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(54) Abstract Title: **Assay device and method**

(57) Assay methods and devices for detecting the presence of analytes in an aqueous sample comprising a lateral flow device, a liquid sample, a sensor which may be an electrode to make an electrochemical measurement and particles susceptible to magnetic manipulation with an affinity to a target chemical moiety. The particles and captured chemical moiety capable of manipulation by a magnetic field though the sample to the sensor for detection. The assay may also involve a second liquid to form a liquid/liquid interface. Also shown is a microanalysis system and a disposable single use test device.

FIGURE 1

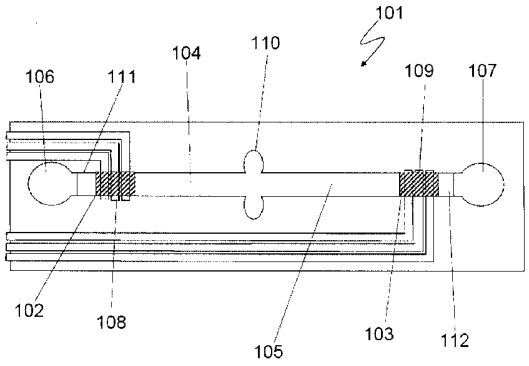

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FIGURE 1

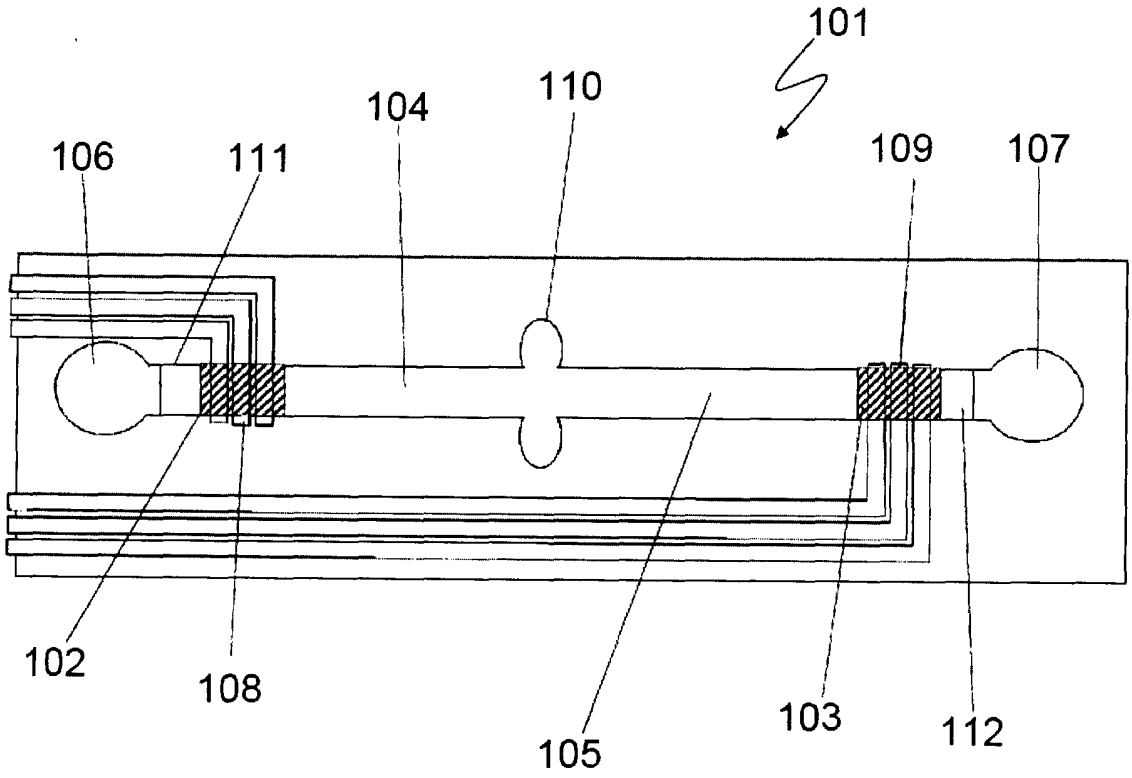

FIGURE 2

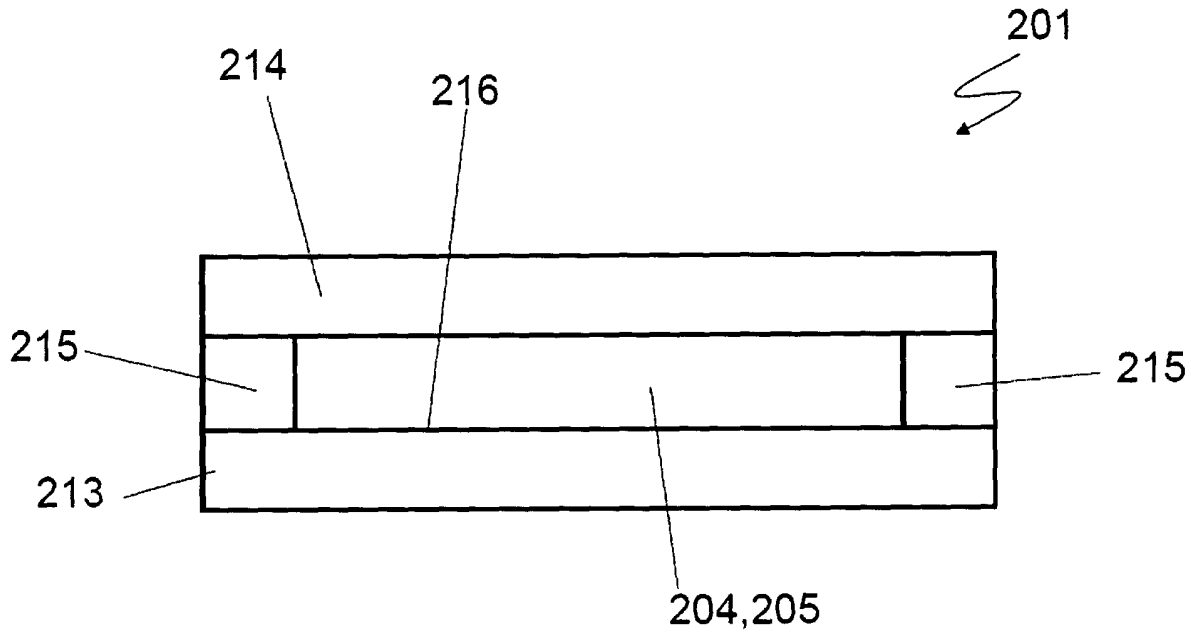

FIGURE 3

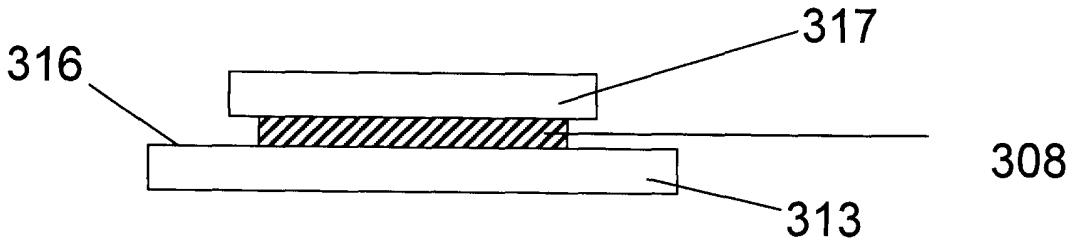

FIG.3A

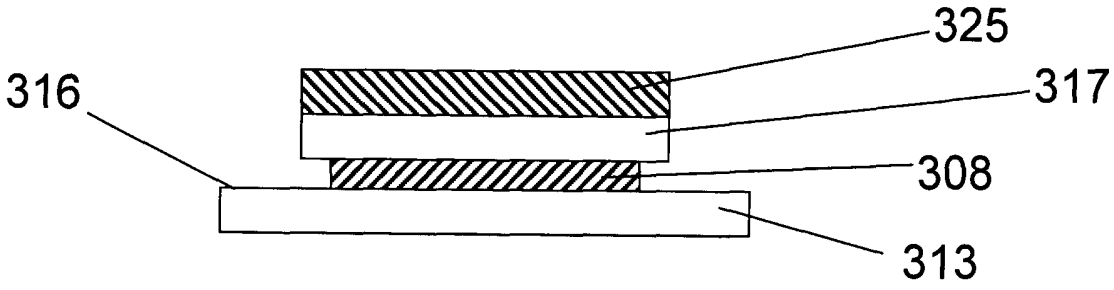

FIG.3B

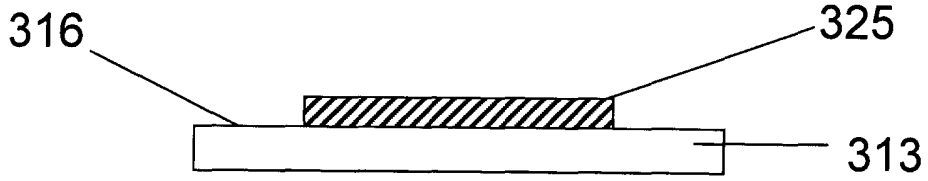

FIG.3C

FIGURE 4

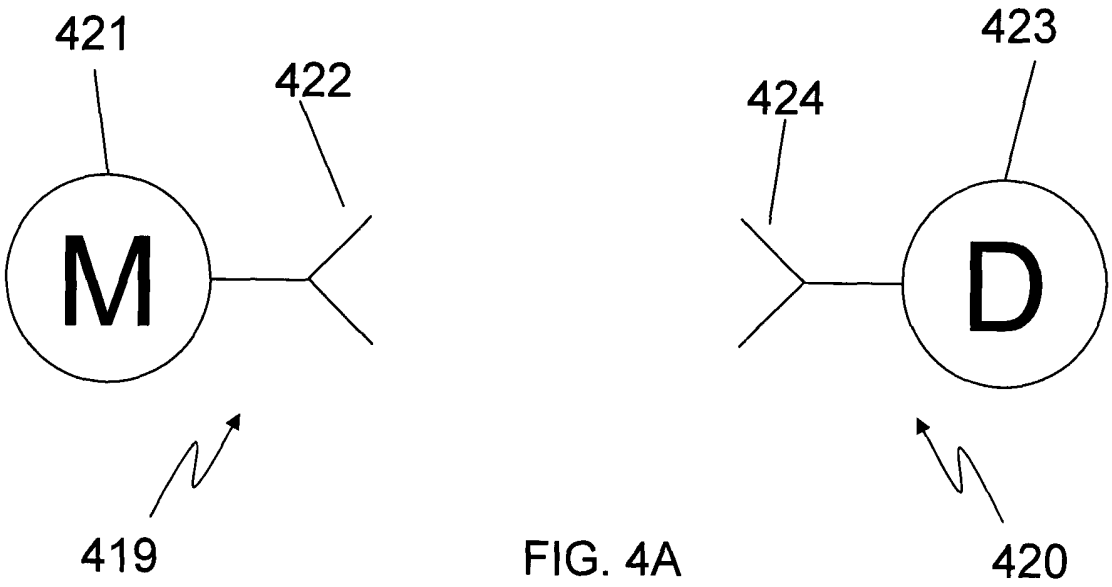

FIG. 4A

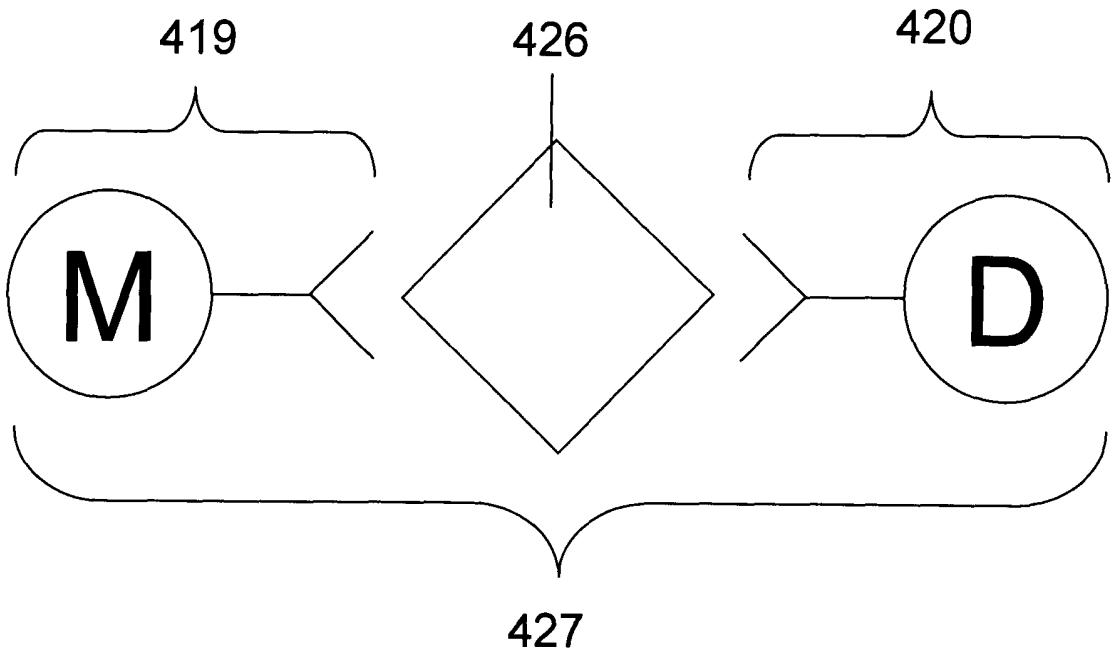

FIG. 4B

FIGURE 5

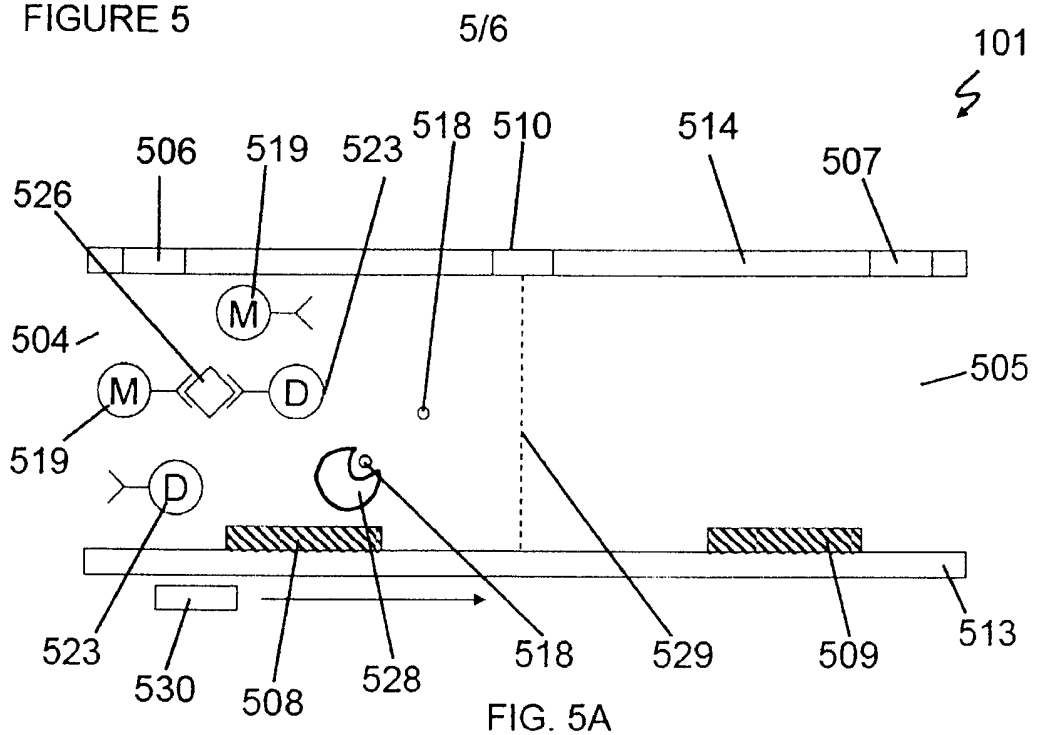

FIG. 5A

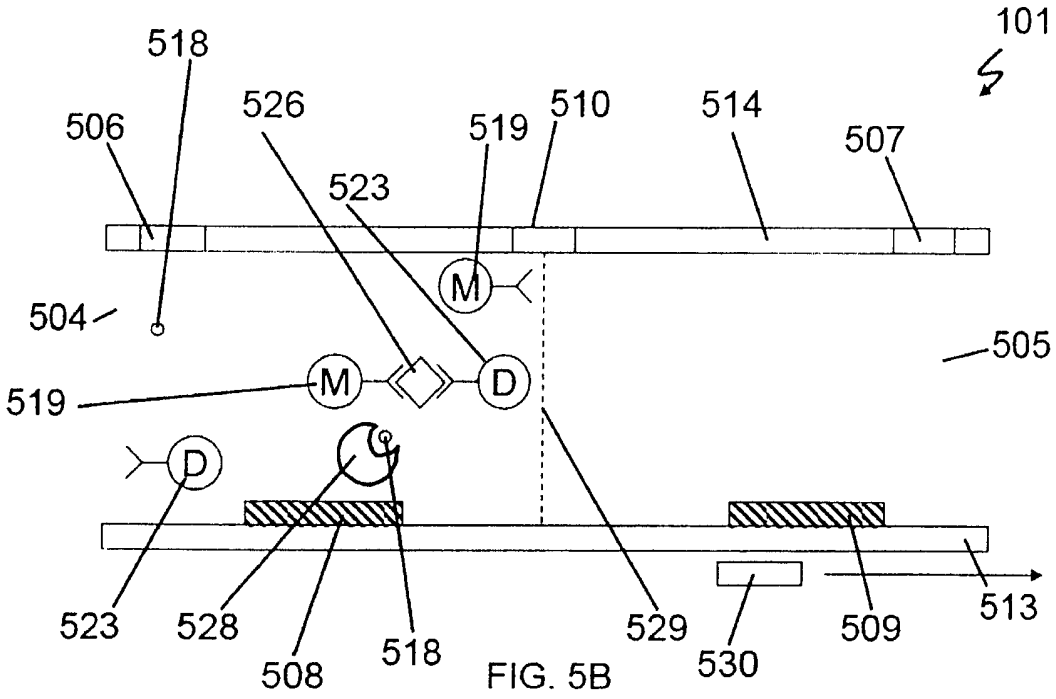

FIG. 5B

FIGURE 6

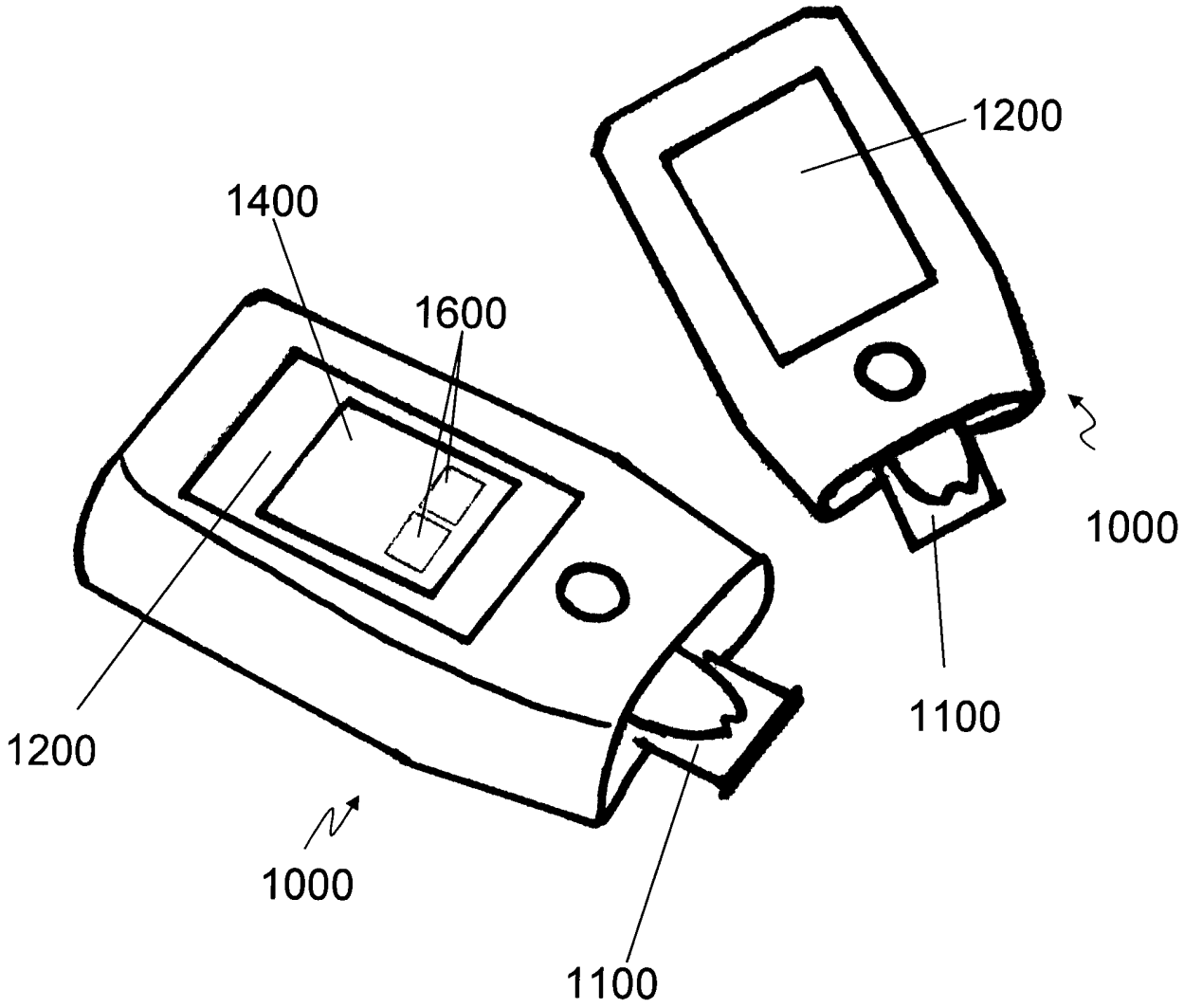

FIG. 6

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Assay methods and devices

Field of Invention

The present invention relates to methods and devices for performing assays. In particular the present invention relates to methods and devices for detecting the presence of analytes.

Background of Invention

Assays are used to qualitatively and/or quantitatively detect analytes. As such, assays have found numerous applications in many industries, and can be performed using various different scientific techniques.

An example of one application that employs the use of assays to detect analytes is the analysis of physiological fluid samples, such as blood samples. In particular, it has become increasingly common to analyse blood samples for analytes that may be indicative of disease or illness. Such analyses can be performed using an assay that directly or indirectly detects an analyte of interest.

Physiological fluids, and blood in particular, are complex mixtures containing many different species in the form of solids, liquids, and solvated solids and gases. When performing an assay on a complex mixture, such as blood, these additional species can interfere with the detection taking place, which may lead to inaccurate results. For example, contamination of assay reagents with non-specific reactions, and physical occlusions of target molecules with cellular debris represent typical problems to be addressed in analysis of biological material, and in particular blood.

It is normal to obtain a physiological sample from a subject prior to performing an assay. In most cases, obtaining large amounts of physiological sample is best avoided, especially when the physiological sample is blood. Therefore, great care must be taken to utilise efficiently the small sample volumes that are typically obtained from a subject. In particular, home testing kits for analysing species present in the blood often rely on the "finger stick" or "finger prick" procedure; a method in which a finger is pricked with a lancet to obtain a small amount of capillary blood. The quantity of blood obtained from this procedure is typically in the order of less than 1 mL, and more typically in the order of 10 μL.

Near-patient testing devices, such as home testing kits, must be capable of accepting such small volumes of fluid sample. Moreover, home-testing kits should be capable of accepting small fluid samples in a simple step, and should be able to present small fluid samples for immediate testing in a reliable and reproducible fashion.

An efficient way to utilise obtained blood samples in a home testing kit is to carry out a series of tests on the same sample. However, this can prove difficult to achieve when only a small volume of blood is available. In particular, it is often necessary to divide a sample into separate portions to minimise any interference effects when different tests are performed. Therefore, it is clear that it is difficult to carry out more than one assay on the same blood sample when only a limited volume of said sample is available.

In order to facilitate the execution of more than one assay on the same blood sample it is desirable to separate or isolate an analyte of interest, within a complex mixture, thereby enabling its visualisation by a detection procedure. In particular, it is desirable to use a specific reagent for visualising a marker related to an analyte of interest and to reliably quantify its presence to inform on a disease state in a subject. As alluded to, it is very difficult to perform this type of procedure for more than one analyte in a small sample volume. Therefore, it is also very difficult to perform more than one accurate assay of different analytes in the same (small volume) blood sample.

The invention to be more particularly described hereinafter obviates or mitigates at least some of the aforementioned problems and offers methods and devices for accurately performing at least one assay, and for detecting at least one analyte.

Summary of the Invention

The subject invention concerns the measurement of analytes in complex mixtures. The amount of analyte present can be detected indirectly and accurately, and in turn can be used to signal the occurrence or non-occurrence of a medical event in a subject. The invention enables efficient use of small sample volumes for analysis of differing analytes of interest upon a single small device by a variety of techniques. Particularly the invention enables capture of analytes in physiological fluids, manipulation of the captured analytes for treatment with assay reagents in simultaneous or successive assay procedures in a manner intended to obviate or mitigate problems normally associated with other components of the physiological fluid.

The invention as defined in the claims hereinafter provides assay methods and devices useful in a variety of applications where a complex liquid has to be analysed. According to one aspect of the invention there is provided an assay for selectively determining a plurality of characteristics of an aqueous liquid sample containing at least one chemical moiety of interest amongst other sample components. A lateral flow device suitable for use in performing the assay comprises at least one lateral flow channel, a sample collection site, at least one reagent deposit zone proximate to the lateral flow channel and sensor means functionally juxtaposed with respect to the lateral flow channel. One reagent used in the assay comprises particles adapted to exhibit a selective affinity towards a target chemical moiety to be

determined in the assay, said particles further being susceptible to manipulation by means of a magnetic field.

Typically, a liquid sample is applied to the sample collection site in a sufficient amount to permit flow thereof into the lateral flow channel and the reagent deposit zone, for a period sufficient to permit adequate interaction of the particles with chemical moiety present in the sample to capture same.

A magnetic field is applied in a controlled manner to localise the particles and captured chemical moiety e.g. to allow transferring of the particles and captured chemical moiety through a surface of the liquid sample so that the particles and captured chemical moiety are separated from other sample components remaining in the liquid sample.

One way of achieving the separation is to transfer the particles and captured chemical moiety into another medium e.g. another liquid. This would be achievable if a further liquid is introduced to the lateral flow device after the sample is applied to the sample collection site and permitted to flow into the lateral flow channel, the further liquid being introduced to the lateral flow channel at a point remote from the sample collection site to permit flow towards the latter such that an interface is formed between the liquid sample and the further liquid at a predictable position in the lateral flow channel.

During the above procedure, it is possible to select a suitable sensor means to detect at least one of the

following characteristics of a component of the sample, namely an optical characteristic, an electrochemical characteristic, a radiation characteristic and an immunological characteristic. An electrochemical characteristic may be measured initially or later, whereas another characteristic may be better measured after the particles are separated from the sample.

Broadly, an assay method of this invention comprises the steps of introducing to a liquid sample, a quantity of particles exhibiting a preferential affinity towards a component of the liquid sample, said particles further being susceptible to manipulation by means of a magnetic field; causing the liquid sample to flow in a lateral flow channel to a predetermined point at which a liquid meniscus is formed; manipulating the particles by means of an applied magnetic field to localise the particles at the liquid meniscus; and optionally introducing a further liquid by lateral flow up to the liquid meniscus of the sample liquid to form a liquid/liquid interface; and manipulating the localised particles by means of an applied magnetic field to transfer the localised particles through the liquid/liquid interface.

Accordingly the invention permits an assay to be designed for determining the presence in a physiological fluid of biomarkers indicative of a potential cardiovascular dysfunction in a patient. Such an assay comprises the steps of providing a lateral flow device in which a shallow well is available for receipt of a liquid and in which at least one dry reagent is deposited, said reagent being one capable of interacting with a first biomarker in a predictable way to serve as an aid to detection of the biomarker; introducing to the well a sample of the

physiological fluid, and particles susceptible to manipulation under magnetic influence, wherein said particles have a selective affinity towards a biomarker to the extent that any biomarker present in the sample is liable to become associated with the particles, subsequently applying a magnetic field to the device to localise the particles in a selected position, and using sensor means sensitive to the reagent-biomarker combination to detect presence of biomarker; and further introducing a liquid to the well to flow fill up to the sample and form a liquid-sample interface; applying a magnetic field to the device to manipulate the particles and transfer the particles from the sample across the liquid-sample interface into the liquid, and conducting a further test for another biomarker in that liquid.

In such an assay, the first biomarker may be ischemia modified albumen (IMA), and the first assay step may be an electrochemical test using an electrode to indirectly determine IMA.

Furthermore, in such an assay a further biomarker may be NTprohormone-brain natriuretic peptide (NTproBNP), and the further test would comprise introducing a reagent to permit formation of a reagent-modified NTproBNP species the presence of which presents a distinctive detectable characteristic such as an optical characteristic, an electromagnetic characteristic, an electrochemical characteristic, a radiation characteristic and an immunological characteristic.

According to a further aspect of the invention, there is provided a method for conducting a plurality of determinations of characteristics selected from the group

consisting of biological, biochemical, chemical and physical characteristics, upon a sample in a liquid form, comprising providing a portable lateral flow device in which at least one shallow covered channel is available for receipt of a liquid, the channel being configured to provide for bidirectional lateral flow of liquid therethrough and having a plurality of reagent treatment zones spaced at intervals in the channel, each such zone having a dry reagent deposited thereon for the purpose of promoting or visualising at least one of the characteristics to be determined, the device further comprising means for controlling flow of liquid to said zones by selectively inhibiting or extending lateral flow of liquid therein, and sensor means configured upon the device and juxtaposed with respect to said channel such that, in use of the device with a liquid sample, flowing of said liquid to said zones permits a characteristic of the liquid sample to be sensed selectively at more than one of said reagent treatment zones.

According to a further aspect of the invention there is provided a method comprising forming a liquid-liquid interface between first and second different liquids, the first liquid comprising first and second analytes, determining the first analyte within the first liquid, moving the second analyte across the liquid-liquid interface into the second liquid, and determining the second analyte within the second liquid.

Determining the first analyte can comprise indirectly determining the first analyte. Also, the first liquid can further comprise a first reagent capable of forming a complex with the first analyte and the first analyte can

be indirectly determined by determining the first reagent within the first liquid. Furthermore, determining the first reagent can comprise electrochemically determining the first reagent.

The first reagent may be, although is not limited to, a metal or ion thereof. In some embodiments the first reagent may be cobalt and the first analyte may be albumin.

Prior to forming the liquid-liquid interface, the method can further comprise introducing sample material to a microfluidic device which comprises a microfluidic network, wherein the sample material comprises the first and second analytes. The first liquid can comprise at least some of the sample material, and forming the liquid-liquid interface can comprise forming said interface within the microfluidic network.

The sample material may comprise blood or a liquid derived from blood. When the sample material comprises blood, introducing the sample material to the microfluidic device can comprise introducing blood to the microfluidic device. Furthermore, when the sample material comprises blood, the method can further comprise a plasma separation step.

After the introducing the sample material, the method can further comprise combining the sample material with the first reagent and a second reagent, the second reagent having an affinity for the second analyte. The second reagent can be a particulate reagent comprising a first

portion having an affinity for the second analyte, and a particle.

Prior to combining, the first and second reagents can be present in a dried state within the microfluidic network, and the method can comprise wetting the dried first and second reagents with the first liquid.

Moving the second analyte across the liquid-liquid interface can comprise applying a force to the second reagent, the second analyte having associated with the second reagent. The particle of the second reagent may be magnetic and the moving the second analyte can comprise subjecting the second reagent to a magnetic field sufficient to move the second reagent across the liquid-liquid interface.

Determining the second analyte may comprise electrochemically determining the second analyte.

Combining the sample material with the first reagent and a second reagent can further comprise combining the sample material with a third reagent. The second analyte, the second reagent, and the third reagent are capable of forming a complex, and the third reagent participates in the electrochemical determination of the second analyte.

The third reagent may be an enzyme. Electrochemically determining the second analyte can comprise contacting the enzyme with a substrate for the enzyme. The enzyme may be, although is not limited to, glucose oxidase and

the substrate may be, although is not limited to, glucose.

Determining the first analyte can be performed before the moving the second analyte across the liquid-liquid interface. Also, forming the liquid-liquid interface can comprise introducing the first liquid at a first location of a channel within a substrate and introducing the second liquid at a second location of the channel, the second channel being spaced apart from the first location, and the liquid-liquid interface being formed between the first and second locations.

The maximum cross-sectional area of the channel between the first and second locations may be about 5 mm^2 or less. In some embodiments the maximum cross-sectional area may be about 1 mm^2 or less.

Forming a liquid interface can comprise moving at least one of the first and second liquids along the channel by capillary action.

According to a further aspect of the invention there is provided a method comprising contacting a first reagent and a second reagent with a liquid sample material comprising first and second analytes, the second reagent comprising a magnetic particle, and mixing the liquid sample material, the first reagent, and the second reagent by subjecting the second reagent to a magnetic field.

According to a still further aspect of the invention there is provided a method comprising forming a mixture

comprising sample material, a metal ion, and an enzymatic reagent, the sample material comprising a protein and a second biological analyte, the metal ion being capable of forming a complex with the protein, the enzymatic reagent being capable of forming a complex with the biological analyte; detecting an amount of the metal ion not complexed with the protein; determining an amount of the protein based on the amount of metal ion not complexed with the protein; separating enzymatic reagent complexed with the biological analyte and enzymatic reagent not complexed with the biological analyte; contacting enzymatic reagent complexed with the biological analyte with a second reagent capable of participating in an enzymatic reaction with the enzymatic reagent; detecting an amount of a product of the enzymatic reaction; and determining an amount of the biological reagent based on the amount of product.

The protein may be, although is not limited to, albumin and the metal ion may be, although is not limited to, cobalt ion. The sample material may comprise blood or a liquid derived from blood, and the biological analyte may be, although is not limited to, a natriuretic peptide. The enzymatic reagent may be, although is not limited to, an enzyme and the second reagent may be a substrate for the enzyme.

Detecting an amount of the product of the enzymatic reaction can comprise indirectly detecting the product.

In one embodiment, the enzymatic reagent may be a glucose oxidase, the second reagent may be a substrate for the

glucose oxidase, and the product may be an oxidized form of the substrate.

The mixture can further comprise a magnetic particulate reagent capable of forming a complex with the enzymatic reagent and the biological analyte, and the separating can comprise subjecting the mixture to a magnetic field.

Subjecting the mixture to a magnetic field can move the complex with the enzymatic reagent, the biological analyte, and the magnetic particulate reagent from a first location to a second location spaced apart from the first location.

Separating can comprise moving enzymatic reagent complexed with the biological analyte across a liquid-liquid interface between first and second liquids, the first liquid comprising the mixture, and the second liquid being different from the first liquid.

Brief Description of the Drawings

The present invention will now be described by way of illustrative example only, with reference to the accompanying drawings in which:

Figure 1 is a perspective view of a schematic representation of a test strip for use with a hand-held electrochemical analysis apparatus;

Figure 2 is a schematic end view of an assembled test strip;

Figures 3A-3C are schematic views of a cross-section parallel to the shortest side of an assembled test strip;

Figures 4A-4B are schematic depictions of reagents and analytes;

Figures 5A-5B are schematic views of a cross-section parallel to the longest side of an assembled test strip; and

Figure 6 is an illustration of a hand-held assay device reader.

Detailed Description of the Invention

The modes for performance of the invention according to the currently envisaged embodiments of the present invention are described below.

According to a general embodiment of the invention there is provided an assay device for performing more than one assay. The assay device comprises a test strip that has at least two detection zones, and at least one linear channel therebetween. The channel has at least one application zone at which a sample (such as blood), or a buffer, can be added to the device. The detection zones are equipped with electrodes (or other apparatus) suitable for detecting a component of the sample. At a point substantially equidistant from the two detection areas there is provided a fusable vent. The vent acts to prevent or promote flow of the sample in the channel.

In the channel there is provided dried reagents that are resuspended on the addition of a fluid such as blood or

buffer. At least one of the dried reagents contains magnetic particles attached to an antibody, which will bind with an antigen in the blood sample.

The assay device is further provided with a magnet, which acts on the magnetic particles in the channel. The magnet is used to move the magnetic particles, and anything bound to them, from one area of the test strip to another. The test strip is suitable for insertion into a reader, which presents to the user the results of the two assays.

The assay device is suitable for performing a first assay which detects a first analyte present in a sample, and a second assay which detects a second analyte present in the sample. The first and second analytes can be different species and the first and second assays can be carried out using the same or different techniques (e.g., electrochemistry and photochemistry).

In a general embodiment of the method, a sample of blood is added to a first application zone on the test strip of the assay device. A first reagent dried onto a first channel in the test strip is resuspended on addition of the blood sample. The first reagent interacts with a first analyte in the sample and the first analyte is indirectly detected by way of, for example, electrochemistry. A first enzyme linked to an antibody and a magnetic particle linked to an antibody, both which are dried onto the first channel in the test strip, are also resuspended on addition of the blood sample. The antibodies recognise antigens on a second analyte and act to form a ternary complex of the second analyte with

antibody bound magnetic particle and antibody bound enzyme.

The magnetic particles, and all that is bound to them, are moved along the first channel, using the magnet, to the vent where flow of the sample constituents ceases. The magnet is moved past the vent, but the magnetic particles and all that is bound to them remains at the meniscus formed at the vent.

A second fluid, which may be a buffer, is introduced at a second application zone connected to a second channel. The second fluid acts to resuspend further reagents, such as a redox mediator and a substrate for the first enzyme, that are dried onto the second channel. The second fluid flows along the second channel to the vent at which point the first and second fluids form a fluid-fluid interface.

The formation of the fluid-fluid interface facilitates the movement of the magnetic particles selectively from the first fluid (blood) to the second fluid (buffer), leaving interferents and analytes that are not of interest in the first fluid. That is, only the magnetic particles and all that is bound to them, such as the second analyte of interest (in the form of a ternary complex of the second analyte with antibody bound magnetic particle and antibody bound enzyme) are transferred to the second fluid in the second capillary channel. The magnetic particles are moved to a second detection zone where the second analyte is indirectly detected by way of, for example, electrochemistry.

The assay device can be a home testing kit and the assays can provide information relating to the absence or presence of a medical condition such as heart disease.

In more detail, the assay device (e.g., a cartridge or test strip) generally includes a base and a lid. A void between the base and the lid defines at least a first capillary flow channel of specific volume, through which a fluid can flow. Alternatively, a third component between the base and lid can provide walls to define the void. The configuration of the device is such that introduction of a fluid at selected points results in inevitable fluid flow to or from points connected by fluid pathways. Thus the capillary flow channel is fluidly connected to at least one application zone and at least one detection zone, to facilitate the flow of applied fluid. There may also be at least one reagent zone fluidly connected to the capillary flow channel. The application zone includes an inlet for accepting fluids. The application zone is fluidly connected to the capillary flow channel, and to the detection zone, to facilitate the flow of the applied sample. Optionally, the assay device can also include at least one reference zone. The reagent zone, application zone, detection zone and reference zone may be combined in different ways such that at least two of said zones are incorporated into the same zone. The base and lid also define at least one vent adapted to selectively inhibit or extend capillary flow within the capillary flow channel. The vent can be a fusable vent, and can take the form of a weir.

Optionally the assay device can include, on a surface of the base, lid, or both, the at least one reagent zone,

reference zone, detection zone, application zone or a combination of these. Alternatively, on at least a third component between the base and lid there is located at least one of the reagent zone, reference zone, detection zone and application zone. In some embodiments, the assay device includes a plurality of reagent zones, a reference zone, application zone and a detection zone. The reagent zones can overlap with one another or with the reference, detection or application zones; or the reagent zones can be separated from each other or from the reference, detection or application zones. Also, the flow channel can be configured such that it is unsuitable for supporting capillary flow. The flow in the channel can be induced by, for example, a pump or a combination of magnet and magnetic particles.

Typically the reference and detection zones will be separated from each other. The detection zone and reference zone can be located such that a sample in the capillary flow channel contacts the detection zone and reference zone. A reagent zone can be located such that a sample will contact the reagent zone after the sample is applied to the sample inlet. For example, the reagent zone can be in the application zone, the detection zone, the reference zone or the capillary channel.

The assay device may comprise a second reagent zone, a second detection zone for performing a second assay. Optionally, the assay device further comprises a second application zone and a second reference zone. These zones can be fluidly connected as described above. The second application zone can be used to add a second fluid to the assay device. Optionally the second fluid is a

solution such as a salt solution. The salt solution can be a buffer. Alternatively the second fluid is a physiological fluid. The physiological fluid can be blood or a fluid derived at least in part therefrom.

The assay device may comprise a second capillary flow channel fluidly connected to a vent. The vent can provide a demarcation between the first and second capillary channels. The second capillary flow channel can be fluidly connected to the first capillary flow channel. The second capillary flow channel can also be fluidly connected to the second reagent zone, the second detection zone, the second application zone and the second reference zone.

At least one reagent zone includes a first reagent capable of recognizing a desired analyte. Recognition can include binding the analyte. For example, recognition includes selectively binding the analyte; that is, binding the analyte with a higher affinity than other components in the sample. This recognition reagent can be, for example, a protein, a peptide, an antibody, a nucleic acid, a small molecule, a modified antibody, a chimeric antibody, a soluble receptor, an aptamer, or other species capable of binding the analyte.

The recognition reagent can optionally produce a detectable change. For example, the recognition reagent can be an element, or one of its corresponding ions, that binds to at least one epitope of the analyte. Alternatively, or in addition, the recognition reagent is linked (e.g., by covalent bond, electrostatic interaction, adsorption, or other chemical or physical

linkage) to a further reagent that can produce a detectable change. The detectable change can be, for example, a change in electrical properties (e.g., redox potential, a voltage, a current, or the like), or optical properties (e.g., a change in absorption, reflectance, refraction, transmittance, or emission of light).

The analyte can be a biomarker for a condition that afflicts the mammalian body. The term "biomarker" refers to a biochemical in the body that has a particular molecular trait to make it useful for diagnosing a condition, disorder, or disease and for measuring or indicating the effects or progress of a condition, disorder, or disease. For example, common biomarkers found in a person's bodily fluids (i.e., breath or blood), and the respective diagnostic conditions of the person providing such biomarkers include, but are not limited to, ischemia modified albumin "IMA" (source: lack of oxygen to the blood; diagnosis: coronary artery disease), N-terminal truncated pro-brain natriuretic peptide "NT pro-BNP" (source: stretching of myocytes; diagnosis: congestive heart failure), acetaldehyde (source: ethanol; diagnosis: intoxication), acetone (source: acetoacetate; diagnosis: diet; ketogenic/diabetes), ammonia (source: deamination of amino acids; diagnosis: uremia and liver disease), CO (carbon monoxide) (source: CH_2Cl_2, elevated % COH; diagnosis: indoor air pollution), chloroform (source: halogenated compounds), dichlorobenzene (source: halogenated compounds), diethylamine (source: choline; diagnosis: intestinal bacterial overgrowth), H (hydrogen) (source: intestines; diagnosis: lactose intolerance), isoprene (source: fatty acid; diagnosis: metabolic

stress), methanethiol (source: methionine; diagnosis: intestinal bacterial overgrowth), methylethylketone (source: fatty acid; diagnosis: indoor air pollution/diet), O-toluidine (source: carcinoma metabolite; diagnosis: bronchogenic carcinoma), pentane sulfides and sulfides (source: lipid peroxidation; diagnosis: myocardial infarction), H_2S (source: metabolism; diagnosis: periodontal disease/ovulation), MeS (source: metabolism; diagnosis: cirrhosis), and Me_2S (source: infection; diagnosis: trench mouth).

A reagent zone can also include a second reagent capable of recognizing a desired analyte. The second reagent can recognize the same or a different analyte. The first and second recognition reagents can be selected to recognize the same analyte simultaneously. For example the first and second recognition reagents can each be an antibody that recognizes distinct epitopes of the analyte. In this way, a ternary (i.e., three-component) complex of analyte, first recognition reagent and second recognition reagent can be formed. In general, the first and second recognition reagents do not associate with one another in the absence of analyte. The presence of analyte, however, can associate the first and second recognition reagents together, in a ternary complex. The reagent zones can include further reagents such as redox mediators, substrates for particular enzymes and salts suitable for forming buffer solutions.

The second recognition reagent can be linked to a particle that can induce mobility on the so-formed ternary complex. The particle can be, for example, a polymer microsphere, a metal nanoparticle, or a magnetic

particle. A magnetic particle is a particle that is influenced by a magnetic field. The magnetic particle can be, for example, a magnetic particle described, in U.S. Patent Application Publication Nos. 20050147963 or 20050100930, or U.S. Patent No. 5,348,876, each of which is incorporated by reference in its entirety, or commercially available beads, for example, those produced by Dynal AS under the trade name DYNABEADS™. In particular, antibodies linked to magnetic particles are described in, for example, United States Patent Application Nos. 20050149169, 20050148096, 20050142549, 20050074748, 20050148096, 20050106652, and 20050100930, and U.S. Patent No. 5,348,876, the teachings of each of which is incorporated by reference in its entirety.

Generally, the detection zones collect the analytes and are the sites of detectable changes. The extent of the detectable changes can be measured at the detection zones. Usually, greater amounts of analytes will result in greater detectable changes; however, the assays can also be configured to produce smaller changes when the analytes are present in greater quantities. The detection zones can collect the analytes by immobilizing them (for example, with a reagent immobilized in the detection zone, where the immobilized reagent binds to the analyte). Alternatively, the detection zone can attract or immobilize a component associated with the analyte. For example, a recognition reagent that binds an analyte and is linked to a magnetic particle can be attracted to a particular detection zone by a magnetic field provided in one or more detection zones.

In some embodiments, one or more of the detection zones include one or more electrodes. The electrodes can be

formed of a material selected for electrical conductivity and low reactivity with sample components, for example, silver, gold, aluminum, palladium, platinum, iridium, a conductive carbon, a doped tin oxide, stainless steel, or a conductive polymer. The electrodes in the detection zones (the working electrodes), in conjunction with second electrodes in the reference zones (the reference electrodes) can measure an electrical property of the sample, such as a voltage or a current. Alternatively, the detection zones and the reference zones can each have at least one working electrode and counter electrode. That is, the detection and reference zones can make independent measurements. Optionally, counter electrodes are also included in the assay device. Assay devices including electrodes for measuring electrical properties of a sample are described in, for example, U.S. Patent Nos. 5,708,247, 6,241,862, and 6,733,655, each of which is incorporated by reference in its entirety.

In some embodiments, the assay device base, assay device lid, or both have a translucent or transparent window aligned with the detection zone. An optical change that occurs in the detection zone can be detected through the window. Detection can be done visually (i.e., the change is measured by the user's eye) or measured by an instrument (e.g., a photodiode, photomultiplier, or the like). In general, the reference zone is similar in nature to the detection zone. In other words, when the detection zone includes an electrode, the reference can likewise include an electrode. When the detection zone is aligned with a window for optical measurement, the reference zone can similarly be aligned with a window for optical measurement. In some embodiments, the reference

zone is not adapted to collect analyte. Alternatively, the reference zone is adapted to collect analyte, but performs a different analysis on said analyte. Thus, the detectable change measured in the reference zone can be considered a background measurement to be accounted for when determining the amount of analyte present in the sample.

The sample can be any biological fluid, such as, for example, blood, blood plasma, serum, urine, saliva, mucous, tears, or other bodily fluid. The analyte can be any component that is found (or may potentially be found) in the sample, such as, for example, a protein, a peptide, a nucleic acid, a metabolite, a saccharide or polysaccharide, a lipid, a drug or drug metabolite, or other component. The assay device can optionally be supplied with a blood separation membrane arranged between a sample inlet and the detection zone, such that when whole blood is available as a sample, only blood plasma reaches the detection zone.

The assay device and included reagents are typically provided in a dry state. Addition of a liquid sample to the assay device (i.e., to the capillary channel) can resuspend dry reagents.

Description of particular embodiments

Referring now to Figure 1, a test strip suitable for use with the assay device is generally depicted at 101. The test strip has a first detection zone 102 and a second detection zone 103 fluidly connected by a first linear channel 104 and a second linear channel 105. The first linear channel 104 is fluidly connected to a first

application zone 106 and the second linear channel 105 is fluidly connected to a second application zone 107. The first and second detection zones, 102 and 103, are equipped with a first set of electrodes 108 and a second set of electrodes 109 respectively. The electrodes are suitable for directly or indirectly detecting a component of the sample. At a point substantially equidistant from the two detection zones, 102 and 103, there is provided a fusable vent 110 fluidly connected to, and forming a coupling between, the first channel 104 and the second channel 105. The vent 110 acts to prevent or promote the flow of fluids in the first and second channels, 104 and 105.

Fluidly connected to the first channel 104, situated between the first application zone 106 and the first detection zone 102, there is provided a first reagent zone 111. Similarly, fluidly connected to the second channel 105, situated between the second application zone 107 and the second detection zone 103, there is provided a second reagent zone 112. The first reagent zone 111 includes a substrate (for example, cobalt) for binding to an analyte of interest (for example, IMA). The first reagent zone 111 also includes a first recognition reagent linked to an enzyme capable of oxidizing or reducing a redox active enzyme substrate. For example, when the redox active enzyme substrate is glucose, the enzyme can be a glucose oxidase (GOD). The first reagent zone 111 further comprises a second recognition reagent selected to bind the desired analyte. In particular, the second recognition reagent is selected to bind the desired analyte simultaneously with the first recognition reagent to form a ternary complex. The second

recognition reagent is linked to a magnetic particle. The second reagent zone 112 includes a redox active enzyme substrate (e.g., glucose) and a redox mediator (e.g., potassium ferricyanide, $K_3Fe(CN)_6$). Reagents are dried onto the reagent zones and may be resuspended on the addition of a fluid such as blood or buffer.

The assay device is further provided with a magnet (not shown), which acts on the magnetic particles in the channel. The magnet is used to move the magnetic particles, and anything bound to them, from one area of the test strip to another. The test strip is suitable for insertion into a reader, which presents to the user the results of any assays performed.

In a detailed embodiment of the method, there is first provided an assay device comprising a test strip, suitable for reading by an electronic reader. To the test strip is added a sample of mammalian blood suspected of containing ischemia modified albumin "IMA" (the first analyte) and N-terminal truncated pro-brain natriuretic peptide "NTproBNP" (the second analyte). The sample of blood mixes with cobalt which has been dried onto the test strip, resuspending the cobalt in solution, and forming a mixture under conditions suitable for interaction of the first analyte with cobalt. In this mixture, some cobalt binds to IMA in the blood to form a complex, whilst some cobalt remains unbound. The sample of blood also mixes with magnetic particles bound to anti-NTproBNP antibody 7206 (the antibody bound magnetic particle) and horse radish peroxidase "HRP" conjugated to anti-NTproBNP antibody 15F11 (the antibody bound enzyme), which have been dried onto the test strip, resuspending

these components in solution, and forming a mixture under conditions suitable for interaction of the second analyte with the antibody bound magnetic particle and the antibody bound enzyme, thereby forming a ternary complex.

An electrochemical analysis is then performed on the first mixture. This analysis provides an indication of the amount of unbound cobalt present in the first mixture. In turn, the amount of IMA present in the sample can be determined. This test procedure for detecting IMA may be optimized in accordance with our co-pending Application **GB 0603049.8**, which is incorporated herein by reference.

This step of the method as described generally allows the indirect detection of any analyte in a complex mixture, although it will be appreciated that the method is also suitable for the indirect detection of an analyte in simple mixtures. The method has applications in any assay where the interaction between a detectable material and an analyte modifies the detectability of said detectable material.

After the first assay is complete, a magnet is moved along the test strip, moving the magnetic particles, and all components bound to them (as the ternary complex or otherwise) along a first channel to an air vent. The magnet is moved approximately 5mm beyond the air vent, towards a second channel where it is held. This holds the magnetic particles at the fluid-air interface, as they cannot pass through the so-formed meniscus.

A second fluid is added to the test strip at the second application zone. The second fluid contains sodium acetate buffer, hydrogen peroxide substrate, and ABTS redox mediator. The second fluid flows along the second channel to the vent where the second fluid contacts the blood sample to form a liquid-liquid interface. The formation of the fluid-fluid interface facilitates the movement of the magnetic particles (and all that is bound to them) from the blood to the second fluid, leaving interferents and analytes that are not of interest in the blood in the first channel. Only the magnetic particles and all that is bound to them, including the NTproBNP (in the form of a ternary complex of NTproBNP with antibody bound magnetic particle and antibody bound enzyme) are transferred to the second fluid in the second channel. The magnetic particles are moved to a second detection zone using the magnet. The magnetic particles are held at the second detection zone, where the second analyte is indirectly detected electrochemically.

In this embodiment the first, second and any further assays are optionally performed sequentially. In an alternative embodiment, at least two assays are performed simultaneously.

Referring now to Figure 2, assembled test strip 201 includes base 213 separated from lid 214 by spacers 215. Spacers 215 can be formed as an integral part of base 213 or lid 214. Alternatively, base 213, lid 214 and spacers 215 can be formed separately and assembled together. When assembled, together, connections between base 213, lid 214 and spacers 215 can be sealed, for example with an adhesive or by welding. Base 213, lid 214 and spacers

215 can define liquid-tight channels 204, 205 where a liquid sample is allowed to contact interior surfaces that define the channels 204, 205, such as surface 216 of base 213. Between the liquid tight channels there is located a vent (not shown) that can promote or prevent capillary flow. The dimensions of spacer 215 can be selected such that surfaces of base 213 and lid 214 facing the interior the channels 204, 205 form a capillary, i.e., the base and lid provide capillary action to a liquid inside channels 204, 205. Alternatively, base 213 or lid 214 can provide capillary action independently of each other. Channels 204, 205 can have a volume of less than 100 microliters, less than 20 microliters, less than 10 microliters, or 5 microliters or less.

Referring now to Figure 3 there is illustrated alternate configurations of reagent deposition on base 313, as a cross-section parallel to the short side of the test strip. In Figure 3A, first electrode set 308 is arranged on surface 316 of base 313. First reagent mixture 317 is deposited over at least one electrode in first electrode set 308. First reagent mixture 317 includes first reagent, second reagent and third reagent, second reagent and third reagent are illustrated in Figure 4A. The first reagent includes cobalt and can interact with a first analyte. Referring to Figure 4A, second reagent 419 includes magnetic particle 421 linked to a first antibody 422. Third reagent 420 includes detectable component 423 linked to a second antibody 424.

An alternate configuration is shown in Figure 3B, in which at least one electrode from electrode set 308 is

arranged on surface 316 of base 313, overlayed with first reagent mixture 317, which in turn is overlayed with second reagent mixture 325. First reagent mixture 317 includes first reagent. Second reagent mixture 325 includes second reagent and third reagent. It will be apparent that alternative combinations of different reagents can be incorporated into one or more layers. Selecting the order in which reagents are deposited can allow selective or timed release of the reagent upon contact with a sample, in order to suit assay kinetics and improve sensitivity.

The reagents may be deposited on one or more electrode and on one or more electrode set. The reagents can be deposited on any part of the channels that facilitates interaction with analytes in the sample before detection takes place.

Alternatively, referring now to Figure 3C, second reagent mixture 325 is deposited on surface 316 of base 313.

When a sample, or other fluid, is introduced to the channels, (for example, by contacting the sample with a sample inlet), liquid can fill the channels and contact the surface of the base, resuspending the reagents deposited on the surface.

If the sample contains the first analyte to which the first reagent binds, the first reagent will bind to the first analyte. The first reagent is chosen to include cobalt, which binds to albumin and IMA. The binding of cobalt can be assayed electrochemically or photochemically, among other techniques.

Referring again to Figure 4, if the sample contains the second analyte 426 recognized by the first and second antibodies 422 and 424, then the antibodies 422, 424 will bind to the second analyte. The antibodies 422, 424 are chosen to bind to different epitopes of the analyte 426, allowing the formation of a ternary complex 427 of reagent 419, analyte 426, and reagent 420, as illustrated in Figure 4B.

Figure 5A and 5B illustrate the assay device, for example, cartridge or test strip 101, during operation. In Figure 5A, there is a side view into the first channel 504 and the second channel 505. The base 513 and lid 514 confine a liquid sample which includes dissolved first reagent 518, second reagent 519 and third reagent 520 and a first analyte 528 and second analyte 526. The reagents 518, 519, 520 can be supplied in excess relative to the amount of analytes 528, 526 present in the sample, such that all analytes 528, 526 are bound, while a portion of the reagents 518, 519, 520 can remain unbound. On the lid 514 there is located a first application zone 506 and a second application zone 507, and a vent 510. A blood sample is introduced to the assay device 101, and the reagents are resuspended by the sample. The sample flows along the first channel 504 to the vent 510 where capillary flow stops, forming a meniscus 529 with air. Reagents, analytes, and complexes can be distributed by diffusion near the location in channel 504 or 505 where the reagents originated. An analysis of the first reagent 518 is performed at a first set of electrodes 508 to give an indication of the presence of the first analyte 528.

After the first assay is complete a magnetic field source 530, located underneath the base 513 and proximate to the first application zone 506, is configured to move the antibody bound magnetic particles 519 and also the second analyte 526 and detectable component 523 where they form a ternary complex with the antibody bound magnetic particles 519, toward the meniscus 529. The magnetic field source 530 is held proximate to the second channel 505. A buffer solution (not shown) containing a substrate (not shown) for the detectable component 523, and a redox mediator (not shown), is added to the second channel 505 via the second application zone 507. The buffer solution travels along the second channel 505 to the meniscus 529 where it forms a liquid-liquid interface with the sample fluid. On formation of the liquid-liquid interface, the magnetic particle bound antibodies 519, and all that is bound to them, moves rapidly from the sample fluid to the buffer solution; the magnetic particles being attracted to the magnetic field source 530 situated proximate to the second channel 505. The rapid movement of the magnetic particles across the liquid-liquid interface prevents impurities from being dragged into the second channel 505. This allows an accurate second assay to be performed at the second electrode set 509 in the second channel 505. The magnetic field source 530 is moved towards the second electrode set 509 to localize the second analyte 526 over said electrodes 509.

The magnetic field source can be configured to provide a shaped magnetic field. A shaped magnetic field can have magnetic field lines designed to direct magnetic

particles toward the first or second detection zones. Such a shaped magnetic field can be useful to control the diffusion or migration of magnetic particles and label particles. More than one magnetic field source can be provided, particularly when a shaped magnetic field is desired. For example, magnetic field sources can be provided at either end of an assay device, where one is configured to attract magnetic particles and the other to repel magnetic particles. Such a configuration can favour the location of all magnetic particles at one end of the assay device.

Referring once more to Figures 4, detectable component 423 can be directly detectable (e.g., a colored particle detected by observation of a colour change, or component 423 can be detected indirectly. Component 423 can produce a product that is directly detected, such that detection of the product is an indirect detection of component 423. For example, component 423 can be an enzyme whose product is detected directly (e.g., optically or electrochemically). The amount of product formed, or rate of product formation, can be related to the amount of detectable component 423.

Glucose oxidase (GOD) is one enzyme that can be used as the detectable component 423. In the presence of glucose and mediator, the GOD (whether or not the associated particle is bound to a magnetic particle 421 via the analyte 426) converts glucose to gluconic acid and converts the mediator (e.g., ferricyanide) from an oxidized form to a reduced from.

Referring again to Figure 5, after a predetermined period of time has elapsed, a working electrode 509 in the second detection zone (not shown) can be turned on. The amount of reduced mediator in the bulk fluid is measured as a current at the working electrode or electrodes 509. This current, produced when the GOD is distributed homogeneously in the sample, is the background signal. When magnetic field source 530 applies a magnetic field in the vicinity of second detection zone (not shown), antibody bound magnetic particles 519, and all reagents bound to them, become localized near the second detection zone. The magnetic field localizes particles whether the particles are bound to reagent or not. The application of a magnetic field by source 530 causes an increase in the concentration of enzyme 523 near the second detection zone. Enzyme 523 in turn produces a change detectable in the second detection zone.

When enzyme 523 is GOD, the increased concentration of reduced mediator at the surface of working electrode 509 is reflected as a higher current at that electrode when the magnetic field is applied. The higher the analyte 526 concentration, the larger the current will be.

The magnetic field can be applied and removed a number of times, and a series of magnetized and non-magnetized working electrode currents can be measured. The data collected allow the concentration of analyte in the sample to be measured. In some embodiments, two working electrodes can be used, one with a magnet and one without, each on opposite internal faces of the channel. In this case, one electrode is magnetized while the other is not, and both electrodes are activated simultaneously.

The currents at the two working electrodes are then compared. The detectable components can be selected to produce an optical change. For example, a detectable change in chemiluminescent signal can be produced when an analyte molecule in a sample brings two particles (or beads) together in close proximity. A first particle, called a donor particle, is linked to a first antibody, and a second particle (an acceptor particle) is linked to a second antibody. The first and second antibodies bind to different epitopes of the same antigen, such that a ternary complex of donor particle antigen acceptor particle can be formed. A cascade of chemical reactions that depends on the proximity of the beads (and therefore on the presence of the analyte) can produce greatly amplified signal. Detection of an analyte at attomolar (i.e., on the order of 10^{-18} molar) concentrations is possible.

Photosensitizer particles (donor particles) including a phthalocyanine can generate singlet oxygen when irradiated with light having a wavelength of 680 nm. The singlet oxygen produced has a very short half-life - about 4 microseconds - and hence it decays rapidly to a ground state. Because of the short half-life, singlet oxygen can only diffuse to a distance of a few hundred microns from the surface of the particles before it decays to ground state. The singlet state survives long enough, however, to enter a second particle held in close proximity. The second particles (acceptor particles) include a dye that is activated by singlet oxygen to produce chemiluminescent emission. This chemiluminescent emission can activate further fluorophores contained in the same particle, subsequently causing emission of light

at 520-620 nm. See, for example, *Proc. Natl. Acad. Sci.* 91:5426-5430 1994; and U.S. Patent No. 6,143,514, each of which is incorporated by reference in its entirety. An optical change can also be produced by a bead linked to an antibody. The bead can include a polymeric material, for example, latex or polystyrene. To produce the optical change, the bead can include a light-absorbing or light-emitting compound. For example, a latex bead can include a dye or a fluorescent compound. The reagent can include a plurality of beads. The beads in the plurality can be linked to one or more distinct antibodies. A single bead can be linked to two or more distinct antibodies, or each bead can have only one distinct antibody linked to it. The reagent can have more than one distinct antibody each capable of binding to the same analyte, or antibodies that recognizes different analytes. When the bead includes a light absorbing compound, the optical measurement can be a measurement of transmittance, absorbance or reflectance. With a fluorescent compound, the intensity of emitted light can be measured. The extent of the measured optical change can be correlated to the concentration of analyte in the sample.

A detectable change can be produced by the enzyme multiplied immunoassay technique (EMIT). In an EMIT assay format, an enzyme-analyte conjugate is used. A first reagent can include an antibody specific for the analyte, an enzyme substrate, and (optionally) a coenzyme. A second reagent can include a labeled analyte: a modified analyte that is linked to an enzyme. For example, the enzyme can be a glucose-6-phosphate dehydrogenase (G-6-PDH). G-6-PDH can catalyze the

reaction of glucose-6-phosphate with NAD(P) to yield 6-phosphoglucono-D-lactone and NAD(P)H. NAD(P)H absorbs light with a wavelength of 340 nm, whereas NAD(P) does not. Thus, a change in absorption of 340 nm light as a result of the G-6-PDH catalyzed reaction can be a detectable change. When the first reagent is mixed with a sample, the analyte is bound by the antibody in the first reagent.

The second reagent is added, and any free antibody binding sites are occupied by the enzyme-linked analyte of the second reagent. Any remaining free antibodies bind the labeled analyte, inactivating the linked enzyme. Labeled analyte bound by the antibody is inactive, i.e., it does not contribute to the detectable change. Labeled analyte that is not bound by antibody (a quantity proportional to amount of analyte in sample) reacts with the substrate to form a detectable product (e.g., NAD(P)H).

Another assay format is the cloned enzyme donor immunoassay (CEDIA). CEDIA is a homogeneous immunoassay based on the bacterial enzyme E-galactosidase of *E. coli* which has been genetically engineered into two inactive fragments. These two inactive fragments can recombine to form an active enzyme. One fragment consists of an analyte-fragment conjugate, and the other consists of an antibody-fragment 5 conjugate. The amount of active enzyme that generates the signal is proportional to the analyte concentration. See, for example, Khanna, P.L. and Coty, W.A. (1993) In: *Methods of Immunological Analysis, volume 1* (Masseyeff, R.F., Albert, W.H., and Staines, N.A., eds.) Weinheim, FRG: VCH Verlagsgesellschaft MbH,

1993: 416-426; Coty, W.A., Loor, R., Powell, M., and Khanna, P.L. (1994) *J. Clin. Immunoassay* 17(3): 144-150; and Coty, W.A., Shindelman, J., Rouhani, R. and Powell, M.J. (1999) *Genetic Engineering News* 19(7), each of which is incorporated by reference in its entirety.

The assay device can be used in combination with a reader configured to measure the detectable change. The reader can include an optical system to detect light from the analysis region. The light to be detected can be, for example, emitted, transmitted, reflected, or scattered from the detection zone. Emitted light can result from, for example, chemiluminescent or fluorescent emission. The optical system can include an illumination source, for example, to be used in the detection of a change in fluorescence, absorbance, or reflection of light. For an assay device configured for an electrochemical measurement, the reader can be in electrical contact with the working electrode and reference electrode. The assay device electrodes can have electrical leads connecting the electrodes to contacts outside the assay void. The contacts register with and contact corresponding contacts of the assay device to provide electrical contact. The reader can also include an output display configured to display the results of the measurement to a user.

The assay device reader can include magnetic field source. The assay device reader can be configured to apply a magnetic field via source at predetermined times, such as after a predetermined period of time has elapsed after a sample has been applied to the assay device. Magnetic field source can be, for example, an electromagnet or a permanent magnet. An electromagnet

can selectively apply a field when a current is supplied to the electromagnet. A permanent magnet can be moved toward or away from the detection zone in order to control the strength of the field at that site.

Referring to Figure 6, reader instrument 1000 accepts test assay device 1100 and includes display 1200. The display 1200 may be used to display images in various formats, for example, text, joint photographic experts group (JPEG) format, tagged image file format (TIFF), graphics interchange format (GIF), or bitmap. Display 1200 can also be used to display text messages, help messages, instructions, queries, test results, and various information to patients. Display 1200 can provide a user with an input region 1400. Input region 1400 can include keys 1600. In one embodiment, input region 1400 can be implemented as symbols displayed on the display 1200, for example when display 1200 is a touch-sensitive screen. User instructions and queries are presented to the user on display 1200. The user can respond to the queries via the input region.

Reader 1000 also includes an assay device reader, which accepts diagnostic test assay devices 1100 for reading. The assay device reader can measure the level of an analyte based on, for example, the magnitude of an optical change, an electrical change, or other detectable change that occurs on a test assay device 1100. For reading assay devices that produce an optical change in response to analyte, the assay device reader can include optical systems for measuring the detectable change, for example, a light source, filter, and photon detector, e.g., a photodiode, photomultiplier, or Avalance photo

diode. For reading assay devices that produce an electrical change in response to analyte, the assay device reader can include electrical systems for measuring the detectable change, including, for example, a voltameter or amperometer.

Device 1000 further can include a communication port (not pictured). The communication port can be, for example, a connection to a telephone line or computer network. Device 1000 can communicate the results of a measurement to an output device, remote computer, or to a health care provider from a remote location. A patient, health care provider, or other user can use reader 1000 for testing and recording the levels of various analytes, such as, for example, a biomarker, a metabolite, or a drug of abuse.

Various implementations of diagnostic device 1000 may access programs and/or data stored on a storage medium (e.g., a hard disk drive (HDD), flash memory, video cassette recorder (VCR) tape or digital video disc (DVD); compact disc (CD); or floppy disk). Additionally, various implementations may access programs and/or data accessed stored on another computer system through a communication medium including a direct cable connection, a computer network, a wireless network, a satellite network, or the like.

The software controlling the reader can be in the form of a software application running on any processing device, such as, a general-purpose computing device, a personal digital assistant (PDA), a special-purpose computing device, a laptop computer, a handheld computer, or a

network appliance. The reader may be implemented using a hardware configuration including a processor, one or more input devices, one or more output devices, a computer-readable medium, and a computer memory device. The processor may be implemented using any computer processing device, such as, a general-purpose microprocessor or an application specific integrated circuit (ASIC).

The processor can be integrated with input/output (I/O) devices to provide a mechanism to receive sensor data and/or input data and to provide a mechanism to display or otherwise output queries and results to a service technician. Input device may include, for example, one or more of the following: a mouse, a keyboard, a touch-screen display, a button, a sensor, and a counter. The display 1200 may be implemented using any output technology, including a liquid crystal display (LCD), a television, a printer, and a light emitting diode (LED).

The computer-readable medium provides a mechanism for storing programs and data either on a fixed or removable medium. The computer-readable medium may be implemented using a conventional computer hard drive, or other removable medium. Finally, the system uses a computer memory device, such as a random access memory (RAM), to assist in operating the reader. Implementations of the reader can include software that directs the user in using the device, stores the results of measurements. The reader 1000 can provide access to applications such as a medical records database or other systems used in the care of patients. In one example, the device connects to a medical records database via the communication port.

Device 1000 may also have the ability to go online, integrating existing databases and linking other websites.

Example 1

According to one embodiment of the present invention the method is performed using wet assays. The instrumentation used includes an Eco Chemie™ Autolab™ with a six-way multistat and GPES™ software. The electrodes used were screen printed in-house. The working and counter electrodes were prepared using carbon D2 (GEM™ Ltd), silver/silver chloride electrodes were prepared using AgCl 70:30 (GEM™ Ltd or DuPont™), and dielectric electrodes were prepared using dielectric D1 (GEM™ Ltd)

The materials used for the test strip include a hydrophobic polyester base and a hydrophilic antifog lid, with a double-sided adhesive spacer (200μm) forming channel therebetween. The antifog lid is preblocked with 40mg/ml bovine serum albumin, 1.5% Tween™ in phosphate buffered saline, pH7.3, before it is rinsed and dried. Alternatively the substrate comprises alumina ceramic or polyester cards.

In this embodiment the reagents used in the first assay include, cobalt chloride, 4-morpholinepropanesulfonic acid (MOPS), potassium chloride. A buffer of pH 7.4 is prepared using 100 mM MOPS and 150 mM potassium chloride and a cobalt chloride standard for 45 mM in 1.5 M potassium chloride is also prepared. The reagents used in the second assay include 5mM hydrogen peroxide, 5mM

2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) in 125mM sodium acetate buffer, pH4.5. Horse radish peroxidise (HRP) conjugated to antibody 15F11. 1µm magnetic particles (Chemicell™ with COOH on surface) bound to antibody 7206.

The samples used for analysis include frozen serums and whole blood samples from volunteers.

5 µL of the cobalt standard is added to 100 µL of the blood sample (serum, plasma or blood) in a tube. The so-formed mixture is mixed for 10 seconds using a vortexer, before being allowed to incubate for 2 minutes. Cobalt binds to albumin and, to a lesser extent, IMA in the blood. Magnetic particles (with anti-NTproBNP antibody 7206 bound) and HRP conjugated to anti-NTproBNP antibody 15F11 are added to the sample and the sample is mixed for 30min at 600rpm. Between 7.5µL and 15 µL of the mixture is then removed and applied to a first channel via the first application zone in a test strip.

The sample mixture travels along the first channel and is stopped at a specific point where air vents are positioned at either side of said first channel. These air vents remain open to a second channel.

A first measurement, to detect the amount of IMA present in the sample fluid, is performed at the first electrode set. The working electrode is poised at +1 Volt for 40 seconds before a linear sweep is applied from + 1 Volt to - 0.5 Volt at a scan rate of 0.7 V/second. The measurements made may be optimised in accordance with our

co-pending Application **GB 0603049.8**, referred to herein previously.

The cobalt 2^+ ions are oxidised and adsorbed as cobalt 3^+ hydroxy species at the electrode surface at + 1 Volt. During the scan the cobalt 3^+ is reduced back to cobalt 2^+ giving a cathodic signal peak at around +0.7 Volts. To calibrate the test, the performance of the electrodes is tested for a range of cobalt concentrations in buffer. To determine whether the amount of IMA in the sample, the value recorded is correlated with the Albumin Cobalt Binding (ACB™) test for IMA.

The magnetic particles (and everything bound to them) are dragged to the liquid/air interface at the air vents using a magnet. The magnet is pulled 5mm past liquid-air interface and held is over the empty second channel. This holds the magnetic particles at the liquid-air interface as they cannot pass through the so-formed meniscus.

Around 11ul of reaction buffer containing 125mM sodium acetate pH4.5, 5mM ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) and 5mM hydrogen peroxide is added to the second channel via the second application zone. This flows towards the liquid-air interface, the flow being facilitated by the presence of a vent positioned at said interface. The reaction buffer forms a liquid-liquid interface with the blood sample. At this point the magnetic particles 'jump' across the liquid-liquid interface, as they are attracted by the magnet which is located proximate to the second channel. This 'jump' minimises the loss of particles at the

interface and minimises the carry over of blood into the reaction buffer zone.

The magnet is then moved at a controlled speed (minimising particle loss) to a position over the working electrode of the second electrode set. The magnet drags the particles along the underside of the blocked lid. This drags the magnetic particles over the working electrode of the second electrode set, whilst separating them from any remaining unbound HRP conjugate. Upon arrival over the second electrode set, the magnetic particles are held in place by the magnet, and a further 50ul of reaction buffer (to further wash the magnetic particles) is added to the second channel via the second application zone. Once this is delivered, the magnet is removed and the reaction is allowed to proceed for 10 minutes with the magnetic particles on the working electrode of the second electrode set. In this setup, a 3 carbon electrode system is used.

After 10 minutes reaction, the test strip is attached to a potentiostat, and the potential stepped from open circuit to +0.0V. The current is measured after 3s and compared to calibration curve to give NTproBNP concentration. The oxidised ABTS ions produced by reaction between reduced ABTS, HRP and hydrogen peroxide, are converted to reduced ABTS species at the electrode surface at +0.0 Volts.

Example 2

According to an alternative embodiment of the present invention the method is again performed using wet assays.

The instrumentation used includes an Eco Chemie™ Autolab™ with a six-way multistat and GPES™ software. The electrodes used were screen printed in-house. The working and counter electrodes were prepared using carbon D2 (GEM™ Ltd), silver/silver chloride electrodes were prepared using AgCl 70:30 (GEM™ Ltd or DuPont™), and dielectric electrodes were prepared using dielectric D1 (GEM™ Ltd)

The materials used for the test strip include a hydrophobic polyester base and a hydrophilic antifog lid, with a double-sided adhesive spacer (200µm) forming channel therebetween. The antifog lid is preblocked with 40mg/ml bovine serum albumin, 1.5% Tween™ in phosphate buffered saline, pH 7.3, before it is rinsed and dried. Alternatively the substrate comprises alumina ceramic or polyester cards.

In this embodiment the reagents used in the first assay include, cobalt chloride, 4-morpholinepropanesulfonic acid (MOPS), potassium chloride. A buffer of pH 7.4 is prepared using 100 mM MOPS and 150 mM potassium chloride and a cobalt chloride standard for 45 mM in 1.5 M potassium chloride is also prepared. The reagents used in the second assay include 200 mM glucose, 100 mM potassium ferricyanide in 5 M ammonium acetate buffer, pH 7.3, Glucose oxidase (GOD) conjugated to antibody 15F11 and 1um magnetic particles (Chemicell with COOH on surface) bound to antibody 7206.

The samples used for analysis include frozen serums and whole blood samples from volunteers.

5 µL of the cobalt standard is added to 100 µL of the blood sample (serum, plasma or blood) in a tube. The so-formed mixture is mixed for 10 seconds using a vortexer, before being allowed to incubate for 2 minutes. Cobalt binds to albumin and, to a lesser extent, IMA in the blood. Magnetic particles (with anti-NTproBNP antibody 7206 bound) GOD conjugated to anti-NTproBNP antibody 15F11 are added to the sample and the sample is mixed for 30min at 600rpm. Between 7.5µL and 15 µL of the mixture is then removed and applied to a first channel via the first application zone in a test strip.

The sample mixture travels along the first channel and is stopped at a specific point where air vents are positioned at either side of said first channel. These air vents remain open to a second channel.

A first measurement, to detect the amount of IMA present in the sample fluid, is performed at the first electrode set. The working electrode is poised at +1 Volt for 40 seconds before a linear sweep is applied from + 1 Volt to - 0.5 Volt at a scan rate of 0.7 V/second. The measurements made may be optimised in accordance with our co-pending Application **GB 0603049.8**, referred to herein previously.

The cobalt 2^+ ions are oxidised and adsorbed as cobalt 3^+ hydroxy species at the electrode surface at + 1 Volt. During the scan the cobalt 3^+ is reduced back to cobalt 2^+ giving a cathodic signal peak at around +0.7 Volts. To calibrate the test, the performance of the electrodes is tested for a range of cobalt concentrations in buffer. To determine whether the amount of IMA in the sample, the

value recorded is correlated with the Albumin Cobalt Binding (ACB™) test for IMA.

The magnetic particles (and everything bound to them) are dragged to the liquid/air interface at the air vents using a magnet. The magnet is pulled 5mm past liquid-air interface and held is over the empty second channel. This holds the magnetic particles at the liquid-air interface as they cannot pass through the so-formed meniscus.

Around 11ul of reaction buffer containing 5M ammonium acetate pH 7.3, 200mM glucose and 100mM ferricyanide is added to the second channel via the second application zone. This flows towards the liquid-air interface, the flow being facilitated by the presence of a vent positioned at said interface. The reaction buffer forms a liquid-liquid interface with the blood sample. At this point the magnetic particles 'jump' across the liquid-liquid interface, as they are attracted by the magnet which is located proximate to the second channel. This 'jump' minimises the loss of particles at the interface and minimises the carry over of blood into the reaction buffer zone.

The magnet is then moved at a controlled speed (minimising particle loss) to a position over the working electrode of the second electrode set. The magnet drags the particles along the underside of the blocked lid. This drags the magnetic particles over the working electrode of the second electrode set, whilst separating them from any remaining unbound GOD conjugate. Upon arrival over the second electrode set, the magnetic

particles are held in place by the magnet, and a further 50ul of reaction buffer (to further wash the magnetic particles) is added to the second channel via the second application zone. Once this is delivered, the magnet is removed and the reaction is allowed to proceed for 10 minutes with the magnetic particles on the working electrode of the second electrode set. In this setup, a three carbon electrode system is used.

After 10 minutes reaction, the device is attached to a potentiostat, and the potential stepped from open circuit to +0.4V. The current is measured after 10 seconds and compared to calibration curve to give NTproBNP concentration. The ferrocyanide ions produced by reaction between ferricyanide, GOD and glucose, are converted to ferricyanide species at the electrode surface at +0.4 Volts.

In an alternative embodiment of the present invention both assays are carried out in whole blood. In this embodiment, IMA binding reagent, magnetic particles and enzyme conjugate are provided in dry form in the first channel, whilst reaction substrates and mediators are provided in dry form in the second channel. The dried reagents are resuspended by the addition of blood. The resuspended IMA binding reagent binds IMA in solution and an assay is performed at a first set of electrodes. The magnetic particles and the enzyme label are mixed with the NTproBNP in the blood. The magnetic particles, and its conjugates, are then moved by magnetic manipulation to the second set of electrodes, separating the magnetic from the unbound enzyme. The second reaction would then proceed over the second electrode set.

In a further alternative the magnetic particles are used as a 'filter'. Magnetic particles and enzyme conjugate are dried onto a test strip and are resuspended by the addition of blood. With antibody bound, they could be positioned above a centrally located electrode in the blood sample. The blood sample could then be pumped back and forward passed the magnetic particles, allowing maximal binding of NTproBNP to the magnetic particle antibody complex and enzyme conjugate, whilst they are held in position. The buffer pouch would then be used to wash the blood away from the beads, into a sink area. A second assay, for IMA, can be performed in the sink area, where there are further sets of electrodes. The reagents for the IMA assay can be dried onto the test strip, or may be present in the buffer fluid. The buffer in which the magnetic particles are left contains substrate and mediator for reaction with enzyme conjugate which occurs over the electrode and which can be measured electrochemically.

In a still further embodiment, the IMA assay can be carried out as described and the second assay uses magnetic particles coated with streptavidin, and a biotinylated antibody (eg 7206). The biotinylated antibody binds NTproBNP, which also binds the enzyme conjugate in whole blood. This has preferential binding kinetics in the absence of bound magnetic particles. The magnetic particles can then be mixed with the binding complexes and bound to the antibody through a streptavidin-biotin association. The magnetic particle complexes are then dragged to the electrodes as described. It is also possible to use a streptavidin-

biotin association between the label and anti-NTproBNP antibody (e.g., 15F11) instead. Also, streptavidin, can be coupled to antibodies and biotin coupled to magnetic particles.

In another embodiment, there is planar capture of magnetic particles bound to NTproBNP on the electrode surface. Anti-NTproBNP antibody is attached either to the electrode, or to the lid above the electrode. Magnetic beads have another anti-NTproBNP antibody as well as an enzyme label bound to their surface. These beads are bound to NTproBNP in the blood sample as in the previous examples, and are dragged over the electrodes and allowed to bind the surface-bound antibodies. Unbound magnetic beads (without NTproBNP bound) are washed away by a wash with a reaction buffer. A signal is then produced by reaction of the enzyme label bound to the beads, proportional to the NTproBNP concentration.

This planar capture can also involve biotin-streptavidin associations to bind the magnetic particle to the antibody (e.g., 7206) where the magnetic particle, as well as having enzyme label bound to its surface, has streptavidin bound also. The anti NTproBNP antibody (e.g. 7206) is biotinylated. The NTproBNP binding to biotinylated antibody and surface-bound antibody occurs prior to attachment of streptavidin-coated magnetic particle to the biotinylated antibody. In a variation of this system, the surface bound antibody can be biotinylated and the surface to which it is attached can be streptavidin coated. In this way, after the magnetic particles with antibody and attached enzyme bind NTproBNP, and NTproBNP binds biotinylated antibody, this

complex can be attached to the surface via the streptavidin-biotin association. Also, the streptavidin and biotin coupling can be reversed, for example the streptavidin can be coupled to antibodies and biotin coupled to magnetic particles or surfaces.

In a still further embodiment, the first assay is performed as described in the examples given. In the second assay, the working electrode is positioned at the point where the magnetic particles jump to after the liquid-liquid interface is formed. This allows a stationary magnet to be used that positions the beads over the working electrode. This requires the reaction buffer to wash past the beads, washing the blood sample into a 'sink' area, whilst the beads are held in position against this flow. This can also be performed using two or three magnets set up in a see-saw arrangement, collecting the beads at specific regions along the channel. As one magnet is lowered towards the device to manipulate the particles, a connected magnet is simultaneously removed, removing its effect on the particles. Electromagnets can also be used instead of permanent magnets. Multiple stationary electromagnets can be switched on/off in sequence to control to positioning of the magnetic particles.

It will be apparent that any suitable antibody pairings can be used including, but not limited to, 15F11 - 24E11, 15C4 - 29D12, 15C4 - 13G12, 15C4 - 18H5, 7206 - 15F11. Also, various sizes, makes and surface coatings of magnetic particle can be used including, but not limited to, 0.1-1um diameter particles from Chemicell™, Bangs™, Spherotech™, Ademtech™, Polymicrospheres™, Chemagen™,

Dynal™, Coprtex™, Micromod™, Polysciences™, Estapor™, Seradyn™ or Bioclone™, with surface coatings of carboxyl, amine, aldehyde, epoxide, N-hydroxysuccinimide, choromethyl, polyglutaraldehyde, thiol, cyanuric, tosyl, hydrazide, hydroxyl, protein, protein G, streptavidin or biotin).

The method can be performed using different labels such as other enzymes including, but not limited to, glucose oxidase, alkaline phosphatase, glucose dehydrogenase, glucose-6-phosphate dehydrogenase, and acetylcholine esterase. Other labels that can be used include fluorescent molecules/particles (e.g., TRF™ latex beads), absorbance labels (e.g., Goldsol™), and radiolabels. To amplify the signal multiple labels such as poly HRP dextran conjugates, or beads coated in glucose oxidase and anti-NTproBNP antibody, can be used.

The fluid stopping point can be controlled by other suitable mechanisms such as introducing a step change in channel height/depth or using fusable vents. Also, mixing can be performed within the assay device using magnetic, thermal and (ultra)sonic mixing techniques. A blood separator can be introduced to separate the red blood cells and allow only plasma into the device channel.

A buffer pouch incorporated into the test strip can deliver the reaction buffer, and the composition of the buffer can be varied (e.g., sodium acetate, phosphate-citrate, sodium citrate or any other buffer at any suitable concentration or pH). Any suitable liquid can be used instead of a buffer.

The concentrations of the redox mediator and the enzyme substrate can be varied. Other mediators such as TMB (tetramethyl benzene), ferrocene and its derivatives, or Ru(phenylimidazole)(phenanthroline) PF_6, or indophane blue could be used for HRP, and other substrates such as sodium perborate or urea peroxide. Depending on the enzyme labels being used, reaction buffers containing relevant substrate/mediator/solution conditions are used. Other labels, such as fluorescent particles, only require solutions that are compatible with, for example, fluorescent measurement (such as water, buffer, salt solution, oil or other organic or aqueous solvents). When a non-electrochemical detection method is used the magnetic particles do not require to be deposited over an electrode. Other methods of detection include absorbance, fluorescence, surface plasmon resonance, scintillation counting, radiography, and luminescence.

The test strip can be equipped with a longer channel, mitigating the use of the extra 50µL wash. A simple drag of the magnetic particles over the electrode is sufficient to remove interferents.

The magnet used can be located less than or greater than 5mm away from the interface, as long as the magnetic particles are still influenced by the magnetic field. The magnetic particles can be positioned within the blood sample (not at interface) until after the liquid-liquid interface has been formed. Also, the magnetic particles can be dragged through the liquid-liquid interface, after it is formed, by moving the magnet from the first channel

to the second channel, across the liquid-liquid interface.

The magnet can hold the magnetic particles in place over the electrode during the reaction and/or measurement. Also, the magnet can drag the magnetic particles along the base of the channel, or in mid-channel. The magnet can be moved in non-linear directions (e.g., the beads can be moved in any shaped channel, such as linear, circular or spiral by, for example, a rotating magnet) and/or in sweeping movements before dragging the particles to the electrode.

The label used can be allowed to react over the electrode for a longer or shorter period of time. If another label was used (such as fluorescence or absorbance), detection of the signal can be performed without an incubation period.

A three or two electrode system can be used, with either gold or carbon electrodes. The electrodes can be positioned in a pit or depression or side channel in order to allow easy positioning of magnetic particles upon it. When deposited in a pit or depression, the lid of the device can be pushed down enclosing the beads in the pit/depression to reduce reaction volume, increasing relative reaction concentrations. Electrodes can also be positioned on either side of the liquid-liquid interface and can act as fill indicators so that the formation of the interface can be monitored.

The magnetic particles can position the beads on the lid above the electrode, or anywhere in the vicinity of the

electrode. The magnetic particles can be mixed during the reaction to increase access of substrate/mediator to the enzyme.

The geometry of the channel and interface dimensions can be varied to increase mixing of reagents, decrease interfacial mixing, and maximise the signal produced over the electrodes, for example, a narrowing of the channel at the interface reduces diffusion mixing of the two separate fluids in the separate channels.

In the electrochemical assay step, any voltage that reduces oxidised species, or that oxidises reduced species, can be used. For example, other potentials are be used for measurement of other species. When other labels are used, such as fluorescence or absorbance, appropriate optical measurements are made.

Although in the examples given the sample is derived from blood it will be appreciated that the method is suitable for detecting other analytes contained in other mediums. For example the first analyte may be, although is not limited to, a protein, a blood protein, albumin, ischemia modified albumin, a mixture of albumin and ischemia modified albumin, and any other chemical or biological species suitable for analysis and/or detection. In some embodiments the first analyte may comprise ischemia modified albumin.

In the example above the reagent in the first assay is cobalt. The reagent used can be any reagent suitable for interacting with the analyte. For example the reagent may be, although is not limited to, a metal, a divalent

cation, a transition metal, cobalt, and any other reagent that is suitable for interacting with the analyte. In some embodiments the reagent may comprise cobalt.

In the electrochemical examples given the electrochemical analysis may involve a voltammetric sweep (single or multiple) during which the detectable components are quantified by the magnitude of their oxidation and/or reduction currents. In addition, the assay period may involve a preliminary period of electrochemical oxidation or reduction, as described previously. However, it will be appreciated that there are many electrochemical amperometric and voltammetric techniques that can be used in combination with the method of the present invention.

Various embodiments of the device are envisaged, including an assay device comprising more than two assay areas in series, in parallel or a combination of both. Such devices can have a plurality of channels, which can diverge and recombine such that a single sample may run in separate channels. Other embodiments of the device can comprise a corkscrew, spiral or zig-zag channel along which assays can be performed.

In a further embodiment of the device there is provided a central sample application area from which flow channels radiate. The radiating flow channels can have assay detection zones and further sample application zones, as well as other additional features.

All of the devices, test strips and flow channels described can have any the features of the devices, test

strips and flow channels described in more detail previously.

In general, the assay device can be made by depositing reagents on a base and sealing a lid over the base. The base can be a micro-molded platform or a laminate platform.

Micro-molded platform

For an assay device prepared for optical detection, the base, the lid, or both base and lid can be transparent to a desired wavelength of light. Typically both base and lid are transparent to visible wavelengths of light, e.g., 400-700 nm. The base and lid can be transparent to near UV and near IR wavelengths, for example, to provide a range of wavelengths that can be used for detection, such as 200 nm to 1000 nm, or 300 nm to 900 nm.

For an assay device that will use electrochemical detection, electrodes are deposited on a surface of the base. The electrodes can be deposited by screen printing on the base with a carbon or silver ink, followed by an insulation ink; by evaporation or sputtering of a conductive material (such as, for example, gold, silver or aluminum) on the base, followed by laser ablation; or evaporation or sputtering of a conductive material (such as, for example, gold, silver or aluminum) on the base, followed by photolithographic masking and a wet or dry etch.

An electrode can be formed on the lid in one of two ways. A rigid lid can be prepared with one or more through holes, mounted to a vacuum base, and screen-printing used

to deposit carbon or silver ink. Drawing a vacuum on the underside of the rigid lid while screen printing draws the conductive ink into the through holes, creating electrical contact between the topside and underside of the lid, and sealing the hole to ensure that no liquid can leak out.

Alternatively, the lid can be manufactured without any through holes and placed, inverted, on a screen-printing platform, where carbon or silver ink is printed. Once the electrodes have been prepared, the micro-molded bases are loaded and registered to a known location for reagent deposition. Deposition of reagents can be accomplished by dispensing or aspirating from a nozzle, using an electromagnetic valve and servo- or stepper-driven syringe. These methods can deposit droplets or lines of reagents in a contact or non-contact mode. Other methods for depositing reagents include pad printing, screen printing, piezoelectric print head (e.g., ink-jet printing), or depositing from a pouch which is compressed to release reagent (a "cake icer"). Deposition can preferably be performed in a humidity- and temperature-controlled environment. Different reagents can be dispensed at the same or at a different station. Fluorescent or colored additives can optionally be added to the reagents to allow detection of cross contamination or overspill of the reagents outside the desired deposition zone. Product performance can be impaired by cross-contamination. Deposition zones can be in close proximity or a distance apart. The fluorescent or colored additives are selected so as not to interfere with the operation of the assay device, particularly with detection of the analyte.

After deposition, the reagents are dried. Drying can be achieved by ambient air-drying, infrared drying, infrared drying assisted by forced air, ultraviolet light drying, forced warm, controlled relative humidity drying, or a combination of these. Micro-molded bases can then be lidded by bonding a flexible or rigid lid on top. Registration of the base and lid occurs before the two are bonded together. The base and lid can be bonded by heat sealing (using a heat activated adhesive previously applied to lid or base, by ultrasonic welding to join two similar materials, by laser welding (mask or line laser to join two similar materials), by cyanoacrylate adhesive, by epoxy adhesive previously applied to the lid or base, or by a pressure sensitive adhesive previously applied to the lid or base. After lidding, some or all of the assembled assay devices can be inspected for critical dimensions, to ensure that the assay device will perform as designed. Inspection can include visual inspection, laser inspection, contact measurement, or a combination of these.

The assay device can include a buffer pouch. The buffer pouch can be a molded well having a bottom and a top opening. The lower opening can be sealed with a rupturable foil or plastic, and the well filled with buffer. A stronger foil or laminate is then sealed over the top opening. Alternatively, a preformed blister pouch filled with buffer is placed in and bonded in the well. The blister pouch can include 50 to 200 µL of buffer and is formed, filled, and sealed using standard blister methods. The blister material can be foil or

plastic. The blister can be bonded to the well with pressure sensitive adhesive or a cyanoacrylate adhesive.

Laminate platform

Three or more laminates, fed on a roll form at a specified width, can be used to construct an assay device. The base laminate is a plastic material and is coated on one surface with a hydrophilic material. This laminate is fed into a printing station for deposition of conductive electrodes and insulation inks. The base laminate is registered (cross web) and the conductive electrodes deposited on the hydrophilic surface, by the techniques described previously. The base laminate is then fed to a deposition station and one or more reagents applied to the laminate. Registration, both cross web and down web, occurs before reagents are deposited by the methods described above. The reagents are dried following deposition by the methods described above. A middle laminate is fed in roll form at a specified width. There can be more than one middle laminate in an assay device. The term middle serves to indicate that it is not a base laminate or lid laminate. A middle laminate can be a plastic spacer with either a pressure sensitive adhesive or a heat seal adhesive on either face of the laminate. A pressure sensitive adhesive is provided with a protective liner on either side to protect the adhesive. Variations in the thickness of the middle laminate and its adhesives are less than 15%, or less than 10%.

Channels and features are cut into the middle laminate using a laser source (e.g., a CO_2 laser, a YAG laser, an excimer laser, or other). Channels and features can be

cut all the way through the thickness of the middle laminate, or the features and channels can be ablated to a controlled depth from one face of the laminate. The middle and base laminates are registered in both the cross web and down web directions, and bonded together. If a pressure sensitive adhesive is used, the lower liner is removed from the middle laminate and pressure is applied to bond the base to the middle laminate. If a heat seal adhesive is used, the base and middle laminate are bonded using heat and pressure.

The top laminate, which forms the lid of the assay device, is fed in roll form at a specified width. The top laminate can be a plastic material. Features can be cut into the top laminate using a laser source as described above. The top laminate is registered (cross web and down web) to the base and middle laminates, and bonded by pressure lamination or by heat and pressure lamination, depending on the adhesive used. After the laminate is registered in cross and down web directions, discrete assay devices or test strips are cut from the laminate using a high powered laser (such as, for example, a CO_2 laser, a YAG laser, an excimer laser, or other).

Some, or all, of the assembled assay devices can be inspected for critical dimensions, to ensure that the assay device will fit perform as designed. Inspection can include visual inspection, laser inspection, contact measurement, or a combination of these.

An example of one application that employs the use of assays to detect analytes is the analysis of physiological fluid samples, such as blood samples. In particular, it has become increasingly common to analyse blood samples for analytes that may be indicative of

disease or illness. Such analyses can be performed using an assay that directly or indirectly detects an analyte of interest.

The present invention provides a device and method for performing more than one assay on a single small volume blood sample, or other biological materials or complex mixtures. Also, the device and method of the present invention provides allows the detection of at least a second analyte without contamination of assay reagents with non-specific reactions, and physical occlusions of target molecules with cellular debris.

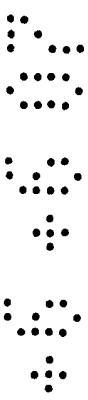
The assay method and device of the present invention can be used in home testing kits for analysing species present in the blood. In particular, as the present invention facilitates the performance of more than one assay on a small sample volume, the assay device and method are suitable for use with home testing kits that utilise the "finger stick" or "finger prick" procedure.

The assay device and method of the present invention is capable of accepting small fluid samples in a simple step, and is able to present small fluid samples for immediate testing in a reliable and reproducible fashion. The present invention provides an efficient way to utilise obtained blood samples in a home testing kit by allowing the performance of a series of tests on the same sample.

Finally, the device and method of the present invention facilitate the execution of more than one assay on the same blood sample by separating and isolating analytes of

interest, within a complex mixture. This enables the visualisation of the analytes by a detection procedure. In particular, the present invention affords the use a specific reagent for visualising a marker related to an analyte of interest and the reliable quantification of its presence to inform on a disease state in a subject. In particular, the present invention allows several analytes, indicative of disease states in a subject, to be detected.

Improvements and modifications may be incorporated herein without deviating from the scope of the invention. Other embodiments are within the scope of the following claims.



CLAIMS

What we claim is:

1. An assay for selectively determining a plurality of characteristics of an aqueous liquid sample containing at least one chemical moiety of interest amongst other sample components, the assay comprising,
providing a lateral flow device for use in performing the assay, the flow device comprising at least one lateral flow channel, a sample collection site, at least one reagent deposit zone proximate to the lateral flow channel and sensor means functionally juxtaposed with respect to the lateral flow channel;
providing particles adapted to exhibit a selective affinity towards a target chemical moiety to be determined in an assay, said particles further being susceptible to manipulation by means of a magnetic field,;
applying a liquid sample to the sample collection site in a sufficient amount to permit flow thereof into the lateral flow channel and said at least one reagent deposit zone, and for a period sufficient to permit adequate interaction of the particles with chemical moiety present in the sample to capture same;
applying a magnetic field in a controlled manner to localise the particles and captured chemical moiety;
transferring the particles and captured chemical moiety by manipulation with the applied magnetic field through a surface of the liquid sample

whereby the particles and captured chemical moiety are separated from other sample components remaining in the liquid sample; and
using the sensor means to detect at least one of the following characteristics selected from the group consisting of an optical characteristic, an electrochemical characteristic, a radiation characteristic and an immunological characteristic.

2. An assay as claimed in claim 1, wherein a further liquid is introduced to the lateral flow device after the sample is applied to the sample collection site and permitted to flow into the lateral flow channel, the further liquid being introduced to the lateral flow channel at a point remote from the sample collection site to permit flow towards the latter such that an interface is formed between the liquid sample and the further liquid at a predictable position in the lateral flow channel.

3. An assay as claimed in claim 1 or claim 2, wherein the step following applying of the sample comprises conducting an electrochemical measurement using sensor means comprising an electrode.

4. An assay as claimed in claim 1 or claim 2, wherein the step of applying of the sample includes a mixing of sample with said particles.

5. An assay as claimed in claim 1 or claim 2, wherein the step of providing said

particles comprises deposition of same at the sample collection site.

6. An assay as claimed in claim 1 or claim 2, wherein the sample is treated with at least one reagent selected from the group consisting of an optically detectable label, an immuno-responsive label, a radioactive label, and conjugates of the aforesaid labels.

7. An assay as claimed in claim 6, wherein the label is selected from the group consisting of enzymes, carrier-hapten conjugates, aptamers, antibodies, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds and bioluminescent compounds.

8. An assay as claimed in claim 6, wherein the label is bound to an insoluble solid support particle.

9. An assay as claimed in claim 8, wherein the insoluble solid support particle is a resin bead.

10. An assay for selectively determining a plurality of characteristics of a liquid sample containing several differing components, the assay comprising the steps of introducing to a liquid sample, a quantity of particles exhibiting a preferential affinity towards a component of the liquid sample, said particles further being susceptible to manipulation by means of a magnetic field; causing the liquid sample to flow in a lateral flow channel to a predetermined point at which a

liquid meniscus is formed;
manipulating the particles by means of an applied magnetic field to localise the particles at the liquid meniscus;
introducing a further liquid by lateral flow up to the liquid meniscus of the sample liquid to form a liquid/liquid interface; and
manipulating the localised particles by means of an applied magnetic field to transfer the localised particles through the liquid/liquid interface.

11. An assay as claimed in claim 10, wherein an electrochemical measurement is carried out on the liquid sample before the particles are introduced to the liquid sample.

12. An assay as claimed in claim 10, wherein an electrochemical measurement is carried out on the liquid sample after the particles are introduced to the liquid sample.

13. An assay as claimed in claim 10, wherein the particles present a surface which is functionalised to interact with a biomolecular species in the liquid sample.

14. An assay as claimed in claim 13, wherein the functionalised surface comprises an antibody or functional binding fragment thereof, capable of binding with the biomolecular species.

15. An assay as claimed in claim 13, wherein the functionalised surface comprises at least one species selected from the group consisting of a protein, an oligopeptide, a peptide, a lipoprotein, a polysaccharide, a sugar

residue, a vitamin, an enzyme, enzyme conjugate, and a ligand.

16. An assay as claimed in claim 15, wherein the functionalised surface comprises a protein selected from the group consisting of a cell-surface associated protein, an immunoglobulin-binding protein, streptavidin and biotin.

17. An assay as claimed in claim 10, wherein the particles present a surface which is functionalised to interact with a chemical moiety in the liquid sample.

18. An assay as claimed in claim 16, wherein the surface is functionalised with a functionality selected from the group consisting of carboxyl, amine, aldehyde, epoxide, N-hydroxy-succinimide, chloromethyl, polyglutaraldehyde, thiol, cyanuric, tosyl, hydrazide, and hydroxide.

19. An assay as claimed in claim 1 or claim 10, wherein the lateral flow channel comprises at least a portion adapted to facilitate capillary flow of an aqueous liquid.

20. An assay for selectively determining a plurality of characteristics of an aqueous liquid sample containing at least one chemical moiety of interest amongst other sample components, the assay comprising,
providing a lateral flow device for use in performing the assay, the flow device comprising at least one capillary flow channel, a sample collection site, at least one reagent deposit zone proximate to the capillary flow channel and an

electrode functionally juxtaposed with respect to the capillary flow channel;
providing particles adapted to exhibit a selective affinity towards a target chemical moiety to be determined in an assay, said particles further being susceptible to manipulation by means of a magnetic field,;
applying a liquid sample to the sample collection site in a sufficient amount to permit flow thereof into the capillary flow channel and said at least one reagent deposit zone, and for a period sufficient to permit adequate interaction of the particles with chemical moiety present in the sample to capture same;
applying a magnetic field in a controlled manner to localise the particles and captured chemical moiety;
transferring the particles and captured chemical moiety by manipulation with the applied magnetic field through a surface of the liquid sample whereby the particles and captured chemical moiety are separated from other sample components remaining in the liquid sample; and
using the electrode to perform an electrochemical analysis step.

21. An assay as claimed in claim 1 or claim 10 or claim 19, wherein a redox mediator is introduced to the sample liquid to facilitate determination of a characteristic of the sample.

22. An assay as claimed in claim 1 or claim 10 or claim 19, wherein the liquid sample is a fresh physiological fluid applied directly to a sample collection site in the lateral flow device,

and the sample collection site is provided with a deposit of reagents which include said particles, and labelling means adapted to selectively discriminate one component of the sample from another.

23. An assay as claimed in claim 22, wherein mixing of the sample and reagents is promoted by applying a magnetic field and manipulating the particles with the applied field to move the particles in the sample.

24. A portable lateral flow assay device for use with liquid samples which may contain an analyte of interest, the device comprising a support configured to provide a shallow liquid flow channel adapted to receive liquid from more than one point, wherein at least a substantial part of said channel is covered, and at least one further part of said channel is adapted to control liquid flow up to at least one intermediate position within the length of said channel; wherein at least one surface accessible to the liquid flow channel has a dry reagent deposited thereon, and wherein said device is provided with sensor means configured upon the device and juxtaposed with respect to said channel such that, in use of the device with a liquid sample, a characteristic of the liquid sample may be sensed.

25. A device as claimed in claim 24, wherein the sensor means includes an electrode positioned at one end of the flow channel.

26. A device as claimed in claim 24 or claim 25, wherein the surface where dry reagent is deposited is remote from the sensor means.

27. A device as claimed in claim 24 or claim 25, wherein the surface where dry reagent is deposited is adjacent the sensor means.

28. An assay device as claimed in claim 24 or claim 25, wherein the channel is adapted to control capillary flow by the presence of at least one air vent to provide a stop at an intermediate position within the length of the channel.

29. An assay device as claimed in claim 24 or claim 25, wherein the channel is adapted to control capillary flow by the presence of a plurality of fusible vents whereby capillary flow may be selectively inhibited or extended.

30. A device as claimed in claim 24 or claim 25, wherein a step change in configuration of the channel is provided to control lateral flow within the channel.

31. A device as claimed in claim 24 or claim 25, wherein the channel has wide and narrow portions, a narrow portion being provided between wide portions.

32. A device as claimed in claim 24 or claim 25, wherein the channel follows a substantially straight linear path throughout.

33. A device as claimed in claim 32, wherein the device is a rectilinear planar device configured such that a sample application site is

provided at a proximal end of the channel and a port is provided at a distal end of the channel.

34. A device as claimed in claim 33, wherein the port is adapted to receive a liquid to be introduced into the channel.

35. A device as claimed in claim 24 or claim 25, wherein the channel follows a path which is configured to provide a plurality of straight sections, the path overall lying within a single plane of the device.

36. A device as claimed in claim 35, wherein the channel is bifurcated.

37. A device as claimed in claim 35, wherein the channel consists of a plurality of successive sections separable by capillary flow control means selected from the group consisting of air vents, and step changes in a dimension of the channel.

38. A device as claimed in claim 35, wherein each section of the channel is adapted to a different analytical step purpose by one or more adaptations selected from the group consisting of, the presence of a selected reagent deposited on a surface in the section, the presence of sensor means, and the presence of ports for admitting or venting fluids.

39. A device as claimed in claim 24 or claim 25, wherein the channel follows a curved linear path.

40. A device as claimed in claim 39, wherein the curved linear path is a helical path.

41. A device as claimed in claim 24 or claim 25, comprising a hydrophobic base part and a hydrophilic cover part configured to define therebetween said flow channel, wherein said sensor means configured upon the device comprises a screen-printed electrode positioned on a surface of the base part at one end of the flow channel, said one end of the flow channel being adapted to serve as a site for application of a sample liquid, said one end of the flow channel also being exposed to a first surface upon which assay reagents are dry-deposited, said reagents comprising the particles, and a label for identifying presence of an analyte of interest in a sample liquid, the flow channel having a second surface upon which assay reagents are dry-deposited, said second surface being laterally spaced from said first surface and wherein said further assay reagents comprising at least a REDOX mediator, and the flow channel further being provided with a port close to said second surface for introduction of a reaction buffer.

42. A device as claimed in claim 41, wherein said second surface is adjacent a further sensor means for sensing a characteristic of the sample after exposure to said further assay reagents.

43. A device as claimed in claim 24 or claim 41, wherein a pouch is provided in fluid communication with the channel for application of a liquid into the channel.

44. A device as claimed in claim 24 or claim 41, wherein the sensor means is positioned within a recess.

45. A device as claimed in claim 44, wherein the sensor means comprises an electrode.

46. A device as claimed in claim 24 or claim 41, wherein at least a portion of the cover over the channel is sufficiently transparent to permit observation of the channel.

47. A device as claimed in claim 46, wherein the channel is covered by an antifog material.

48. An assay for determining the presence in a physiological fluid of biomarkers indicative of a potential cardiovascular dysfunction in a patient, comprising the steps of providing a lateral flow device in which a shallow well is available for receipt of a liquid and in which at least one dry reagent is deposited, said reagent being one capable of interacting with a first biomarker in a predictable way to serve as an aid to detection of the biomarker;
introducing to the well a sample of the physiological fluid, and particles susceptible to manipulation under magnetic influence, wherein said particles have a selective affinity towards a biomarker to the extent that any biomarker present in the sample is liable to become associated with the particles, subsequently
applying a magnetic field to the device to localise the particles in a selected position, and
using sensor means sensitive to the reagent-

biomarker combination to detect presence of biomarker; and further

introducing a liquid to the well to flow fill up to the sample and form a liquid-sample interface; applying a magnetic field to the device to manipulate the particles and transfer the particles from the sample across the liquid-sample interface into the liquid, and conducting a further test for another biomarker in that liquid.

49. An assay as claimed in claim 48, wherein the first biomarker is ischemia modified albumen (IMA), and the first assay step is an electrochemical test using an electrode to indirectly determine IMA.

50. An assay as claimed in claim 48 or claim 49, wherein a further biomarker is NTprohormone-brain natriuretic peptide (NTproBNP), and the further test comprises introducing a reagent to permit formation of a reagent-modified NTproBNP species the presence of which presents a distinctive characteristic which is selected from the group consisting of an optical characteristic, an electromagnetic characteristic, an electrochemical characteristic, a radiation characteristic and an immunological characteristic.

51. An assay as claimed in claim 48 or claim 49, wherein a further biomarker is NTprohormone-brain natriuretic peptide (NTproBNP), and the further test comprises introducing a reagent to permit formation of a reagent-modified NTproBNP species the formation of which suppresses a distinctive characteristic of the reagent which

characteristic is selected from the group consisting of an optical characteristic, an electromagnetic characteristic, an electrochemical characteristic, a radiation characteristic and an immunological characteristic

52. An assay as claimed in claim 48 or claim 49, wherein the modified NTproBNP species is formed using a reagent which comprises a labelled binding partner for NTproBNP.

53. An assay as claimed in claim 48 or claim 49, wherein the modified NTproBNP species is formed using a reagent which is selected from the group consisting of a labelled molecular probe capable of covalently bonding to NTproBNP, a labelled NTproBNP antibody, a labelled binding fragment of an NTproBNP antibody, and an insoluble resin capture bead functionalised to adsorb NTproBNP.

54. A method for conducting a plurality of determinations of characteristics selected from the group consisting of biological, biochemical, chemical and physical characteristics, upon a sample in a liquid form, comprising providing a portable lateral flow device in which at least one shallow covered channel is available for receipt of a liquid, the channel being configured to provide for bidirectional lateral flow of liquid therethrough and having a plurality of reagent treatment zones spaced at intervals in the channel, each such zone having a dry reagent deposited thereon for the purpose of promoting or visualising at least one of the characteristics to be determined, the device further comprising means

for controlling flow of liquid to said zones by selectively inhibiting or extending lateral flow of liquid therein, and sensor means configured upon the device and juxtaposed with respect to said channel such that, in use of the device with a liquid sample, flowing of said liquid to said zones permits a characteristic of the liquid sample to be sensed selectively at more than one of said reagent treatment zones.

55. A microanalysis system comprising a planar device comprising a base part and a cover part which in combination provide walls defining a lateral flow path for a liquid, and at least one of said parts comprises fusible vent means for selectively controlling the flow of liquid within the device by excluding or admitting air to the flow path.

56. A microanalysis system as claimed in claim 55, wherein a reservoir of liquid is associated with the flow path and provided with means operable to control liquid flow in the flow path by transfer of liquid between the reservoir and the flow path.

57. A microanalysis system as claimed in claim 56, wherein the reservoir comprises a compressible surface to effect transfer of liquid.

58. A micro analysis system as claimed in claim 55, wherein at least one dry reagent is deposited upon a surface of at least part of one of the walls defining the lateral flow path to define a reagent treatment zone; and further comprising a magnetic field source juxtaposed with

the device and operable to apply a magnetic field to the device in a localised selected position.

59. A microanalysis system as claimed in claim 55, wherein the sensor means comprises an electrode recessed into a well in the base part and juxtaposed with respect to said channel such that, in use of the device with a liquid, an electrochemical characteristic of the liquid may be determined.

60. An electrochemical lateral flow device comprising a base part and a cover part, at least one of said parts having configured thereon a first electrode set including an electrode adapted to detect an analyte in a liquid, and a counter electrode, the base part and cover part being configured to provide at least one well therebetween and ports for introducing a liquid to the well and for venting liquid therefrom, the well having deposited therein at least one dry reagent and being positioned with respect to the first electrode set such that when liquid is introduced to the well it reaches the electrode set, and dry reagent is taken up into the liquid, such that presence of the analyte in the liquid can be detected, said parts of the device further having formed therebetween a covered channel having a proximal end opening at the well whereby said channel is adapted to be filled with a liquid by lateral flow, and at least one of said parts has a further electrode set spaced from the first and positioned at a distal end of the channel for the purposes of conducting a further electrochemical test.

61. A method of determining the presence of analytes in a liquid medium which contains at least one analyte of interest (AOI), the method comprising the steps of

providing magnetic particles adapted to capture said at least one AOI to form a detectable capture particle species,

introducing the liquid medium including said at least one AOI, with said magnetic particles to a capillary and allowing the capillary to flow fill to a predetermined lateral flow limit point,

applying a magnetic field to the capillary to gradually localise the magnetic particles at a selected point within the capillary, thereby isolating said detectable capture particle species at the selected point, and

conducting an analytical test on the capture particle species at the selected point.

62. A method of separating analyte(s) from a liquid medium which contains at least one analyte of interest (AOI), the method comprising the steps of

providing magnetic particles adapted to capture said at least one AOI to form a detectable capture particle species,

introducing the liquid medium including said at least one AOI, with said magnetic particles to a capillary and allowing the capillary to flow fill to a predetermined lateral flow limit point,

applying a magnetic field to the capillary to gradually localise the magnetic particles and

detectable capture particle species at a selected point proximate to said lateral flow limit point,

introducing a second liquid to said capillary to form a liquid-liquid interface at said lateral flow limit point,

applying a magnetic field to the capillary to transfer the localised magnetic particles and detectable capture particle species through the liquid-liquid interface into the second liquid.

63. A test device for conducting a plurality of determinations of characteristics selected from the group consisting of biological, biochemical, chemical and physical characteristics, upon a liquid sample, said device comprising a generally planar base part and a corresponding cover part superposed upon the base part, and configured to define at least one shallow well therebetween for receiving a liquid sample at a first zone, said well being dimensioned to facilitate lateral flow of liquid between said first zone and a plurality of discrete distal zones spaced apart from each other,

wherein at least some of said discrete distal zones each have a dry reagent deposited therein, and sensor means configured upon the device and juxtaposed with respect to said distal zones such that, in use of the device with a liquid sample, a characteristic of the liquid sample may be sensed selectively at more than one of said distal zones.

64. A disposable single use test device for detecting an analyte of interest in a candidate liquid sample, said device comprising a

planar substrate having a sample deposition zone defined at a first location, a reagent dry-deposited proximate to said first location, said reagent containing releasable magnetic particles adapted to capture the analyte of interest when contacted by the candidate liquid sample, a liquid impermeable membrane positionable so as to overly the planar substrate to form a lateral flow region for liquid, and a detection zone remote from the sample deposition zone and juxtaposed with an edge of said membrane to receive in use liquid flowed from the lateral flow region which may contain captured analyte of interest for detection thereof.

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Application No:	GB0606263.2	**Examiner:**	Dr Jonathan Corden
Claims searched:	62	**Date of search:**	18 December 2006

Patents Act 1977
Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	WO 99/26067 A1 (BIO-RAD LABORATORIES) see whole document

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
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&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application

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Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

G1B

Worldwide search of patent documents classified in the following areas of the IPC

G01N

The following online and other databases have been used in the preparation of this search report

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Application No:	GB0606263.2	**Examiner:**	Dr Jonathan Corden
Claims searched:	48-53	**Date of search:**	18 December 2006

Patents Act 1977
Further Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	WO 2005/001475 A2 (VOORHEES et al) see whole document
A	-	WO 99/26067 A1 (BIO-RAD LABORATORIES) see whole document

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Application No:	GB0606263.2	**Examiner:**	Dr Jonathan Corden
Claims searched:	1-9, 19-23	**Date of search:**	27 July 2006

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 4, 6, 7, 19, 20, 23	WO 2003/102546 A2 (UNIV. OF CALIFORNIA) see figures, abstract and whole document
X	1, 4, 6, 7, 19, 20, 23	WO 92/14138 A1 (IGEN INC) see abstract, figures and whole document
X	1, 4, 6, 7, 19, 20, 23	WO 2004/011942 A1 (QUANTUM DESIGN) see abstract, figures and whole document
X	1, 4, 6, 7, 19	US 5145784 A (CAMBRIDGE BIOTECH) see abstract, figures and whole document
X	1, 4, 6, 7, 19	US 2004/0043507 A1 (KIMBERLEY-CLARK) see abstract, figures and whole document
X	1, 4, 6, 7, 19	US 2004/0053423 A1 (QUANTUM DESIGN) see abstract, figures and whole document
X	1, 4, 6, 7	US 2002/0094548 A1 (WAVESENSE) see abstract, figures and whole document

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application

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