromatographic element which is positioned on separator which in turn is positioned above an absorbent pad. The each chromatographic element consists of

three parts: sample receiving end A, reaction zone B & B1 and reagent releasing end C & C1. The sample receiving end is common and positioned at the centre of device and placed on the separator, so that once sample is added it flows in both directions over reaction zones. The chromatographic elements are supported by plastic backing and has three zone sample absorption (X), reaction zone (Y) and reagent releasing zone (Z). In this embodiment of the present invention, an optional filter pad is attached to and constitutes the part of sample receiving and while optional reagents bearing pad is impregnated with releasable reagents. Reaction zones contain colored indicators as well as immobilized capturing partners. Optionally, reaction zones also contain known protein (e.g. Protein-A or Protein-L) as an indicator of the validity of the device.

[0077] The assay device of the present invention is able to provide improved sensitivity for analyte detection over current available rapid chromatographic assays without compromising specificity. This advancement is demonstrated in the example section of the present invention. However, the advantages of the assay device of the present invention are not limited to the functional aspects of the assay device, but address the practical aspects as well. Regardless of the particular embodiment employed, the instant assay device does not need to include additional filtration devices, such as filters with special coatings, to handle a wide variety of biological fluids. This versatility is achieved by the design of the assay device, which allows staged reactions and sufficient washing without involving additional steps.

[0078] An additional advantage of the assay devices of the present invention is in the ease of manufacture of the assay device. The devices of the present invention employ a generic construct, which can be modified with minimal alteration from one application to another.

[0079] This generic platform is versatile enough to accommodate the needs and requirements for multiple product lines. A product specific for detection of a particular analyte can be easily adapted to another product for a different analyte with minimal modification of the overall design of the assay device, such as replacement of the binding partner to one particular analyte. Accordingly, it is not necessary to develop additional specific detecting reagents for each specific product. Rather, the specificity of the reaction is determined by the first binding partner while the labeled binding partner can be a multipurpose generic construct (for example, anti-human antibodies or anti-GST antibodies labeled with a detectable label such as a naturally colored particle). This is a great advantage as compared to the development of traditional rapid assays, in which a specific detector for a specific product must be developed for each assay, in order to

maintain an acceptable sensitivity. The assay device of the present invention, therefore, reduces both the time and the cost used for product development. Furthermore, since the major components of the assay device can be used across a variety of assays, production parameters can be maintained without need for change. A production facility manufacturing a series of products based on the present invention would use a single set of manufacturing equipment and a minimal array of inventories of raw materials, which in turn significantly reduces the costs of operation.

[0080] The present invention is now described by the following non-limiting Examples.

EXAMPLE 1 (MULTISCAN Blood TEST)

[0081] For the detection of human antibodies profiles against different blood borne infections (HIV, HCV, HBV and Syphilis) both antibodies and antigens against each pathogen can be detected simultaneously and is shown in Fig. 4: Detection of both signature molecules against each pathogen could be used as **One Stop** confirmatory assay.

EXAMPLE 2 (ToRCH Panel)

[0082] The Double Chamber Bi-directional Reverse Flow Technique will be designed to detect two different types of antibody molecule into two different chambers as shown in Fig. 5. The detection of two types antibody molecules (IgG and IgM) against multifactorial infections like ToRCH (Toxo, Rubella, CMV and Herpes) is very important for the screening of pregnant women samples in order to prevent abortion and/or birth related complication of mother and new born.

EXAMPLE 3 (Cardiac Markers)

[0083] Another example of application of double chamber Bi-directional Reverse Flow Technique could be Cardiac markers are used in the diagnosis and risk stratification of patients with chest pain and suspected acute coronary syndrome (ACS). The cardiac troponins, in particular, have become the cardiac markers of choice for patients with ACS. Indeed, cardiac troponin is central to the definition of acute myocardial infarction (AMI) in the consensus guidelines from the American College of Cardiology (ACC) and the European Society of Cardiology (ESC). However, several cardiac markers including creatine kinase MB (CK-MB), myoglobin, troponin I, and troponin T and inflammatory markers like CRP and MYO that yield qualitative and quantitative results comparable to traditional central lab assays are important in emergency room patients with chest pain. The double chamber Bi-directional Reverse Flow device may improve triage and clinical outcomes where all necessary bio-markers can be detected in two separate chambers as shown in Fig. 6.

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CLAIMS:

1. An assay device or test kit comprising:
 - (a) a chromatographic element comprising a central sample receiving area and a multiplicity of reagent releasing ends and reaction chambers;
 - (b) an absorbent pad adjacent the central sample receiving area; and
 - (c) a removable separator positioned between the portion of the chromatographic element and the absorbent pad.
2. The assay device or kit of Claim 1 wherein the chromatographic element comprises one sample releasing end, two reagent releasing ends and a double reaction chamber.
3. The assay device or kit of Claim 2, wherein the central sample receiving area comprises with elements which absorb and release first binding partner in two opposite directions.
4. The assay device or kit of Claim 3, wherein the releasable binding partner comprises a detectable label.
5. The assay device or kit of Claim 2, wherein the central sample receiving area comprises a first releasable binding partner, and the reagent releasing ends comprise a second releasable binding partner.
6. The assay device or kit of Claim 2, wherein the reaction chambers comprise immobilized binding partners for a specific analytes.
7. The assay device or kit of Claim 6, wherein the specific analyte comprises one or more capturing agents.
8. The assay device or kit of Claim 6, wherein in one of the reaction chambers comprises one or more antigens and/or antibodies as capturing agents.
9. The assay device or kit of Claim 6, wherein the specific analyte in the other reaction chamber comprises antigens and/or antibodies as antigen and/or antibodies capturing agents.
10. The assay device or kit of Claim 2, wherein the separator protrudes beyond the

chromatographic element and the absorbent pad at the central zone of the device.

11. The assay device or kit of Claim 1 or 2, further comprising

(d) a casing surrounding at least a portion of the chromatographic element, absorbent pad and separator.

12. The assay device or kit of Claim 11, wherein a portion of the separator protrudes from one side from the casing.

13. The assay device or kit of Claim 1 or 2, further comprising an aqueous solution.

14. The assay device or kit of Claim 1 or 2, further comprising one or more of: two containers for holding chromatographic elements, the separator, the absorbent pad, or a combination thereof; packaging materials for packaging the chromatographic element, the separator, the absorbent pad, or a combination thereof; and an instruction set.

15. A method for detecting an analyte in a sample, the method comprising:

(a) adding the sample to the central sample receiving area of the chromatographic element of the assay device or kit of Claim 1 or 2;

(b) allowing the sample to flow into two opposite directions from the central sample receiving area and through at least a portion of the reaction chambers in both sides of the chromatographic element;

(c) reacting the analyte within the sample with a first binding partner immobilized within the reaction chambers to form a first complex;

(d) adding an aqueous solution to the reagent releasing ends of the chromatographic elements and solubilizing a releasable second binding partner incorporated therein, wherein the releasable second binding partner comprises a label;

(e) removing the separator from the assay device to bring the absorbent pad into contact with the portion of the chromatographic element;

(f) allowing the releasable second binding partner to flow from the releasing ends and through at least the portion of the reaction chambers of the chromatographic element;

(g) forming a second complex between the releasable second binding partner and a substrate selected from the group consisting of the analyte, the first binding partner, and the first complex; and

(h) detecting the second complex through the development of color.

16. The method of Claim 15, wherein the adding the aqueous solution and

solubilizing a releasable second binding partner is performed prior to the removing the separator from the assay device.

17. The method of Claim 15, wherein removing the separator from the assay device is performed prior to the adding the aqueous solution and solubilizing a releasable second binding partner.

18. The method of Claim 15, wherein removing the separator from the assay device is performed concomitant with the adding the aqueous solution and solubilizing a releasable second binding partner from the both end of device.

19. The method of Claim 15, wherein the separator is removed by pulling out of the separator from between the chromatographic element and the absorbent pad.

20. The method of Claim 15, wherein the analyte comprises an antibody, an antigen, or both an antigen and an antibody.

21. A method for detecting an analyte in a sample, the method comprising:

(a) adding a sample to the central sample receiving area of the chromatographic element of the assay device or kit of Claim 1 or 2;

(b) allowing the analyte to flow from the sample receiving area into both and opposite directions and through at least a portion of a reaction chamber of the chromatographic element;

(c) reacting the first complex with an immobilized agent within the reaction chamber to form a first immuno-complex;

(d) adding to the reagent releasing ends of the chromatographic element an aqueous solution and solubilizing a releasable second binding partner incorporated therein;

(e) removing the separator from the assay device to bring the absorbent pad into contact with the chromatographic element which allows absorbent pad to absorb fluid from the sample pad;

(f) allowing the releasable second binding partner to flow in reverse direction through at least the portion of the reaction chamber;

(g) forming a second complex between the releasable second binding partner and a substrate selected from the group consisting of the analyte, the releasable first binding partner, and the first complex; and

(h) detecting the second complex through the development of color.

22. The method of Claim 21, wherein the adding the aqueous solution and solubilizing a releasable second binding partner is performed prior to the removing the separator from the assay device.

23. The method of Claim 21, wherein the removing the separator from the assay device is performed prior to the adding the aqueous solution and solubilizing a releasable second binding partner.

24. The method of Claim 21, wherein the removing the separator from the assay device is performed concomitant with the adding the aqueous solution and solubilizing a releasable second binding partner.

25. The method of Claim 21, wherein the separator is removed by pulling the separator from between the chromatographic element and the absorbent pad.

26. The method of Claim 21, wherein the analyte comprises an antibody, an antigen, an antibody, or both an antigen and an antibody.

27. A method for detecting an analyte in a sample, the method comprising:

(a) adding the sample to the central sample receiving area of the chromatographic element of the assay device or kit of Claim 1 or 2;

(b) allowing the sample to flow from the central sample receiving area in both and opposite directions and through at least a portion of the reaction chamber of the chromatographic element;

(c) reacting the analyte within the sample with a first binding partner immobilized within the reaction chamber to form a first complex;

(d) adding an aqueous solution to the reagent releasing ends of the chromatographic element and solubilizing a releasable second binding partner and a labeled releasable third binding partner incorporated therein;

(e) removing the separator from the assay device to bring the absorbent into contact with the chromatographic element;

(f) binding the releasable second binding partner to the releasable third binding partner to form a second complex;

(g) allowing the second complex to flow from the reagent releasing end and through at least the portion of the reaction zone of the chromatographic element;

(h) forming a third complex between the first complex and the second complex; and

(i) detecting the third complex through the development of color.

28. The method of Claim 27, wherein the adding the aqueous solution is performed prior to the removing the separator from the assay device.

29. The method of Claim 27, wherein the removing the separator from the assay device is performed prior to the adding the aqueous solution.

30. The method of Claim 27, wherein the removing the separator from the assay device is performed concomitant with the adding the aqueous solution.

31. An assay device or kit comprising:

- (a) a chromatographic element comprising a central sample receiving end, two reagent releasing ends, and a double reaction zone;
- (b) an absorbent pad adjacent to said reaction zone; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier.

32. The assay device or kit of Claim 31, further comprising:

- (d) a casing surrounding at least a portion of the chromatographic element absorbent pad and separator.

33. The assay device or kit of Claim 31, further comprising an aqueous solution.

34. The assay device or kit of Claim 31, further comprising one or more of a container for holding the chromatographic element, the separator, the absorbent pad, or a combination thereof; packaging materials for packaging the chromatographic elements, the separator, the absorbent pad, or a combination thereof; and instructions for use.

35. An assay device or kit comprising:

- (a) a chromatographic element comprising a sample receiving area at the middle of the device, a reagent releasing area at both ends, and two reaction zones or windows;
- (b) an absorbent pad positioned in between said reaction zones; and
- (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier; and wherein the sample receiving end comprises a releasable first binding partner, the reaction zone comprises an immobilized second binding partner, and the reagent releasing zones comprise a labeled releasable third binding partner.

36. The assay device or kit of Claim 35, further comprising:
- (d) a casing surrounding at least a portion of the chromatographic elements, absorbent pad and separator.
37. The assay device or kit of Claim 35, further comprising an aqueous solution.
38. The assay device or kit of Claim 35, further comprising one or more of: a container for holding the chromatographic elements, the separator, the absorbent pad, or a combination thereof; packaging materials for packaging the chromatographic elements, the separator, the absorbent pad, or a combination thereof; and an instruction set.
39. An assay device or kit comprising:
- (a) a chromatographic element comprising a sample receiving area and a multiplicity of reagent releasing ends and reaction zones;
 - (b) an absorbent pad positioned in between said reaction zones; and
 - (c) a removable separator positioned between the chromatographic element and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier; and wherein the reaction zone comprise an immobilized first binding partner, and the reagent releasing end comprises a releasable second binding partner and a labeled third binding partner.
40. The assay device or kit of Claim 39, further comprising:
- (d) a casing surrounding at least a portion of the chromatographic absorbent pad and separator.
41. The assay device or kit of Claim 39, further comprising an aqueous solution.
42. The assay device or kit of Claim 39, further comprising one or more of: a container for holding the chromatographic elements, the separator, the absorbent pad, or a combination thereof; packaging materials for packaging the chromatographic elements, the separator, the absorbent pad, or a combination thereof; and instructions for use.
43. Use of:
- (a) a chromatographic element comprising a central sample receiving area and a multiplicity of reagent releasing ends and reaction zones;
 - (b) an absorbent pad adjacent the central sample receiving area; and
 - (c) a removable separator positioned between the chromatographic element

and the absorbent pad, wherein the separator comprises a fluid-impermeable barrier,
in the manufacture of an assay device or kit for the detection of multiple
signature molecules in a sample.

44. An assay device or test kit configured to detect multiple analytes in a sample
comprising:

(a) a chromatographic element comprising a central sample receiving area
having a releasable first binding partner, a multiplicity of reaction zones having
immobilized one or more second binding partners, and a multiplicity of reagent
releasing ends having a releasable labeled third or more binding partners;

(b) an absorbent pad; and

(c) a separator positioned between the portion of the chromatographic
element and the absorbent pad.