(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 369 473 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
21.01.2009 Bulletin 2009/04

(21) Application number: **02022910.0**

(22) Date of filing: **10.10.2002**

(51) Int Cl.:
C12M 1/34 *(2006.01)* ***G01N 33/53*** *(2006.01)*
G01N 33/543 *(2006.01)* ***G01N 35/00*** *(2006.01)*
C12M 1/40 *(2006.01)*

(54) Automated cassete module for an apparatus for conducting immunoassays and use thereof

Automatisierte Kassette für eine Vorrichtung zur Durchführung von immunoanalytischen Tests und Verfahren zu ihrer Verwendung

Cassette automatique accessoire pour un dispositif des éssais immunoanalytiques et procédé d'utilisation

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

(30) Priority: **07.06.2002 US 387287 P**

(43) Date of publication of application:
10.12.2003 Bulletin 2003/50

(73) Proprietor: **CHOLESTECH CORPORATION**
Hayward, CA 94545-3808 (US)

(72) Inventors:
- **Nugent, Anthony**
  Fremont, CA 94555 (US)
- **Cowley, Leanne**
  Fremont, CA 94538 (US)
- **Bellet, Neal**
  Walnut Creek, CA 94598 (US)
- **Shindelman, Jeffrey**
  Castro Valley, CA 94552 (US)
- **Leos, Michael**
  Fairfield, CA 94534 (US)
- **Worthy, Thomas**
  Walnut Creek, CA 94598 (US)
- **Haley, Kimberly**
  Belmont, CA 94002 (US)

(74) Representative: **Hess, Peter K. G.**
Patent- und Rechtsanwälte
Bardehle . Pagenberg . Dost .
Altenburg . Geissler
Postfach 86 06 20
81633 München (DE)

(56) References cited:
US-A- 5 110 724
US-A- 5 135 873
US-A- 5 611 995
US-A- 5 744 096

EP 1 369 473 B1

Printed by Jouve, 75001 PARIS (FR)

Description

Field of the Invention

[0001] The present invention is directed to an apparatus and method for use in assaying a body fluid sample for a selected analyte, and particularly for use in automated multi-stage assays.

Background of the Invention

[0002] In view of the background of the invention, references are made for De Maat, M.P.M. et al., Fibrinolysis 8 (Suppl 2):50-52 (1994); Grau, A.J. et al., "Clinical and Biochemical Analysis in Infection-Associated Stroke." Stroke 26(9):1520-6 (Sept 1995); Harlow, E. et al., "Antibodies: A Laboratory Manual", Cold Spring Harbor Lab (1988); Hewett, G.E., U.S. Patent No. 5,110,724 (1992); Hewett, G.E. et al., U.S. Patent No. 5,171,688 (1992); Kuller, L.H. et al., Am J Epidemiol. 144:537-547 (Sep 15 1996). Leuvering et al., U.S. Patent No. 4,313,734 (1982); Liuzzo G, M.D. et al., N Engl J Med 331(7): 417-424, Aug. 18, 1994; Mendall, M.A. et al., British Med. J. 312:1061-65 (Apr 27 1996); Thompson, S.G. et al., N Engl J Med 1995;332:635-41; Tracy, R.P. et al., Circulation, 1996, V93, N3 (Feb 1), P8 (Tracy).

[0003] Assays for detecting the presence and level of a variety of analytes in body fluid samples are known. Such assays are often designed for simplicity of use so that they can be reliably conducted in a doctors office or other clinical setting where personnel may have little training in clinical assay procedure or in interpreting assay results. In order to minimize the need for operator involvement, it is preferable that the assay be carried out in an automated or self-contained manner.

[0004] Such self-contained assays have typically been limited, for the sake of simplicity of operation, to one-step assay procedures. A number of useful assays, however, are multistage in nature, requiring more than one reacting or binding step. Further, one or more of the steps may be rate limiting, or affected by localized reagent concentrations. Typically, multistage assays are less readily automated and generally require more input from the user, thus increasing the possibility of error.

[0005] The European patent application 02004339.4 titled "High density lipoprotein assay device and method" discloses a cassette for measuring the concentration of HDL-associated cholesterol in blood fluid samples. For reasons of effectiveness and simplicity, the assay design is such that removal of non-HDL lipoproteins from a sample an assay of HDL cholesterol in the sample occur without interruption of the assay. Thus, the sample flow as well as the reagent contact with the same can not be controlled generating interference in the quantitative and qualitative testing.

[0006] Document US 5,744,096 also assigned to the applicant of the present application discloses an immunoassay cassette useful for carrying out multistage immunoassays in an automated manner. The cassette may assume different operational positions in which solutions are transferred across an absorbent pathway containing assay reagents. To this end the cassette contains a body and a support member, which support member may be pushed towards the base member to establish a contact between two reaction means. The US '096 also discloses an apparatus for automatically move said support and said base member towards each other, to establish a contact between a transfer strip provided on the base member and a control reaction zone provided on the support member. The apparatus does however not repeatedly move the support and the base member toward and away from each other to allow a control of the rate of sample transfer.

[0007] It is therefore the problem of the present invention to provide an automated, self-contained assay device which is able to perform multistage assays, in particular those containing multiple reacting or binding steps in which one or more of the steps is rate limiting or the final assay result is affected by localized reagent of analyte concentrations.

Summary of the Invention

[0008] The above problems are solved by a method according to claim 1 and an apparatus according to claim 6.

[0009] A cassette for use in detecting an analyte in a liquid body-fluid sample is described in the following. The cassette provides a cassette body preferably having a sample well for receiving the sample, a support mounted on the body, for movement toward and away from a transfer position, and a reagent reservoir and a reagent strip carried on the body and support, respectively. The reagent reservoir contains a first reagent composition effective to react with one or more sample components to form a modified sample, as sample migrates from the sample well into the reservoir. The reagent strip contains a second reagent composition effective to react with the modified sample to form a detectable analyte-dependent product. In one embodiment the reagent strip has a transfer zone that is brought into contact with the reservoir, when the support is moved to its transfer position, and a detection zone located downstream of the transfer zone. By controlling the movement of the support toward and away from its transfer position, the volume and rate of sample flow from the reservoir to the strip can be controlled to optimize and/or standardize sample-transfer conditions in the assay.

[0010] For use in detecting a multivalent analyte in a liquid body-fluid sample, the first reagent composition in the reagent reservoir may include a non-immobilized conjugate of an anti-analyte antibody and a detectable reporter group, where the reaction to form a modified sample includes binding of the conjugate to sample analyte, to form an analyte-conjugate complex. The second reagent composition in the reagent strip may include an

anti-analyte antibody immobilized at a detection region in the reagent strip located downstream of the strip's sample-transfer region, where the reaction to form a detectable analyte-dependent product includes binding of complex to the immobilized antibody, to localize the detectable reporter in the complex at the detection zone. The non-immobilized conjugate in this embodiment may be a conjugate of an anti-analyte-antibody and a visible reporter, such as metal particles, particles labeled with colored or fluorescent moieties, polymers labeled with colored of fluorescent moieties, particles, or colored or fluorescent molecules.

[0011] For use in detecting C-reactive protein analyte in a blood sample, the anti-analyte antibody in the non-immobilized conjugate in the reagent reservoir, and the immobilized anti-analyte antibody in the reagent strip may be antibodies specific against a common epitope in C-reactive protein. Alternatively, the two antibodies may be directed against different C-reactive protein epitopes.

[0012] The support preferably comprises a window through which binding of the complex at the detection zone in the reagent strip can be viewed. In addition, the detection zone in the reagent strip may be covered by a reflective film at the strip surface facing away from the window.

[0013] The cassette preferably further comprises an absorbant reservoir carried on the support, downstream of said detection zone, for receiving sample liquid transferred through the reagent strip.

[0014] The invention comprises an apparatus for use in detecting an analyte in a liquid body-fluid sample according to claim 6. The apparatus comprises a cassette of the type described above, and a cassette-handling instrument. The instrument has (a) a cassette holder into which the cassette is removably placed, during a sample assay, (b) an actuator operable to move the support in the cassette toward and away from its sample-transfer position, (c) a detector operable to detect an analyte-specific reaction at the detection zone in the reagent strip, and (d) a processor operably connected to the actuator, for controlling the volume timing and rate of movement of sample material from the reagent reservoir to the reagent strip.

[0015] In one preferred embodiment, the detection zone in the reagent strip in the cassette is covered by a reflective film at the strip's surface facing away from said window, such that flow of sample liquid through the detection zone produces a first change in reflectance measurable through the window, and the presence of analyte-dependent reaction at the detection zone produces a second change in reflectance measurable through the window. The detector may be operable to detect liquid flow through the detection zone, by a first change in measured optical reflectance, and is operable to measure a subsequent analyte-dependent reaction at the detection zone, by a second change in measured optical reflectance.

[0016] The control unit may be operable to control the volume and rate of sample transfer from the reagent reservoir to the reagent strip by controlling one or more of (i) the period of sample incubation before sample is first transferred from the reservoir to the reagent strip, (ii) the cycle frequency with which the actuator moves the support toward and away from its transfer position, (iii) the time of contact that the support is held in its transfer position, during each cycle, and (iv) the total number of transfer cycles. The unit is operable to control the volume and rate of sample transfer from said reservoir to the reagent strip by controlling (i) the cycle frequency with which the actuator moves the support toward and away from its transfer position and (ii) the time of contact that the support is held in its transfer position, during each cycle.

[0017] The invention comprises a method according to claim 1 of conducting an assay for a body-fluid analyte, by the steps of: (a) introducing a body fluid containing the analyte into a absorbent reservoir containing a first reagent composition effective to react with one or more sample components to form a modified sample, (b) repeatedly contacting the reservoir, with such containing an absorbed body-fluid sample, with an absorbent reagent pad containing a second reagent composition effective to react with the modified sample formed in the reservoir to produce a detectable analyte-dependent product, and (c)controlling the frequency and duration of said contacting, thereby to control the volume and rate of transfer of sample fluid from the reservoir to the pad.

[0018] In one generally preferred embodiment of the present invention, the reagent pad is an elongate reagent strip having a sample-transfer zone at which the reservoir makes contact with the strip, and a detection zone located downstream of the transfer zone.

[0019] For detecting a multivalent analyte in a liquid body-fluid sample, such as C-reactive protein in a blood or serum sample, the first reagent composition in the reagent reservoir may include a non-immobilized conjugate of an anti-analyte antibody and a detectable reporter group, and the reagent composition in the reagent strip, an anti-analyte antibody immobilized at a detection region in the reagent strip.

[0020] These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

[0021]

Figs. 1A and 1B are plan views of an immunoassay cassette constructed in accordance with one embodiment of the invention, with the cassette in an initial sample loading position (Fig. 1A), and a sample-transfer position (Fig. 1B);

Fig. 2 is an enlarged section view of the cassette support in the region of the detection zone;

Figs. 3A and 3B illustrate the distribution of sample fluid prior to (Fig. 3A), and following (Fig. 3B) sample-fluid transfer in the cassette.

Fig. 4 is a perspective view of a cassette-handling instrument constructed according to one embodiment of the invention;

Fig. 5 illustrates various functional components of the cassette-handling instrument in relationship to an immunoassay cassette of the invention;

Figs. 6A and 6B illustrate different sample-volume transfer profiles achievable by the invention;

Fig. 7 illustrates reflectance profiles for a C-reactive protein analyte at three different concentrations, during an assay procedure in accordance with one embodiment of the invention; and

Fig. 8 is a plot of measured reflectance as a function of C-reactive protein analyte concentration, generated in accordance with the invention.

Detailed Description of the Invention

I. Immunoassay Cassette

[0022] Figs. 1A and 1B show an immunoassay cassette 10 constructed according to one embodiment of the invention. The cassette contains two plate-like members, a base member or body 12 and a support or support member 14, which may be produced by standard molding or machining methods. The support 14 is mounted on the body 12 for movement in a vertical direction in the figures from an at-rest position seen in Fig. 1A to a sample-transfer position shown in Fig. 1B. More particularly, the support 14 is mounted for movement toward and away from a sample transfer position.

[0023] The structure mounting the support 14 on the body 12 may be compressible blocks, such as elastomeric blocks, as shown at 16, which support opposite ends of the support 14. These blocks 16 become compressed as the support 14 is moved from its at-rest position, where the blocks 16 are substantially uncompressed, to the sample-transfer position, where the blocks 16 are maximally compressed. It will be appreciated that a variety of compressible structures, such as springs or magnets, could be to mount the support 14 on the cassette body 12 for biased movement toward and away from the sample-transfer position.

[0024] Provided within body 12 is a sample well 18 for receiving the body fluid sample to be analyzed. The well 18 is designed to receive a body-fluid sample, such as a blood or serum sample, typically having a volume between 20 and about 60 μl. The sample well 18 transfers sample to a center pad 20. The center pad 20, in turn, communicates through a capillary wick 22, also referred to herein as a spreading layer, with a reagent pad or reservoir 24 containing a first reagent or reagent composition, to be described. When sample liquid is placed in well 18, it migrates by capillarity through the center pad 20, and from the center pad 20, through the spreading layer 22 to the reservoir 24. Where the sample being analyzed is a blood sample, one or more of the elements in the flow path between the sample well 18 and the reservoir 24, and typically the center pad 20 and/or the spreading layer 22, may be effective to remove or retard the flow of red blood cells, so that the sample material reaching the reservoir has been freed of blood cells or other particulate components. Glass fiber and other matrix material suitable for this purpose are well known.

[0025] Alternatively, or in addition, one or more of the elements in the flow path may be effective to remove undesired sample components through the use of immobilized binding agents, e.g., antibodies, specific against the unwanted components. Undesired sample components can also be removed by exposing the sample to a precipitating agent in the flow path effective to selectively precipitate the undesired sample components. For example, dextran sulfate may be used to selectively precipitate certain lipoproteins in a blood sample. The precipitated particles are either blocked from migration through the flow path, or retarded in flow. The center pad 20, sample transfer strip 22, and reservoir 24 are preferably formed of bibulous, fibrous material capable of drawing fluid via capillary flow. A variety of fibrous materials, such as are commonly used in fibrous-mat filters, including cellulose, cellulose acetate, and glass fibrous matrices, are suitable materials for the transfer strip. The fibers may be crosslinked, if desired, by chemical crosslinking, heat fusion, or the like. Also suitable are porous substrates, such as sintered glass, fused polymer beads, and the like, whose wettability and dimension of interstices are such as to promote movement of an aqueous medium into the strip by surface wetting. One exemplary material is a glass fiber filter having a packing density of about 0.2 - 0.5 gm/cm^3. The center glass 20, spreading layer 22 and reservoir 24 may be mounted on the body 12 directly on the body or through a backing made of plastic or other inert support material.

[0026] Although the cassette embodiment 10 shown is designed for a single assay, at the right side of the cassette in the figures, it will be appreciated that the cassette could be adapted for additional assay(s) at the left side of the cassette. Further, the additional assay may have the same fluid-flow format, or may have a different format, e.g., the center pad 20 may communicate with an elongate reaction strip extending along the upper left edge region of the cassette body.

[0027] Completing the description of the cassette body 12, a pair of elastomeric blocks 26 on either side of the center pad serve to cushion and limit the movement of the support as it is moved toward its sample-transfer position.

[0028] Support 14 has a pair of elongate reaction bars,

such as bar 28, extending outwardly from the center of the support, as seen in Figs. 1A and 1B. An elongate reagent strip 30 is affixed to and extends along the lower, inward-facing surface of the support 14, as shown. The strip has an upstream sample-transfer zone 32 and a downstream detection zone 34 located directly below a window 36 formed in the support bar. As seen in Fig. 1B, movement of the support 14 to its sample-transfer position, with compression of blocks 16, brings the sample-transfer zone 32 into contact with the reagent reservoir 24 on the cassette body 12, promoting capillary fluid flow from the reservoir 24 to the reagent strip 30.

[0029] The reagent strip 30 is formed of a material porous or fibrous material which promotes capillary flow therethrough. Preferred materials include porous, fused polymer or microporous polymer membranes, such as polysulfone, polypropylene, nylon, nitrocellulose, Teflon™, or polyvinylchloride microporous membranes. In the present case, nitrocellulose, such as is available from Sartorius, is particularly preferred, with such having length, width, and thickness dimensions between 5-20 mm, 1-5 mm, and 0.1-0.5 mm, respectively.

[0030] The downstream end of strip 30 is in contact with an absorbent pad 38 which functions as a reservoir to draw sample liquid supplied to the strip 30 at the sample-transfer zone, and flowing in a downstream direction (to the right in the figures) into and through the detection zone 34. The pad 38 is formed of a suitable absorbent material such as fibrous glass or cellulose. The absorbance volume of the pad 38 is preferably at least half of the volume of the sample added, e.g., 10 to 30 μl.

[0031] Disposed within the fluid pathway defined by the reagent strip 30 are reagents, described further below, effective to produce a detectable, analyte-dependent reaction product which is detected at the detection zone. Various assays may therefore be carried out using the cassette 10, as described below.

[0032] The outer-facing surface of the reagent strip 30, downstream of the sample-transfer zone 32, is covered by an impermeable reflective film, such as a Mylar film. In particular, the film extends over the detection zone 34 in the reagent strip 30, which is sandwiched between the support window 36 and the reflective film. The purpose of the reflective strip is to enhance the reflectivity of the reagent strip 30, as viewed through the support window 36, and in particular, to enhance the change in reflectivity observed when the strip 30 is wetted, and in response to an analyte-specific reaction occurring in the detection zone 34, as will be discussed further below.

[0033] The construction of the various layers on the support 14 is illustrated in exploded view in Fig. 2. Shown here are support bar 28 having window 36 located therein, and reagent strip 30 having upstream sample-transfer zone 32 and detection zone 34. The strip 30 is attached to the support bar 28 with a double-side adhesive strip 44 initially covered with a removable backing 45. As shown, the adhesive strip 44 is separated by a space corresponding to the support window 36 and detection zone 34. Absorbent pad 38 is positioned to overlap with the downstream end of reagent strip 30, and the reflective film is positioned to extend from a point just downstream of the sample-transfer region in strip 30 to a point beyond the absorbent pad 38. In construction, the components above are arranged as shown, and attached to the support bar 28 and to one another by removing adhesive backing 44 and pressing the adhesive side of the assembly firmly against the support bar 28.

[0034] The cassette 10 is designed particularly for an analyte assay in which (i) sample components, which typically include the analyte itself, react with one or more reagents in the reservoir 24 to form a modified sample, and (ii) the modified sample, typically modified analyte, reacts with a second reagent composition to produce a detectable, analyte-dependent product. That is, both the reagent reservoir 24 and the reagent strip 30 in this preferred embodiment contain one or more reagents for carrying out these reactions. The one or more reagents in the reservoir 24 and strip 30 are also referred to herein as a first and second reagent compositions, respectively, and may include one or more enzymes, antibodies, labeled antibodies, or enzyme substrates, binding agents, and/or precipitation agents, as discussed further below.

[0035] In one exemplary cassette 10, for detection of a multivalent antigen analyte, the reagent composition in the reservoir 24 includes a non-immobilized analyte-specific antibody labeled, e.g., covalently with a detectable reporter, e.g., metal particles, fluorescent or colored molecules, branched polymers containing attached colored or fluorescent moieties, and coated particles, e.g., fluorescent-coated latex particles. By "non-immobilized" is meant that the reagent is freely mobile within the reservoir 24. Thus, when analyte is added to the reservoir 24, it reacts specifically with the antibody reagent to form a mobile, labeled analyte-antibody complex. In other preferred embodiments of the present invention, the reagent compositions may be immobilized or non-immobilized, depending on whether the reagent must co-migrate with the analyte and/or whether the reagent would be expected to interfere with the final analyte determination.

[0036] Methods of labeling a binding agents, such as labeled antibodies, are known in the art, including the use of radiolabels, fluorescent labels, or linked enzymes which convert a separate substrate to a detectable species. A variety of reporter-labeled antibodies, such as enzyme-labeled antibodies, are commercially available or may be readily prepared according to known methods (see, e.g., Harlow, pp. 319-358). Optically detectable labeling methods are preferred for use with the present immunoassay cassette 10. Enzymes which react with a substrate to produce a visible reaction product are widely used for this purpose. A particularly preferred labeled reagent is an analyte-specific antibody conjugated to a visible particle such as a colored latex bead or colloidal gold. Such conjugates are described in U.S. Patent No. 4,313,734 (Leuvering) and may also be obtained from manufacturers such as BB International (Cardiff, UK) and

NanoProbes (Stony Brook, NY).

[0037] The reagent composition in the reagent strip 30, which may include one or more reagents, may be distributed throughout the strip 30, or localized on the strip 30, for example, at the sample-transfer zone 32, just downstream of the transfer zone 32, or in the detection zone 34, in immobilized or non-immobilized form. In the embodiment described above, for detection of a multivalent analyte, the second reagent composition includes an anti-analyte antibody immobilized at the detection zone 34. In this format, labeled analyte-antibody complex transferred from the reservoir 24 to the reagent strip 30 migrates in a downstream direction in the strip 30, where it is captured in the detection zone 34. The step of forming a detectable reaction product includes capturing a detectable complex at the detection zone 34.

[0038] In other preferred embodiments, the second reagent composition may include immobilized or non-immobilized enzymes, substrates, labeled binding reagents, photosensitizer agents, reducing or oxidizing agents, and/or acid or base groups that that can donate protein and hydroxyl ions as part of an analyte-detection reaction, according to known, two-step reaction procedures, where, in the present invention, one of the steps is to be carried out in the reagent reservoir 24, and the second in the reagent strip 30.

[0039] More specifically, the detection zone 34 may contain reagents effective to produce a detectable reaction product with unlabeled antibody-analyte complex. For example, the detection zone 34 may contain an oxidase, a peroxidase, and a compound oxidizable to a detectable species such as a dye. When the analyte-antibody complex includes a substrate for the oxidase, the H_2O_2 generated in the resulting reaction reacts with the oxidizable compound, catalyzed by the peroxidase, to generate the detectable dye. Such assays are described, for example, in Hewett et al.

[0040] The reagents may be incorporated into the reservoir 24 and strip 30 by soaking the reservoir or strip material in a solution of the reagents, followed by drying, or adding a solution of the reagent material to the entire or a localized region of the strip 30, followed by drying. Where the reagent is immobilized, the reservoir or strip region may naturally provide, or be chemically modified according to known methods to have surface reactive groups, such as amine, carboxyl, sulfhydryl, or aldehyde groups, allowing covalent coupling by the use of activating agents or bifunctional coupling agents.

[0041] In one particularly preferred embodiment, the cassette 10 is designed for detection of C-reactive protein, typically measured in a blood or serum sample. In this embodiment, the reagent composition in the reservoir 24 is a non-immobilized monoclonal antibody specific against a C-reactive protein epitope, i.e., an epitope on one of the 5 identical subunits in C-reactive protein. One exemplary anti-C-reactive protein antibody is a monoclonal antibody produced by cell line identified by clone number CC002, and available from Scripps Laboratories (San Diego, CA). The antibody is labeled by conjugation to gold microparticles, according standard methods, e.g., as described in, The place of gold in rapid tests, John Chandler, Tracey Gurmin, and Nicola Robinson, IVD Technology 6, no. 2 (2000): 37-49. The second reagent is an antibody specific against C-reactive protein and immobilized at the detection zone 34. An exemplary antibody is the same as that employed in the first (reservoir) antibody reagent. Details of the assay are given in Example 1.

[0042] Figs. 3A and 3B illustrate the flow of sample liquid into and through the fluid-flow elements of the cassette 10 during cassette operation. As seen in Fig. 3A, liquid sample applied to sample well 18 is drawn by capillarity into center pad 20, and from here, through the spreading layer 22 into reservoir 24. Here the sample may be held for a desired incubation time, in the presence of the first reagent. Typical incubation times may vary from a few seconds up to ten minutes or more.

[0043] When the support 14 in the cassette 10 is moved to its sample-transfer position, shown in Fig. 3B, sample liquid flows by capillarity into the strip transfer zone 32 when the latter is brought into contact with the reservoir 24. From the transfer zone 32, the sample liquid migrates in a downstream direction into and through the detection zone 34 and ultimately into absorbent pad 38. By controlling the delay between addition of sample material to the cassette sample well 18 and initial movement of the support 14 to its sample-transfer position, the cassette 10 can be operated to control the period of sample incubation before sample is first transferred from the reservoir 24 to the reagent strip 30.

[0044] In addition the rate of liquid transfer, and the total sample volume transferred can be controlled by controlling (i) the cycle frequency with which the actuator moves the support 14 toward and away from its transfer position, (ii) the time of contact that the support 14 is held in its transfer position, during each cycle, and (iii) the total number of transfer cycles. As discussed in the next section, this control is conveniently provided by a cassette-handling instrument in an assay apparatus constructed in accordance with another aspect of the invention.

II. Cassette-handling instrument

[0045] Fig. 4 is a perspective view of a cassette-handling instrument 48 constructed in accordance with the invention, and Fig. 5 shows key functional elements of the instrument in diagrammatic form. The instrument includes a cassette holder, represented by drawer 50, adapted to receive and hold the cassette 10 in an operative condition when a sample liquid has been added to the cassette 10. In particular, drawer 50 is biased to engage guide notches, such as notches 52, in the cassette body 12 to anchor the cassette 10 in the holder 50 at a desired position.

[0046] The instrument further includes an actuator 53 having solenoid-activated pistons or pushers, indicated

at 54 operable to engage the cassette support 14 and move the support 14 from its relaxed-state to its sample-transfer position, upon actuation from a control unit 55 in the instrument 48. In particular, the control unit 55 may be programmed or user-adjusted to control the one or more of the following actuator variables:

(i) the period between sample addition to the cassette sample transfer to the strip 30. This time corresponds roughly to the incubation period of sample liquid exposure to and reaction with the first reagent composition, and may vary from an optimum of several seconds up to 10 minutes or longer, depending on the nature of the first sample reaction.

(ii) the cycle frequency with which the actuator 53 moves the support 14 toward and away from its transfer position. The frequency may be varied between one per assay, to a few per minute to one per second.

(iii) the time of contact that the support 14 is held in its transfer position, during each cycle. Together with frequency, this variable determines the total rate at which sample fluid is transferred to the reagent strip 30. This rate can be optimized for different types of assay chemistries. For example, it may be desirable to regulate the flow of material from the sample well 18 through the reagent reservoir 24, to ensure sufficient reaction time in the reagent reservoir 24, or to meter the flow rate through the reagent strip 30, to ensure adequate reaction time in the strip 30.

(iv) the total number of transfer cycles, which, together with contact time in each cycle, will determine the total volume transferred from the cassette body 12 to the strip 30. By controlling the total volume transferred, a more quantitative measure of analyte concentration, expressed as amount of analyte/volume of sample can be determined. In particular, the volume that passes through the detection zone 34 will be the total amount transferred less a predetermined quantity remaining in the portion of the strip 30 upstream of the detection zone 34.

[0047] It will be appreciated that the control unit 55 can be preprogrammed to control liquid transfer in the assay in an optimized manner for any selected type of assay chemistry. Various liquid transfer profiles that can be achieved with the invention will be considered below.

[0048] Also as shown in Fig. 5, the cassette-handling instrument 48 includes a photo-detector 56, operable to detect changes in the reflectance of the detection zone, as observed through window 36. The photo-detector 56 in this case may be a simple device for measuring the light intensity of reflectance at the window 36, when the detection window 36 is illuminated by a light source, e.g., an LED, also forming part of the detector 56. In other embodiments, the detector 56 may include a selected-wavelength fluorescence excitation beam and emitted-light detector, or a selected-wavelength visible light source and photo-detector for measuring light absorption at the detection surface.

[0049] Particularly where the light detector 56 is designed to measure reflectance from the reagent strip surface in the detection zone, the reflective film is effective to enhance, i.e., amplify the reflectance intensity, and thus improve resolution and accuracy. As will be seen in the next section, the enhanced reflectance also allows the detection zone to serve as a "control" to monitor fluid flow through the reagent strip 30.

II. Performance characteristics

[0050] As noted above, one feature of the invention is the ability to control the rate and volume of fluid flow from one reaction area to another, and thus the kinetics of the reactions and the total assay volume. This feature is important where one or more of the assay reactions are rate-limiting or where it is desired to assay a kinetic end point. The feature is also important in controlling the total amount of sample liquid that flows into and through the detection zone 34, for quantitating the concentration of analyte in the zone 34.

[0051] Figs. 6A and 6B illustrate two sample transfer curves that illustrate the different sample transfer characteristics that can be achieved in the invention. In the first case, illustrated in Fig. 6A, sample is added to the cassette 10 at time t_0, and allowed to incubate in the reagent reservoir 24 until a time t_1, when the support bar in the cassette is brought into contact with the reservoir 24. If the support bar 28 is held in contact with the reservoir 24 over an extended period, sample transfer into the strip 30, expressed as sample volume as a function of time, increases linearly until a time t_f when both strip 30 and pad 38 are fully saturated (ignoring sample evaporative effects).

[0052] In Fig. 6B, the sample incubation time, from t_0 to t_1, is the same as in Fig. 6A, but sample transfer is effected by three discrete transfer events, interspersed with intervals in which the support is move out of contact with the reservoir 24 and volume accumulation over time is flat. As can be appreciated, the latter approach allows a more controlled, and typically slower rate of volume transfer than when sample transfer occurs as an unbroken event.

[0053] Fig. 7 shows an exemplary reflectance curve for an assay in which C-reactive protein analyte reacts first with labeled anti-analyte antibody in the cassette reservoir 24, to form a detectable analyte-antibody complex, and the complex is then transferred to the reagent strip 30, where it is captured by an immobilized anti-analyte antibody in the in the detection zone 30. The initial reflectance, in the first few second after sample transfer to the support 14, corresponds to a dry strip reflectance. The precipitous drop in reflectance occurs when the lead-

ing edge of the transferred sample passes through the detection zone 34. The drop in reflectance is due both to the wetting of the strip 30 in the detection zone 34 and the presence of colloidal gold labeled antibodies, either in complexes or non-complexed form.

[0054] With continued flow of sample material through the detection zone 34, the level of reflectance begins to change over time, depending on the relative concentrations of complex and non-complexed antibody conjugate, i.e., depending on the sample analyte concentration. At lower analyte concentration, where relatively more of the conjugate is in non-complexed form, and relatively less of the conjugate is captured at the detection zone 34, the reflectance begins to increase over time as more and more of the conjugate is carried out of the detection zone 34 by sample transfer through the zone 34. This is seen in the "diamond" plot in Fig. 7. Conversely, at higher analyte concentrations, progressively more conjugate is captured in the detection zone 34, with sample flow through the zone 34, acting to decrease reflectance over time, as indicated for the "triangle" plot in the same figure.

[0055] The analyte concentration is measured by comparing the measured reflectance at a selected end point, e.g., 4 minutes, with standard reflectance measurements from known analyte concentrations. The measured reflectance may be expressed, for example, as a ratio of the final percent reflectance to initial percent reflectance. As seen in the plot shown in Fig. 8, a plot of this ratio shows an analyte-dependent curve over a C-reactive protein concentration of 0 to 8 μg/ml.

[0056] From the foregoing, it can be appreciated how various objects and features of the invention are achieved. The cassette 10 provides a dry-strip assay format in which successive analyte-dependent reactions can be carried out in a controlled manner, by controlling volume and rate transfer from one reaction region to another. In addition, the cassette 10 is designed to allow controlled and measured volumes of sample through the detection zone 34, for more quantitative determination of analyte concentration. The cassette format is amenable to multiple assays in the same cassette, and fed from the same sample. Finally, the reflector strip in the support bar 28 acts to enhance reflectance changes, enhancing the reliability and resolution of an assay.

[0057] Preferably, the cassette 10 of the invention is supplied with solutions and reagents preloaded and is thus entirely self-contained, not requiring operator loading of solutions. The reader 48 containing the cassette 10 may be programmed to adjust the cassette to its different operational positions at designated times. A multiple-stage assay may thus be carried out with the cassette 10 contained within a cassette reader 48, requiring no outside operator input.

Example 1

Assay for C-Reactive Protein

[0058] In a specific application of the present device, a blood sample is analyzed for levels of C-reactive protein. Altered levels of this compound have been shown to be diagnostic of disorders characterized by risk factors for cerebral vascular ischemia and stroke, and ischemic heart disease and stroke (see, for example, De Maat, Grau, Kuller, Liuzzo, Mendall, Thompson, and Tracy).

[0059] The cassette 10 for the assay was prepared as follows: The center pad 20 is a glass fiber pad capable of absorbing about 20 μl of liquid. The spreading layer 22 is also glass fiber. The reservoir 24 is a porous plastic material having a total absorption volume of about 6 μl. The reservoir 24 was initially soaked with 6 μl of a 20 O.D. solution of antibody conjugate formed by conjugating colloidal gold with antibody specific against C-reactive protein, obtained from BBInternational (Cardiff, UK). The reservoir 24 was then dried.

[0060] The reagent strip 30 in the cassette 10 is an 11 mm by 3 mm nitrocellulose strip, obtained from Sartorius (Goettingen, GmbH), having a thickness of less than 10 mils. The antibody against C-reactive protein was attached to the detection region 34 through hydrophobic interaction with the nitrocellulose. The detection region 34 was located about 6 mm from the sample-transfer region 32. The absorption pad 38 at the downstream end of the strip 30 is a cellulose fiber material, having a total absorption volume of about 25 μl.

[0061] In the assay method, a 50 μl human blood sample was applied to the sample well 18 in the cassette 10. After an incubation period of 3 minutes, the support 14 was moved to its sample-transfer position for 4 minutes. During flow of sample liquid onto the strip 30, the reflectance at the support-bar window 36 was monitored. At about 4 minutes, a stable end point was reached (Fig. 7). The signal ratio of endpoint reflectance to initial reflectance was determined, and used to calculate analyte concentration from a standard curve generated by samples with known amounts of C-reactive protein.

[0062] While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications may be made without departing from the invention.

Claims

1. A method of conducting an assay for a body-fluid analyte comprising

   a) introducing a body fluid containing the analyte into an absorbent reservoir (24) containing a first reagent composition effective to react with one or more sample components to form a modified sample,

b) repeatedly contacting the reservoir (24), with such reservoir containing an absorbed body-fluid sample, with a reagent strip (30) containing a second reagent composition effective to react with the modified sample formed in said reservoir (24) to produce a detectable analyte-dependent product, and

c) controlling the frequency and duration of said repeated contacting, thereby to control the volume and rate of transfer of sample fluid from the reservoir (24) to the reagent strip (30).

2. The method of claim 1, wherein said reagent strip (30) is an elongate reagent strip (30) having a sample-transfer zone (32) at which the reservoir (24) makes contact with the strip (30), and a detection zone (34) located downstream of the transfer zone (32).

3. The method of claim 2, for detecting a multivalent analyte in a liquid body-fluid sample, wherein the first reagent composition in the reagent reservoir (24) includes a non-immobilized conjugate of an anti-analyte antibody and a detectable reporter group, the reaction to form a modified sample includes binding of the conjugate to sample analyte, to form an analyte-conjugate complex, the reagent composition in the reagent strip (30) includes an anti-analyte antibody immobilized at a detection region in the reagent strip (30), and the reaction to form a detectable analyte-dependent product includes binding of complex to the immobilized antibody, to localize the detectable reporter in the complex at the detection zone (34).

4. The method of claim 3, wherein said non-immobilized conjugate is a conjugate of an anti-analyte antibody and a detectable reporter selected from the group consisting of metal particles, particles labeled with colored or fluorescent moieties, polymers labeled with colored or fluorescent moieties, particles, and colored or fluorescent molecules.

5. The method of claim 4, for use in detecting C-reactive protein analyte in a blood sample, wherein the anti-analyte antibody in the non-immobilized conjugate in the reagent reservoir (24), and the immobilized anti-analyte antibody in the reagent strip (30) are antibodies specific against a common epitope in C-reactive protein.

6. Apparatus for use in detecting an analyte in a liquid body-fluid sample, comprising

i) a cassette (10) having

a) a cassette body (12) comprising a reagent reservoir containing a first reagent composition effective to react with one or more sample components to form a modified sample;

b) a support (14) mounted on said body (12), operable to move toward and away from a transfer position for realizing fluid communication between said reservoir (24) and a reagent strip (30) carried on said support (14); and

c) said reagent strip (30) containing a second reagent composition effective to react with the modified sample formed in said reservoir (24) to form a detectable analyte-dependent product;

ii) a cassette handling instrument (48) having

a) a cassette holder (50) into which the cassette (10) can be removably placed, during a sample assay;

b) an actuator (53) operable to move the support (14) in the cassette (10) toward and away from the transfer position;

c) a detector (56) operable to detect an analyte-specific reaction in the reagent strip; and

d) a control unit (55) operably connected to the actuator (53), for controlling the volume and rate of sample transfer from said reagent reservoir (24) to the reagent strip (30) during an assay procedure, wherein the control unit (55) is operable to control the rate of sample transfer from said reservoir (24) to the reagent strip (30) by repeatedly moving the support (14) in the cassette (10) toward and away from the transfer position and controlling (i) the cycle frequency with which the actuator (53) moves the support (14) toward and away from its transfer position and (ii) the time of contact that the support (14) is held in its transfer position, during each cycle.

7. The apparatus of claim 6, wherein said cassette body (12) having a sample well (18) for receiving said sample so that it can migrate from said sample well (18) into said reservoir (24).

8. The apparatus of claim 7, wherein the first reagent composition in the reagent reservoir (24) includes a non-immobilized conjugate of an anti-analyte antibody and a detectable reporter group, the reaction to form a modified sample includes binding of the conjugate to sample analyte, to form an analyte-conjugate complex, the reagent composition in the reagent strip (30) includes an anti-analyte antibody immobilized at a detection region (34) in the reagent strip (30), and the reaction to form a detectable an-

alyte-dependent product includes binding of complex to the immobilized antibody, to localize the detectable reporter in the complex at the detection zone (34).

9. The apparatus of claim 8, wherein said non-immobilized conjugate is a conjugate of an anti-analyte antibody and a detectable reporter selected from the group consisting of metal particles, particles labeled with colored or fluorescent moieties, polymers labeled with colored or fluorescent moieties, particles, and colored or fluorescent molecules.

10. The apparatus of claim 9, for use in detecting C-reactive protein analyte in a blood sample, wherein the anti-analyte antibody in the non-immobilized conjugate in the reagent reservoir (24), and the immobilized anti-analyte antibody in the reagent strip (30) are antibodies specific against a common epitope in C-reactive protein.

11. The apparatus of claim 8, wherein said support (14) includes a window (36) through which a detectable reaction at the detection zone (34) in the reagent strip (30) be detected by said detector (56).

12. The apparatus of claim 6, wherein said control unit (55) is operable to control the volume and rate of sample transfer from the reagent reservoir (24) to the reagent strip (30) by controlling further the period of sample incubation before sample is first transferred from the reservoir (24) to the reagent strip (30).

Patentansprüche

1. Verfahren zur Durchführung einer Untersuchung eines Körperflüssigkeitsanalyts, umfassend:

a. Einführen einer Körperflüssigkeit, die den Analyt enthält, in ein absorbierendes Reservoir (24), welches eine erste Reagenzzusammensetzung enthält, die wirksam ist, um mit einem oder mehreren Komponenten der Probe zu reagieren um eine modifizierte Probe zu bilden,
b. wiederholtes Kontaktieren des Reservoirs (24), wobei das Reservoir eine absorbierte Probe der Körperflüssigkeit enthält, mit einem Reagenzstreifen (30), der eine zweite Reagenzzusammensetzung enthält, die wirksam ist, um mit der modifizierten Probe zu reagieren, die in dem Reservoir (24) gebildet wurde, um ein detektierbares, analytabhängiges Produkt zu bilden, und
c. Steuern der Frequenz und der Dauer dieses wiederholten Kontaktierens, wodurch das Volumen und die Raten der Übertragung der Probenflüssigkeit von dem Reservoir (24) zu dem Reagenzstreifen (30) gesteuert werden.

2. Das Verfahren nach Anspruch 1, wobei der Reagenzstreifen (30) ein länglicher Reagenzstreifen (30) ist, der eine Probenübertragungszone (32) aufweist, an der das Reservoir (24) in Kontakt mit dem Streifen (30) kommt und eine Detektionszone (34), die stromabwärts von der Übertragungszone (32) angeordnet ist.

3. Das Verfahren nach Anspruch 2 zur Detektion eines multivalenten Analyts in einer flüssigen Probe eines Körperfluids, wobei die erste Reagenzzusammensetzung in dem Reagenzreservoir (24) ein nicht immobilisiertes Konjugat eines anti-Analyt-Antikörpers umfasst und eine detektierbare Reportergruppe, wobei die Reaktion zum Bilden einer modifizierten Probe das Binden des Konjugates zum Probenanalyt umfasst, um einen Analyt-Konjugat-Komplex zu bilden, und die Reagenzzusammensetzung in dem Reagenzstreifen (30) einen anti-Analyt-Antikörper umfasst, der in einer Detektionszone in dem Reagenzstreifen (30) immobilisiert ist, und wobei die Reaktion zum Bilden eines detektierbaren analytabhängigen Produkts das Binden des Komplex zu dem immobilisierten Antikörper umfasst, um den detektierbaren Reporter in dem Komplex in der Detektionszone (34) zu lokalisieren.

4. Das Verfahren nach Anspruch 3, wobei das nicht immobilisierte Konjugat ein Konjugat eines Anti-Analyt-Antikörpers ist, und ein detektierbarer Reporter aus der Gruppe ausgewählt ist, bestehend aus Metallpartikeln, Partikeln die mit gefärbten oder fluoreszierenden Resten markiert sind, Polymere, die mit gefärbten oder fluoreszierenden Resten markiert sind, Partikel und gefärbte oder fluoreszierende Molekühle.

5. Das Verfahren nach Anspruch 4 zur Verwendung beim Detektieren von c-reaktiven Proteinanalyten in einer Blutprobe, wobei der anti-Analyt-Antikörper in dem nicht immobilisierten Konjugat in dem Reagenzreservoir (24) und der immobilisierte anti-Analyt-Antikörper in dem Reagenzstreifen (30) Antikörper sind, die gegen ein Epitop in dem c-reaktiven Protein spezifisch sind.

6. Vorrichtung zur Verwendung beim Detektieren eines Analyts in einer flüssigen Körperflüssigkeitsprobe umfassend:

i.) eine Kassette (10) mit

a. einem Kassettenkörper (12) umfassend ein Reagenzreservoir, welches eine erste Reagenzzusammensetzung enthält, die wirksam ist, um mit einem oder mehreren Probekomponenten zu reagieren, um eine modifizierte Probe zu bilden;

b. einen Träger (14) der an dem Körper (12) montiert ist, und der eingerichtet ist, so das er zu einer Übertragungsposition hin und wieder weg bewegt werden kann, um eine Fluidkommunikation zwischen dem Reservoir (24) und einem Reagenzstreifen (30) zu realisieren, der an dem Träger (14) getragen ist; und

c. wobei der Reagenzstreifen (30) eine zweite Reagenzzusammensetzung enthält, die wirksam ist, um mit der modifizierten Probe zu reagieren, die in dem Reservoir (24) gebildet wurde, um ein detektierbares, analytabhängiges Produkt zu bilden;

ii) ein Kassettenhandhabungsinstrument (48), welches aufweist:

a. einen Kassettenhalter (50), in den die Kassette (10) entfernbar angeordnet werden kann während einer Probenuntersuchung;

b. ein Betätigungsmittel (53), das betrieben werden kann, um den Träger (14) in der Kassette (10) zu der Übertragungsposition und davon weg zu bewegen;

c. einen Detektor (56), der betrieben werden kann, um eine analytspezifische Reaktion in dem Reagenzstreifen zu entdecken; und

d. eine Steuereinheit (55), die funktional mit dem Betätigungsmittel (53) verbunden ist, um das Volumen und die Rate der Probenübertragung von dem Reagenzreservoir (24) zum Reagenzstreifen (30) während eines Untersuchungsverfahrens zu steuern, wobei die Steuereinheit (55) betrieben werden kann, um die Rate der Probenübertragung von dem Reservoir (24) zu dem Reagenzstreifen (30) durch wiederholtes Bewegen des Trägers (14) in der Kassette (10) zu der Übertragungsposition und wieder weg von der Übertragungsposition zu steuern, und (i) die Zyklusfrequenz steuert, mit der das Betätigungsmittel (53) den Träger (14) zu der Übertragungsposition bewegt und von der Übertragungsposition weg bewegt und (ii) die Kontaktzeit steuert, in der der Träger (14) in seiner Übertragungsposition während jedes Zyklus gehalten ist.

7. Die Vorrichtung nach Anspruch 6, wobei der Kassettenkörper (12) eine Probenöffnung (18) zum Aufnehmen der Probe hat, sodass diese von der Probenöffnung (18) in das Reservoir (24) migrieren kann.

8. Die Vorrichtung nach Anspruch 7, wobei die erste Reagenzzusammensetzung in dem Reagenzreservoir (24) ein nicht immobilisiertes Konjugat eines anti-Analyt-Antikörpers umfasst, und eine detektierbare Reportergruppe, wobei die Reaktion zum Bilden einer modifizierten Probe das Binden des Konjugats zu einem Probenanalyt umfasst, um einen Analyt-Konjugat-Komplex zu bilden, wobei die Reagenzzusammensetzung in dem Reagenzstreifen (30) einen anti-Analyt-Antikörper umfasst, der in einer Detektionszone (34) in dem Reagenzstreifen (30) immobilisiert ist, und wobei die Reaktion zum Bilden eines detektierbaren, analytabhänigen Produkts das Binden des Komplexes mit dem immobilisierten Antikörper umfasst, um den detektierbaren Reporter in dem Komplex an der Detektionszone (34) zu lokalisieren.

9. Die Vorrichtung nach Anspruch 8, wobei das nicht immobilisierte Konjugat ein Konjugat eines anti-Analyt-Antikörpers ist und ein detektierbarer Reporter aus der Gruppe bestehend aus: Metallpartikeln, Partikeln, die mit gefärbten oder fluoreszierenden Resten markiert sind, Polymere, die mit gefärbten oder fluoreszierenden Resten markiert sind, Partikel und gefärbte oder fluoreszierende Molekühle, besteht.

10. Die Vorrichtung nach Anspruch 9, zur Verwendung beim Detektieren eines c-reaktiven Proteinanalyts in einer Blutprobe, wobei der anti-Analyt-Antikörper in dem nicht immobilisierten Konjugat in dem Reagenzreservoir (24) und der immobilisierte anti-Analyt-Antiköper in dem Reagenzstreifen (30) Antikörper sind, die spezifisch gegen ein Epitop im c-reaktiven Protein sind.

11. Die Vorrichtung nach Anspruch 8, wobei der Träger (14) ein Fenster (36) umfasst, durch welches eine detektierbare Reaktion in der Detektionszone (34) in dem Reagenzstreifen (30) durch den Detektor (56) entdeckt werden kann.

12. Die Vorrichtung nach Anspruch 6, wobei die Steuereinheit (55) betrieben werden kann, um das Volumen und die Rate der Probenübertragung von dem Reagenzreservoir (24) zu dem Reagenzstreifen (30) zu steuern, in dem weiter die Zeitdauer der Probeninkubation gesteuert wird, bevor die Probe erstmals von dem Reservoir (24) zu dem Reagenzstreifen (30) übertragen wird.

Revendications

1. Un procédé pour mener un dosage pour un analyte de fluide corporel, comprenant :

a) introduire un fluide corporel contenant l'analyte dans un réservoir absorbant (24) contenant

une première composition réactive efficace pour réagir avec un ou plusieurs composants de l'échantillon pour former un échantillon modifié,
b) mettre en contact de façon répétée le réservoir (24), ce réservoir contenant un échantillon de fluide corporel absorbé, avec une bande réactive (30) contenant une seconde composition réactive efficace pour réagir avec l'échantillon modifié formé dans ledit réservoir (24) pour produire un produit détectable dépendant de l'analyte, et
c) contrôler la fréquence et la durée de ladite mise en contacte répétée, pour contrôler ainsi le volume et la vitesse de transfert du fluide échantillon depuis le réservoir (24) vers la bande réactive (30).

2. Le procédé de la revendication 1, dans lequel ladite bande réactive (30) est une bande réactive allongée (30) ayant une zone de transfert d'échantillon (32) à l'endroit de laquelle le réservoir (24) vient en contact avec la bande (30), et une zone de détection (34) située en aval de la zone de transfert (32).

3. Le procédé de la revendication 2, pour détecter un analyte multivalent dans un échantillon de fluide corporel liquide, où la première composition réactive dans le réservoir de réactif (24) comprend un conjugué non-immobilisé d'un anticorps anti-analyte et un groupe rapporteur détectable, la réaction pour former un échantillon modifié inclut une liaison du conjugué à l'analyte de l'échantillon, pour former un complexe analyte-conjugué, la composition réactive de la bande réactive (30) comprend un anticorps anti-analyte immobilisé en une région de détection de la bande réactive (30), et la réaction pour former un produit détectable dépendant de l'analyte comprend la liaison du complexe à l'anticorps immobilisé, pour localiser le rapporteur détectable dans le complexe à l'endroit de la zone de détection (34).

4. Le procédé de la revendication 3, dans lequel ledit conjugué non-immobilisé est un conjugué d'un anticorps anti-analyte et d'un rapporteur détectable sélectionné parmi le groupe constitué par les particules métalliques, les particules marquées par des fractions colorées ou fluorescentes, les polymères marqués par des fractions colorées ou fluorescentes, les particules, et les molécules colorées ou fluorescentes.

5. Le procédé de la revendication 4, pour une utilisation dans la détection d'un analyte de protéine C-réactive dans un échantillon sanguin, dans lequel l'anticorps anti-analyte dans le conjugué non-immobilisé du réservoir de réactif (24), et l'anticorps anti-analyte immobilisé de la bande réactive (30) sont des anticorps spécifiques à l'encontre d'un épitope commun dans la protéine C-réactive.

6. Dispositif utilisable pour détecter un analyte dans un échantillon liquide de fluide corporel, comprenant :

i) une cassette (10) avec

a) un corps de cassette (12) comprenant un réservoir de réactif contenant une première composition réactive efficace pour réagir avec un ou plusieurs composants d'échantillon pour former un échantillon modifié;
b) un support (14) monté sur ledit corps (12), actionnable pour se déplacer en direction et en éloignement d'une position de transfert pour réaliser une communication de fluide entre ledit réservoir (24) et une bande réactive (30) portée sur ledit support (14) ; et
c) ladite bande réactive (30) contenant une seconde composition réactive efficace pour réagir avec l'échantillon modifié formé dans ledit réservoir (24) pour former un produit détectable dépendant de l'analyte ;

ii) un instrument de manipulation de cassette (48) avec :

a) un porte-cassette (50) dans lequel la cassette (10) peut être placée de manière amovible pendant un dosage d'échantillon ;
b) un actionneur (53) actionnable pour déplacer le support (14) dans la cassette (10) en direction et en éloignement de la position de transfert ;
c) un détecteur (56) actionnable pour détecter une réaction spécifique à un analyte dans la bande réactive ; et
d) une unité de contrôle (55) reliée de manière opérante à l'actionneur (53), pour contrôler le volume et la vitesse de transfert d'échantillon depuis ledit réservoir de réactif (24) vers la bande réactive (30) pendant une procédure de dosage, où l'unité de contrôle (50) est actionnable pour contrôler la vitesse de transfert de l'échantillon depuis ledit réservoir (24) vers la bande réactive (30) en déplaçant de façon répétée le support (14) de la cassette (10) en direction et en éloignement de la position de transfert et en contrôlant (i) la fréquence de cycle à laquelle l'actionneur (53) déplace le support (14) en direction et en éloignement de sa position de transfert et (ii) le temps de contact pendant lequel le support (14) est maintenu dans sa position de transfert, pendant chaque cycle.

7. Le dispositif de la revendication 6, dans lequel ledit corps de cassette (12) possède un puits d'échantillon (18) pour recevoir ledit échantillon de manière qu'il puisse migrer depuis ledit puits d'échantillon (18) jusque dans ledit réservoir (24).

8. Le dispositif de la revendication 7, dans lequel la première composition réactive dans le réservoir de réactif (24) comprend un conjugué non-immobilisé d'un anticorps anti-analyte et un groupe rapporteur détectable, la réaction pour former un échantillon modifié inclut une liaison du conjugué à l'analyte de l'échantillon, pour former un complexe analyte-conjugué, la composition réactive de la bande réactive (30) comprend un anticorps anti-analyte immobilisé en une région de détection de la bande réactive (30), et la réaction pour former un produit détectable dépendant de l'analyte comprend la liaison du complexe à l'anticorps immobilisé, pour localiser le rapporteur détectable dans le complexe à l'endroit de la zone de détection (34).

9. Le dispositif de la revendication 8, dans lequel ledit conjugué non-immobilisé est un conjugué d'un anticorps anti-analyte et d'un rapporteur détectable sélectionné parmi le groupe constitué par les particules métalliques, les particules marquées par des fractions colorées ou fluorescentes, les polymères marqués par des fractions colorées ou fluorescentes, les particules, et les molécules colorées ou fluorescentes.

10. Le dispositif de la revendication 9, pour une utilisation dans la détection d'un analyte de protéine C-réactive dans un échantillon sanguin, dans lequel l'anticorps anti-analyte dans le conjugué non-immobilisé du réservoir de réactif (24), et l'anticorps anti-analyte immobilisé de la bande réactive (30) sont des anticorps spécifiques à l'encontre d'un épitope commun dans la protéine C-réactive.

11. Le dispositif de la revendication 8, dans lequel ledit support (14) comprend une fenêtre (36) au travers de laquelle peut être détectée par ledit détecteur (56) une réaction détectable dans la zone de détection (34) de la bande réactive (30).

12. Le dispositif de la revendication 6, dans lequel ladite unité de contrôle (55) est actionnable pour contrôler le volume et la vitesse de transfert d'échantillon depuis le réservoir de réactif (24) vers la bande réactive (30) en contrôlant en outre la période d'incubation d'échantillon avant que l'échantillon soit transféré pour la première fois depuis le réservoir (24) vers la bande réactive (30).

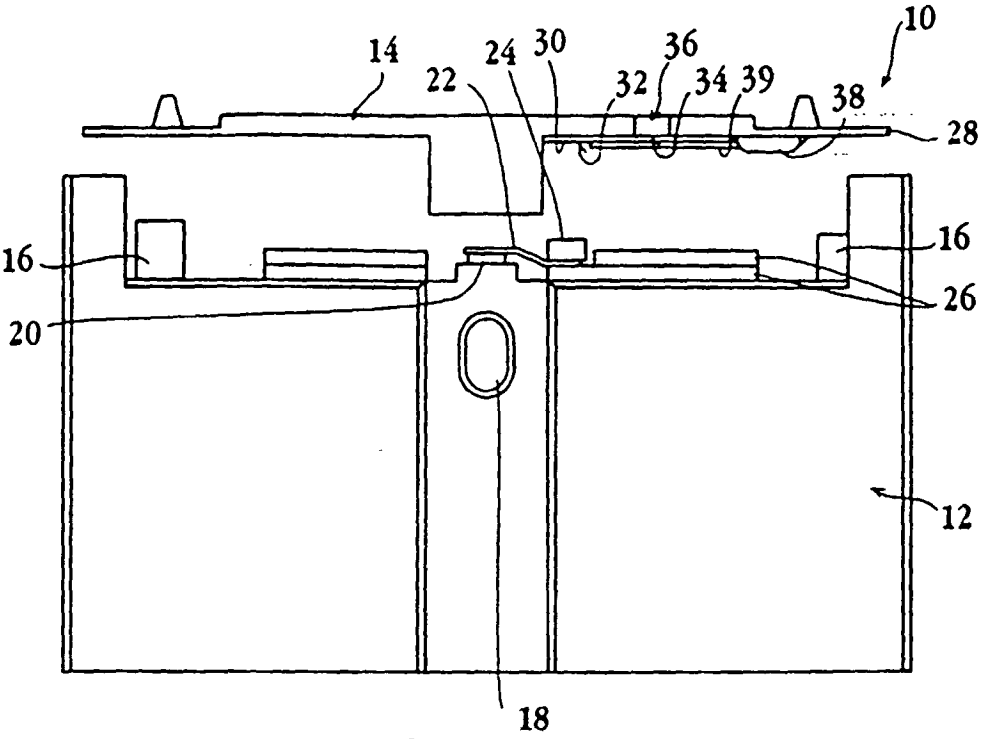
10
14
24
30
36
22
32
34
39
38
28
16
16
26
20
12
18

Fig. 1A

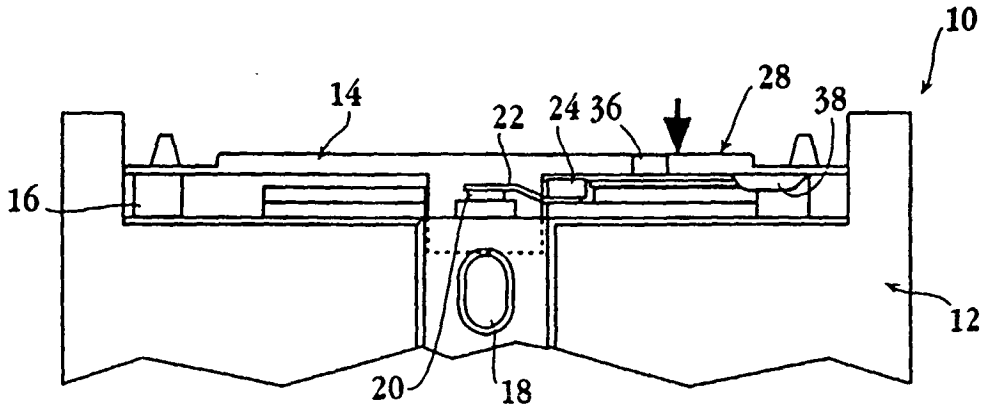
10
14
22
24
36
28
38
16
12
20
18

Fig. 1B

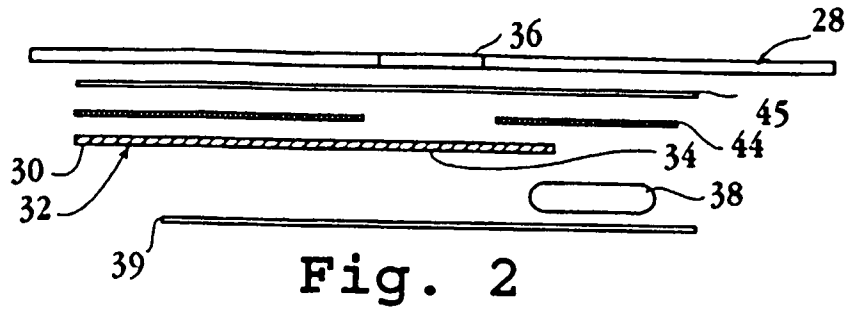

Fig. 2

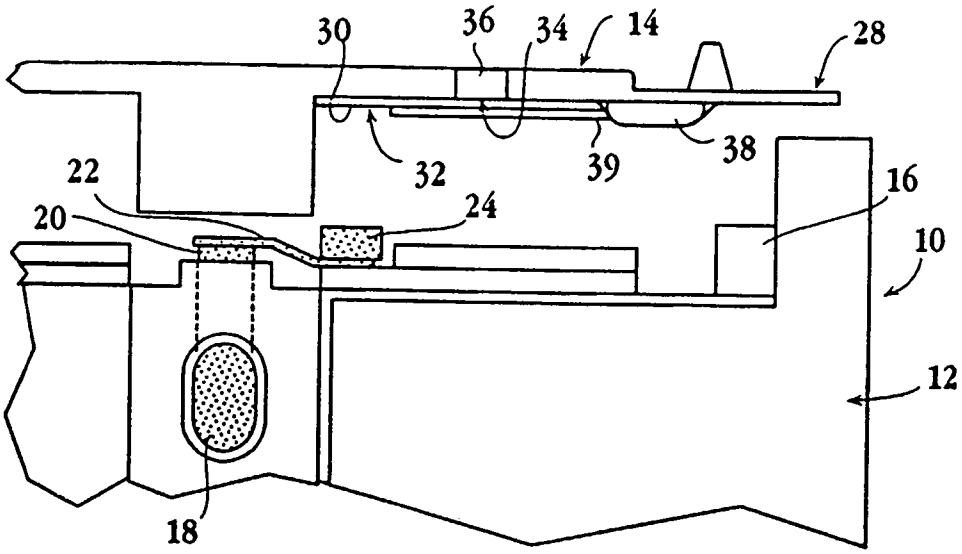

Fig. 3A

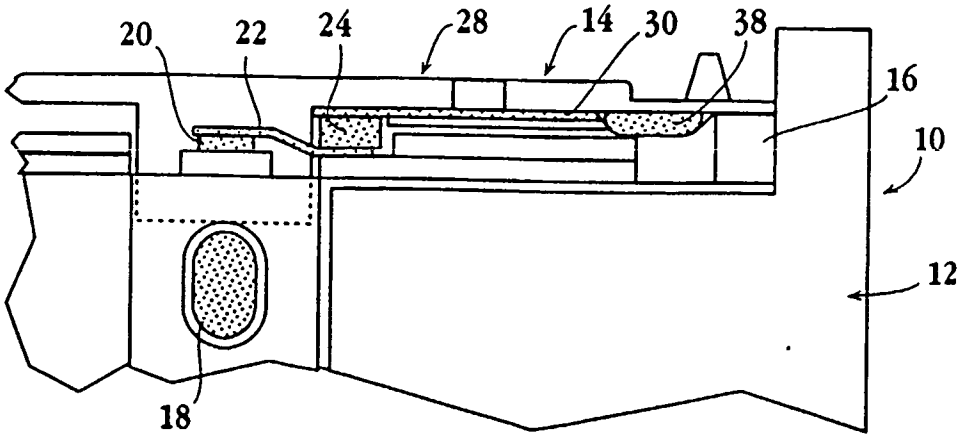

Fig. 3B

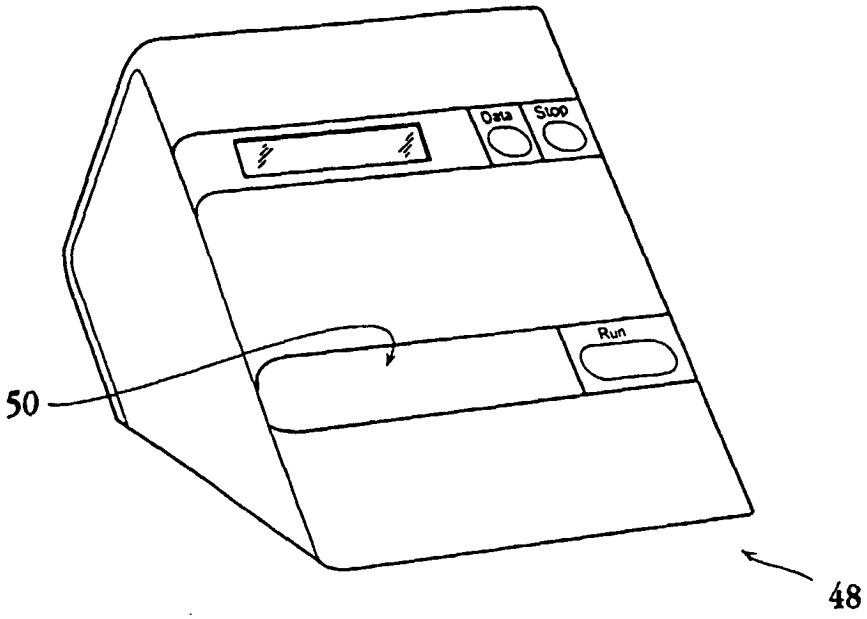
Data
Stop
Run
50
48

Fig. 4

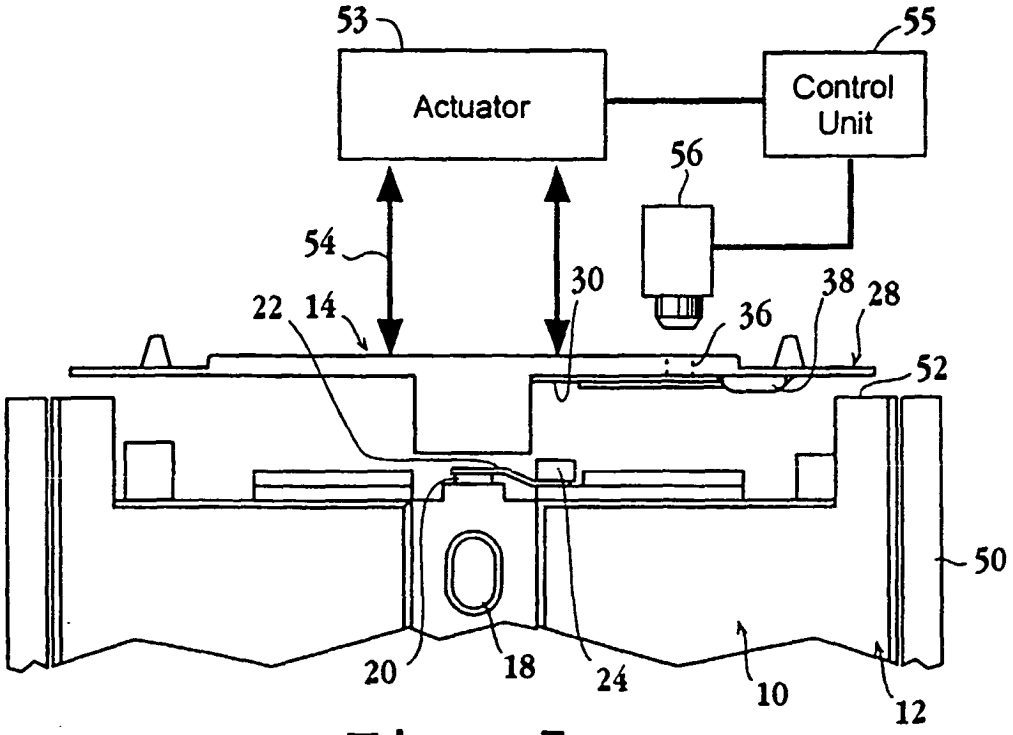
53
55
Actuator
Control Unit
56
54
30
38
28
22
14
36
52
50
20
18
24
10
12

Fig. 5

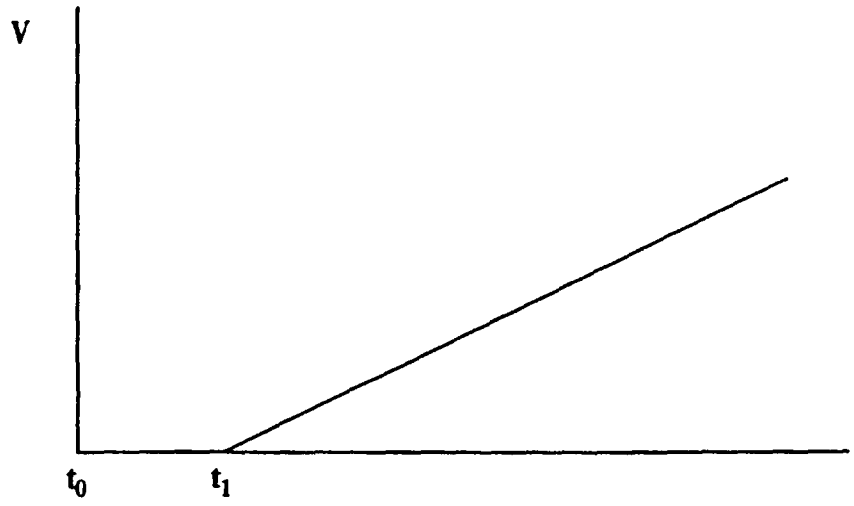

Fig. 6A

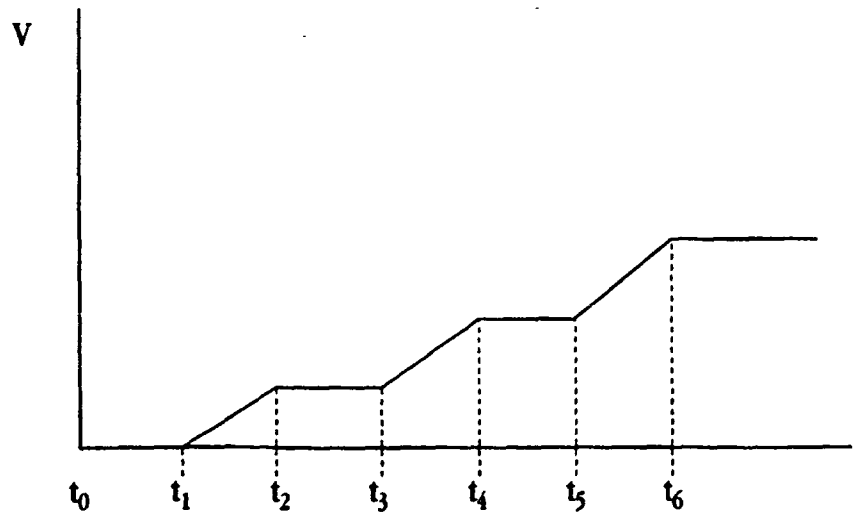

Fig. 6B

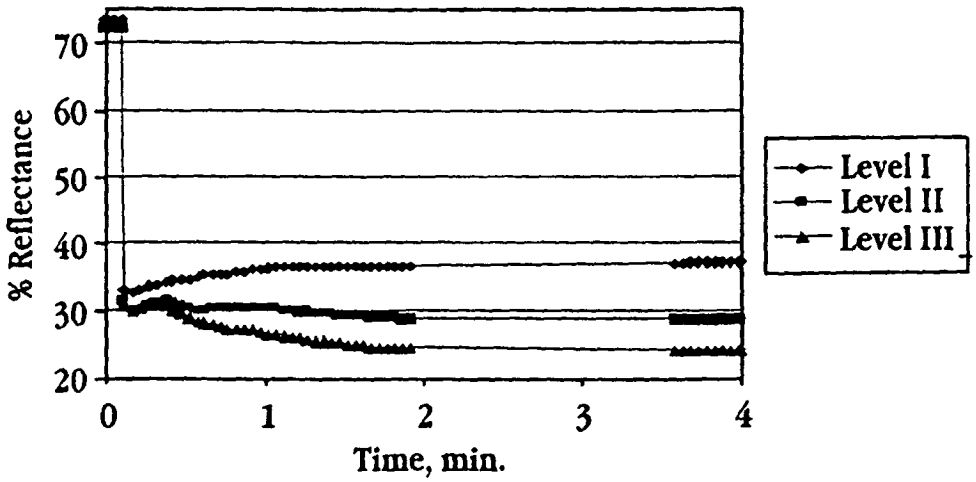
70
60
50
40
30
20
% Reflectance
0
1
2
3
4
Time, min.
Level I
Level II
Level III

Fig. 7

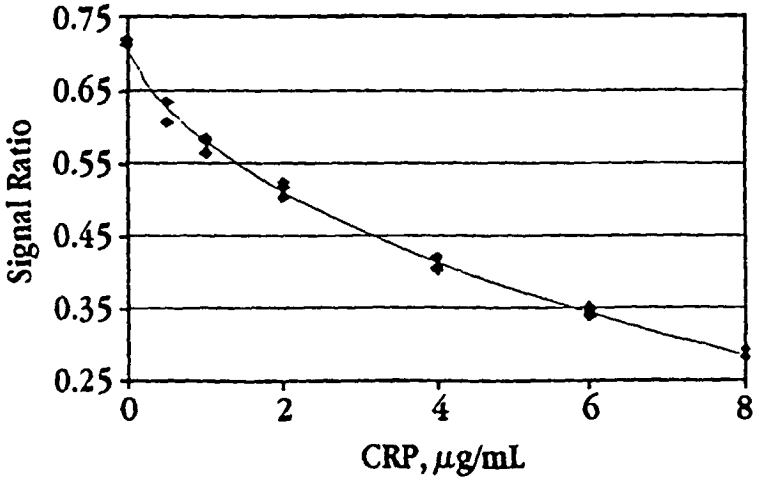
0.75
0.65
0.55
0.45
0.35
0.25
Signal Ratio
0
2
4
6
8
CRP, μg/mL

Fig. 8

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5110724 A, Hewett, G.E. **[0002]**
- US 5171688 A, Hewett, G.E. **[0002]**
- US 4313734 A, Leuvering **[0002] [0036]**
- EP 02004339 A **[0005]**
- US 5744096 A **[0006]**

Non-patent literature cited in the description

- **DE MAAT, M.P.M. et al.** *Fibrinolysis 8,* 1994 **[0002]**
- **GRAU, A.J. et al.** Clinical and Biochemical Analysis in Infection-Associated Stroke. *Stroke,* September 1995, vol. 26 (9), 1520-6 **[0002]**
- **HARLOW, E. et al.** Antibodies: A Laboratory Manual. Cold Spring Harbor Lab, 1988 **[0002]**
- **KULLER, L.H. et al.** *Am J Epidemiol.,* 15 September 1996, vol. 144, 537-547 **[0002]**
- **LIUZZO G, M.D. et al.** *N Engl J Med,* 18 August 1994, vol. 331 (7), 417-424 **[0002]**
- **MENDALL, M.A. et al.** *British Med. J.,* 27 April 1996, vol. 312, 1061-65 **[0002]**
- **THOMPSON, S.G. et al.** *N Engl J Med,* 1995, vol. 332, 635-41 **[0002]**
- **TRACY, R.P. et al.** *Circulation,* 1996, vol. 93 (3), 8 **[0002]**
- **JOHN CHANDLER ; TRACEY GURMIN ; NICOLA ROBINSON.** *IVD Technology,* 2000, vol. 6 (2), 37-49 **[0041]**

专利名称(译)	用于进行免疫测定的装置的自动化cassete模块及其用途		
公开(公告)号	EP1369473B1	公开(公告)日	2009-01-21
申请号	EP2002022910	申请日	2002-10-10
申请(专利权)人(译)	CHOLESTECH CORPORATION		
当前申请(专利权)人(译)	CHOLESTECH CORPORATION		
[标]发明人	NUGENT ANTHONY COWLEY LEANNE BELLET NEAL SHINDELMAN JEFFREY LEOS MICHAEL WORTHY THOMAS HALEY KIMBERLY		
发明人	NUGENT, ANTHONY COWLEY, LEANNE BELLET, NEAL SHINDELMAN, JEFFREY LEOS, MICHAEL WORTHY, THOMAS HALEY, KIMBERLY		
IPC分类号	C12M1/34 G01N33/53 G01N33/543 G01N35/00 C12M1/40 B01L3/00 B01L9/00 G01N33/545 G01N33/553 G01N35/02		
CPC分类号	B01L9/52 B01L3/5023 B01L2300/0825 B01L2300/0887 B01L2400/0406 B01L2400/0633 G01N33/5304 G01N33/54306 G01N33/54366 G01N33/54386 Y10S435/805 Y10S435/81 Y10S435/97 Y10S436/81		
优先权	60/387287 2002-06-07 US		
其他公开文献	EP1369473A2 EP1369473A3		
外部链接	Espacenet		

摘要(译)

公开了一种用于检测液体体液样品中的分析物的免疫测定盒10，装置和方法。盒10具有主体12和安装在主体12上的支撑件，用于朝向和远离样品转移位置移动。提供给盒体12中的样品孔18的样品被含有第一试剂组合物的试剂贮存器24吸收，所述第一试剂组合物有效地与一种或多种样品组分反应以形成改性样品。支撑件12提供试剂条30，其具有转移区32，当支撑件14移动到其转移位置时，转移区32与贮存器24接触，位于转移区32下游的检测区34和第二试剂有效地与改性样品反应形成可检测的分析物依赖性产物的组合物。通过控制支撑件14朝向和远离其转移位置的移动，可以控制样品从贮存器24流到条带30的体积和速率，以优化和/或标准化测定中的样品转移条件。

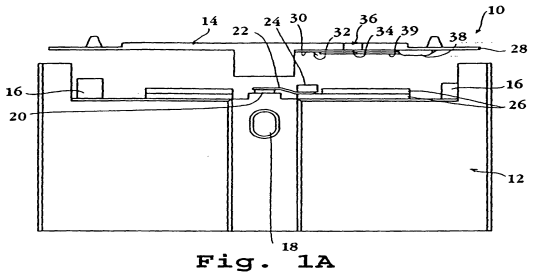

Fig. 1A

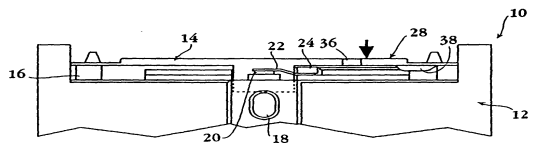

Fig. 1B