ASSAY

FIELD

The present invention relates to an assay apparatus, assay method, assay card and an assay card controller.

BACKGROUND

Assays are known. Such assays typically perform a diagnostic test to provide an indication of whether a particular target agent is present in a test sample. The complexity and functionality of these diagnostic devices varies considerably. For example, highly sophisticated diagnostic devices are typically provided in laboratories or hospitals for high-throughput diagnostic testing but these are typically only used in such environments because of their relatively high maintenance cost and complicated operation procedure.

There are less complicated devices also provided, in a laboratory or in a physician's clinic, which receive typically a blood or urine sample taken from the patient by a trained healthcare professional. The rest of the procedure is carried out automatically and the result of the diagnostic test is typically displayed on a liquid crystal display (LCD) screen or on paper using a printer. Other devices have dried or immobilised or lyophilized reagent inside. When a liquid test sample is mixed, the reagent is rehydrated. In some arrangements, a number of reagents are provided in the reaction chamber or bottle. Typically, each of these reagents is individually provided into the reaction chamber so that the reagents are introduced in the correct order.

For example, a reagent may be required to pretreat any sensor reagent to active it from the storage condition, while other reagents may be required to bind to the test sample to make a detectable signal by, for example, optical or electrochemical means. After the reaction, washing reagents may be provided to wash out the unbound reagents. Also, some substrate reagents may be provided to convert or amplify any signal further. These devices are sometimes called point-of-care (POC) devices since they provide a handy means due to their small size, automatic sample and reagent handling, rapid result and low maintenance cost. Many have replaceable cartridges that contain all disposable parts like reagents, sensor and sampling channel. Changing the cartridge makes the maintenance simple and easy.

Other POC devices exist such as the cellulose membrane based rapid lateral flow kit, often referred to as a 'rapid test kit'. The rapid test kit has been so successful that it has become a de facto standard in the POC market since it has most of the desirable features of a more complex POC device such as low cost, disposability, quick response and simplicity.

Other devices exist for providing an indication of whether a particular target agent, such as a chemical or biological agent, is present in a test sample such as a water sample, a food sample, or a chemical or biological sample.

Although each of these diagnostic devices provides many benefits, they each have their own shortcomings. Accordingly, it is desired to provide an improved diagnostic device or assay.

SUMMARY

According to a first aspect, there is provided an assay apparatus, comprising: an assay card comprising a substrate having at least one compressible reagent reservoir thereon, each compressible reagent reservoir containing an associated reagent and each compressible reagent reservoir being coupled with a reaction chamber operable to receive a test sample; and an assay card controller comprising a compression mechanism operable to compress each of the compressible reagent reservoirs to cause each associated reagent to be injected in a predetermined order into the reaction chamber to react with the test sample.

The first aspect recognises that a problem with rapid test kits is that although they are very simple, they lack the ability to perform simple reagent delivery which makes it difficult to provide a simple device which is sensitive and reproducible. The first aspect also recognises that although other POC devices enable reagent delivery, a problem with these devices is that they are complicated which makes them unnecessarily expensive, difficult to manufacture and may affect their reliability. Similarly, the first aspect recognises that although the highly sophisticated devices provided in laboratories enable reagent delivery, they are highly expensive, have high maintenance costs, and have complex operating procedures which requires highly skilled operators.

Accordingly, an assay apparatus is provided comprising an assay card and assay card controller. The assay card may comprise a substrate and may have a compressible, squeezable or squashable reagent reservoir. The reagent reservoir may contain an associated reagent and may be coupled with a reaction or test chamber into which a test sample may be placed. The

assay card controller may comprise a compression mechanism which may compress the reagent reservoir to cause the reagent contained therein to be displaced therefrom and provided to the reaction chamber to react with the test sample. It will be appreciated that such an arrangement provides compression of the reagent reservoir to enable injection of reagent liquid into the reaction chamber in a pre-programmed manner using a very simple and reliable squeezing mechanism. This simple arrangement enables reagent reactions to occur which can be carefully controlled using a simple, low cost and reliable device which is easy to operate with a low level of skill and provides a sensitive and reproducible assay.

In one embodiment, each of a plurality of compressible reagent reservoirs is located at a predetermined location along a predetermined actuation path along the assay card to be followed by the compression mechanism. The assay card may comprise a substrate and may have a number of compressible, squeezable or squashable reagent reservoirs. Each of the reagent reservoirs may contain an associated reagent and each reservoir may be coupled with a reaction or test chamber into which a test sample may be placed. The assay card controller may comprise a compression mechanism which may compress each of the reagent reservoirs to cause the reagents contained therein to be displaced therefrom and provided to the reaction chamber in a predetermined order to react with the test sample. For example, such ordering may enable one reagent to be provided prior to another, one reagent to be provided whilst another is already being provided or more than one reagent to be provided substantially simultaneously. It will be appreciated that such an arrangement provides a sequential compression of the reagent reservoirs to enable sequential injection of reagent liquids into the reaction chamber in a pre-programmed manner using a very simple and reliable squeezing mechanism. The location of each reservoir on the assay card may be carefully determined in order to ensure that each reagent is delivered in the correct order and at the correct time. The location of the each reservoir may be determined based on the knowledge of the location of the actuation path to be followed by the compression mechanism. Of course, the location of each reservoir may be dependent on the particular arrangement of the compression mechanism and the path which that compression mechanism may follow. This may provide for a simple, convenient and reliable arrangement which supports potentially complicated, multi-staged and time-critical reactions.

In one embodiment, the actuation path is linear. Accordingly, for a very simple compression mechanism which follows a linear actuation path, the each reservoir may be

placed at an appropriate location at different points along the length of this linear actuation path.

In one embodiment, the actuation path extends along an axis of the assay card. Accordingly, should the compression mechanism be arranged to travel along a path which is generally aligned with one of the axes of the assay card, then each reservoir may be placed at a predetermined location along that axis of the assay card to ensure that it is actuated in the correct sequence and at the correct time. Also, any edges of the assay card which are aligned with that axis may be utilised to help guide the assay card when moving relative to the compression mechanism.

In one embodiment, the reaction chamber is located along the actuation path and those of a plurality of compressible reagent reservoirs located furthest away from the reaction chamber along the actuation path are compressible to enable injection of associated reagents prior to those of the plurality of compressible reagent reservoirs located closest to the reaction chamber. Accordingly, the reaction chamber may typically be located furthest away from that reagent reservoir which is intended to be the first which is activated by the compression mechanism, with the remaining reservoirs being actuated in the order which they lie along the actuation path between the first reagent reservoir and the reaction chamber.

In one embodiment, at least two of reagent reservoirs are located at the same predetermined location along the actuation path to enable simultaneous injection of associated reagents into the reaction chamber. Accordingly, more than one separate reagent reservoirs may be provided at the same distance along the actuation path in order to ensure that they are substantially simultaneously compressed to release their respective associated reagents. This helps to support increasingly complex reactions where multiple reagents may be required which cannot be stored in the same reservoir.

In one embodiment, each reservoir chamber comprises a reagent sack operable to receive the associated reagent. The reagent sack or balloon may be composed of a thin plastic bag filled with reagent liquid. The sack may be ruptured by the pressure derived from the roller movement. The ruptured sack may then release the reagent liquid into the reservoir. The sack may also play an important role in protecting the reagent liquid from degradation due to the presence of humidity, oxygen and/or light.

In one embodiment, the reagent sack comprises a stamped metal foil protrusion having a sealed polymer backing.

In one embodiment, the assay card comprises: a waste chamber operable to receive excess from the reaction chamber. Accordingly, a waste chamber may be provided which may receive excess fluid displaced from the reaction chamber. Such excess fluid may typically occur as a result of the actuation of each reservoir. The size of the waste chamber may readily be calculated based on the size of the reagent reservoirs and the reaction chamber to ensure that no fluids are inadvertently emitted by the assay card during operation.

In one embodiment, the waste chamber comprises: material operable to retain the excess. Accordingly, material may be provided to retain excess fluid within the waste chamber and reduce the likelihood of these reagents inadvertently re-entering the reaction chamber. Also, the provision of the material helps to retain the excess in the event of the rupture of the assay card to prevent spillage.

In one embodiment, the assay card comprises: an inlet port coupled with the reaction chamber and operable to receive the test sample. Accordingly, the inlet port may be arranged to receive the test sample to be conveyed to the reaction chamber.

In one embodiment, the inlet port is sealable. By sealing the inlet port, the test sample may be retained within the assay card and the inadvertent release of fluid during processing of the assay card may be prevented.

In one embodiment, the sealable inlet port comprises an adhesive operable to seal the inlet port when compressed. Accordingly, the inlet port may be sealed through simple compression.

In one embodiment, the assay card comprises a vacuum device operable to generate a negative pressure at the inlet port to assist receiving the test sample. Hence, any fluid presented to the inlet port may be drawn into the assay card through negative pressure generated by the vacuum device.

In one embodiment, the vacuum device comprises a resiliently compressible reservoir in fluid communication with the inlet port. Hence, the vacuum device may comprise a reservoir or chamber which may be expanded and contracted to change its volume to generate the required negative pressure to draw in and retain the fluid.

In one embodiment, the resiliently compressible reservoir comprises a reforming device operable, following compression of the resiliently compressible chamber, to assist decompression of the resiliently compressible reservoir. Accordingly, a structure may be provided within the chamber to help re-expand the chamber towards its original configuration.

In one embodiment, the reforming device comprises a sponge operable to receive the test sample. Hence, the reforming device may be a sponge which helps to re-inflate the chamber and retain the sample therein.

In one embodiment, the sponge comprises at least one reagent retained therein. Accordingly, the sample may be pre-treated with a reagent prior to being provided to the reaction chamber. Such a pre-treatment reagent may comprise, for example, an anti-coagulant when testing blood samples.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber. Hence, the test sample may be provided to the reaction chamber from the reservoir. This may occur following sealing of the inlet, upon further compression of the resiliently compressible reservoir.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber via at least one reagent retained within a substrate. Accordingly, the test sample may be passed through or over one or more reagents retained within a substrate when being provided to the reaction chamber. Such reagents may comprise a control reagent.

In one embodiment, the assay card comprises a flow preventer operable to selectively fluidly decouple the reaction chamber from at least one of the compressible reagent reservoirs and the resiliently compressible reservoir. Accordingly, a flow preventer may be provided to de-couple the inlet and resiliently compressible reservoir from the rest of the assay card during, for example, initial loading of the test sample. This helps to create the required pressure differential to facilitate loading of the test sample and/or to prevent any uncontrolled mixing with other reagents within the card.

In one embodiment, the flow preventer is removable to fluidly couple the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir. The flow preventer may be removed once, for example, the inlet port

has been sealed to enable the sample to pass to other parts of the assay card on compression of the resiliently compressible reservoir.

In one embodiment, the flow preventer is operable to compress at least one microfluidic channel coupling the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir. Compressing the microfluidic channels may effectively block those channels.

In one embodiment, the assay card controller is operable to prevent actuation of the compression mechanism on detection of the flow preventer. Accordingly, the processing of the assay card by the assay card controller may be prevented upon detection of the flow preventer in order to prevent damage to the assay card.

In one embodiment, the assay card comprises: at least one microfluidic channel operable to couple the inlet port with the reaction chamber. The provision of the microfluidic channel may conveniently enable the test sample to be conveyed to the reaction chamber under, for example, capillary action.

In one embodiment, the assay card comprises: a plurality of microfluidic channels operable to couple at each of the plurality of compressible reagent reservoirs with the reaction chamber. The provision of microfluidic channels between each of the reservoirs and the reaction chamber may help to minimise any inadvertent premixing or reagents prior to the assay card being used. Also, the use of microfluidic channels may help to minimise the amount of reagent which needs to be provided since very little volume is wasted in these channels and may maximise the rate at which any reagent is injected into the reaction chamber.

In one embodiment, the microfluidic channels are selectively sealable. Hence, the microfluidic channels may be sealed. Such sealing may occur as a result of compression by a user or operator, or as a result of compression performed by the assay card controller itself. It will be appreciated that such sealing can assist in preventing back flow to areas of the card which have been emptied such as, for example, emptied reservoirs.

In one embodiment, the microfluidic channels comprise adhesive on sealable portions. Accordingly, adhesive portions may be provided between sheets forming the microfluidic channels. When the microfluidic channel is then compressed, opposing sides of the channel

may be retained in a compressed state by the adhesive, thereby sealing the microfluidic channel.

In one embodiment, the reaction chamber comprises: an indicator operable to provide an indication of a presence of a target agent within the test sample. Accordingly, the reaction chamber may be provided with an indicator providing any indication of the presence of a particular agent within the test sample. Such an indicator will typically be designed to be compatible with any detector to be utilised.

In one embodiment, the indicator comprises: a window through which the presence of the target agent may be detected.

In one embodiment, the window is transparent to enable optical detection of the presence of the target agent.

In one embodiment, the indicator comprises: an electrochemical detector operable to electrochemically detect the presence of the target agent, the electrochemical detector being coupled with a metallic coupling formed on the assay card to provide an electrical connection between the electrochemical detector and the assay card controller. Hence, an appropriate electrochemical detector may be provided on the assay card and coupled with the assay card controller.

In one embodiment, the reaction chamber comprises: a predetermined reagent. Accordingly, the reaction chamber may be preconfigured to contain a particular reagent to be utilised to detect the presence of the target agent in the test sample. This ensures the reagent is already present within the reaction chamber and reduces the need to provide a separate reservoir containing that reagent.

In one embodiment, the predetermined reagent is provided on substrate within the reaction chamber. Accordingly, the reagent may be provided within a substrate within the reaction chamber in order to retain the reagent within the reaction chamber during the injection of other reagents during the processing of the assay card.

In one embodiment, the assay card is extruded and thermoformed with the aid of a vacuum. Accordingly, the assay card may be manufactured simply using extrusion techniques. This arrangement provides an assay card having no moving parts as such which reduces its complexity and improves reliability.

In one embodiment, the assay card comprises: a plurality of sheets arranged to form a laminate, at least one of the sheets being a thermoplastic having the plurality of compressible reagent reservoirs, the reaction chamber and the microfluidic channels formed thereon. Hence, a number of sheets may be provided which together form the assay card. One of the sheets may be a thermoplastic having the shape of a portion of the reservoirs of the microfluidic channels formed therein. Typically, the complete reservoirs and microfluidic channels may then be formed by attaching a further sheet to the extruded thermoplastic sheet.

In one embodiment, the assay card comprises a heating element provided on one of the sheets operable to heat the assay card. Accordingly, the assay card may comprise at least one heating element provided on at least one of the sheets. The heating element may be used to heat the assay card or portions thereof. In this way, it will be appreciated that the speed of reactions may be carefully controlled. In particular, the speed of reactions may be increased.

In one embodiment, the heating element comprises an electrical heating element and the assay apparatus comprises electrical contacts operable to power the heating element. Accordingly, the assay card may be heated by electrical heating. The heating element may be arranged on the assay card such that the heating element only couples with the electrical contacts at a predetermined time in order to perform heating only when required. Alternatively, the heating element may be in contact with the electrical connectors and power only provided to the electrical connectors at predetermined times which may be determined from control signals generated by indexing detectors which detect the location of the assay card within the assay apparatus.

In one embodiment, the assay apparatus comprises a thermometer operable to control the heating element achieve a predetermined assay card temperature. Accordingly, a thermometer may be provided which detects whether the assay card has reached a particular temperature. Power may then be removed from the heating element when a predetermined temperature has been reached. The thermometer may only monitor portions of the assay card such as the reaction chamber temperature rather than the whole assay card temperature. Furthermore, a detector used in detecting reactions in the reaction chamber may also be used as a thermometer. Conversely, the thermometer may be provided on the assay card.

In one embodiment, the assay card comprises: an indicator operable to provide an indication of at least one of position information of reservoirs and the detection zone, and timing information detailing amounts of time to wait between compression of the reservoirs.

In one embodiment, the assay card comprises: a fluid absorber provided at a junction of a plurality microfluidic channels coupled with a plurality of reservoirs, the fluid absorber having a fluid absorption rate which is higher than a rate at which fluid is supplied from the plurality of reservoirs.

In one embodiment, the compression mechanism comprises: a roller operable to move relative to the assay card, along the actuation path. A roller provides a particularly convenient, reliable and simple mechanism for compressing the reservoirs. The intended direction of travel of the roller may define the actuation path. It will be appreciate that embodiments may be provided in which the roller moves, with the assay card remaining static, or where the roller remains static and the assay card is moved, or a combination of both. As the roller moves relative to the assay card, the reservoirs it travels over may be compressed and the contents therein ejected.

In one embodiment, the compression mechanism comprises: a pair of rollers operable to receive the assay card therebetween and operable to move relative to the assay card, along the actuation path. By providing a pair of rollers, it may be possible to grip the assay card between each roller to improve the controllability and reliability of the movement of the assay card relative to the rollers and to control the force applied during compression of the reservoirs.

In one embodiment, the compression mechanism comprising: a secondary roller operable to move relative to the roller to compress predetermined areas of the assay card. Accordingly, rather than providing a single roller, a secondary roller may also be provided. The two rollers may both then be used to selectively compress reservoirs and microfluidic channels in order to decouple reservoirs from each other during the processing of the assay card in order to prevent undesirable premixing and back flow of fluid around the card. It will be appreciated that the exact arrangement of the rollers will depend on the spatial arrangement of the reservoirs and the microfluidic channels on the assay card. The rollers may have identical lengths or different lengths and may compress mutually exclusive or overlapping

regions of the assay card. Furthermore, each roller may comprise one or more rolling portions having one or more apertures therebetween which fail to contact with the assay card.

In one embodiment, the secondary roller comprises a pair of rollers.

In one embodiment, the assay card comprises microfluidic channels routed to the predetermined areas to enable selective compression of the microfluidic channels. Hence, the microfluidic channels may be arranged to extend into areas which intersect with the path of the rollers to enable selective sealing of those microfluidic channels.

In one embodiment, the compression mechanism comprises: gears operable to couple the pair of rollers. Accordingly, gears may be provided between the rollers to ensure that they are simultaneously activated and to ensure a fixed relationship between the rotation of the two rollers. For example, the gears may provide a unitary gear ratio to ensure that the two rollers operate at the same speed of rotation to avoid any slippage on the assay card.

In one embodiment, the assay card controller comprises: a controller operable to vary speed of movement the compression mechanism along the actuation path. Accordingly, the speed of the relative motion of the compression mechanism and assay card may be varied to suit the reaction times of the reagents. It will be appreciated that this enables, for example, reservoirs to be located at fixed positions and the speed of the compression mechanism varied which provides for a more compact assay card than would be possible if the compression mechanism was moved at a constant speed at the locations of the reservoirs needed to be varied to ensure the correct amount of time between delivery of the different reagents.

In one embodiment, the speed controller is operable to change direction of movement of the compression mechanism the along the actuation path. Accordingly, the direction of movement may be changed in order that, for example, only an initial portion of a reagent is delivered due to a change in direction of the movement during such delivery which causes some of the reagent to remain in the reservoir. Similarly, a change in direction may occur after delivery of a reagent to extend the time until another reagent is delivered. Likewise, such change in direction may enable the initial compression to occur at some point along the card and then enable movement of the compression mechanism away from that starting position towards two different edges of the assay card.

In one embodiment, the assay apparatus comprises an index detector operable to detect an indexing mechanism provided on the assay card to produce at least one control signal to be provided to the controller. Accordingly, an indexing mechanism may be provided on the assay card which, when detected by an index detector, may generate a control signal. More than one indexing mechanism may be provided at different locations on the assay card to provide control signals at different times during processing of the card.

In one embodiment, the index detector is operable to detect a plurality of indexing mechanisms provided on the assay card to produce a plurality of control signals to be provided to the controller. Accordingly, each indexing mechanism may generate one a plurality of different control signals. The indexing mechanism may provide different control signals at different times to cause the assay apparatus to perform different tasks. Accordingly, different functions may be performed at different times dependant on the control signals generated. For example, a control signal may be generated when the card has reached a certain point, such as immediately following release of a pre-reagent. Such a control signal may cause the compression mechanism to pause for a particular time whilst the reaction takes place. Alternatively and/or additionally, the control signal may cause the assay apparatus to vibrate the assay card, to irradiate the assay card and/or to heat the assay card. It will be appreciated that many different indexing mechanisms may be provided which may either themselves encode an indication of the control signal or may select one of a plurality of pre-programmed control signals. It will be appreciated that such indexing may occur through a variety of techniques, such as, for example, mechanical, electrical or optical mechanisms.

In one embodiment, the assay card controller comprises: a detector operable to detect the presence of the target agent. Such a detector may be arranged to detect a predetermined characteristic of the reaction with the test sample.

In one embodiment, the detector comprises: an optical detector operable to optically detect the presence of the target agent.

In one embodiment, the detector comprises: an amplifier operable amplify a signal provided from the electrochemical detector.

In one embodiment, the assay card controller comprises: an indicator operable to indicate the presence of the test agent in response to an indication provided by the detector. Accordingly, the indicator may indicate whether the detector sufficiently indicates the

presence of the target agent in the test sample. Such an indication may be based on a simple threshold amount to provide either a positive or negative result or may provide a quantitative indication of the amount or concentration of the target agent in the test sample.

According to a second aspect, there is provided an assay method, comprising the steps of: providing an assay card comprising a substrate having at least one compressible reagent reservoir, each compressible reagent reservoir containing an associated reagent and each compressible reagent reservoir being coupled with a reaction chamber operable to receive a test sample; and compressing each compressible reagent reservoir to cause each associated reagent to be injected in a predetermined order into the reaction chamber to react with the test sample.

In one embodiment, each of a plurality of compressible reagent reservoirs is located at predetermined locations along a predetermined actuation path to be compressed along the assay card.

In one embodiment, the actuation path is linear.

In one embodiment, the actuation path extends along an axis of the assay card.

In one embodiment, the reaction chamber is located along the actuation path and the step of compressing comprises: compressing those of the plurality of compressible reagent reservoirs located furthest away from the reaction chamber along the actuation path to enable injection of associated reagents prior to those of the plurality of compressible reagent reservoirs located closest to the reaction chamber.

In one embodiment, at least two compressible reagent reservoirs are located at the same predetermined location along the actuation path and the step of compressing comprises: simultaneously compressing the at least two of the plurality of compressible reagent reservoirs to simultaneously inject associated reagents into the reaction chamber.

In one embodiment, the method comprises the step of: receiving the associated reagent in a reagent sack in each reservoir chamber.

In one embodiment, the method comprises the step of: receiving excess from the reaction chamber in a waste chamber.

In one embodiment, the method comprises the step of: retaining excess within material provided within the waste chamber.

In one embodiment, the method comprises the step of: receiving the test sample at an inlet port of the assay card.

In one embodiment, the method comprises the step of: sealing the inlet port.

In one embodiment, the step of sealing comprises sealing the inlet port with an adhesive when compressed.

In one embodiment, the method comprises the step of: generating a negative pressure at the inlet port to assist receiving the test sample.

In one embodiment, the step of generating a negative pressure comprises compressing a resiliently compressible reservoir in fluid communication with the inlet port to generate the negative pressure.

In one embodiment, the step of generating a negative pressure comprises compressing the resiliently compressible reservoir comprising a reforming device operable, following compression of the resiliently compressible chamber, to assist decompression of the resiliently compressible reservoir.

In one embodiment, the method comprises the step of: receiving the test in a sponge provided as the reforming device.

In one embodiment, the sponge comprises at least one reagent retained therein.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber via at least one reagent retained within a substrate.

In one embodiment, the method comprises the step of: selectively fluidly decoupling the reaction chamber from at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the method comprises the step of: fluidly coupling the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the method comprises the step of: compressing microfluidic channels coupling the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the method comprises the step of: preventing actuation of the compression mechanism on detection of a flow preventer.

In one embodiment, the assay card comprises at least one microfluidic channel operable to couple the inlet port with the reaction chamber.

In one embodiment, the assay card comprises a plurality of microfluidic channels operable to couple at each of the plurality of compressible reagent reservoirs with the reaction chamber.

In one embodiment, the method comprises the step of: selectively sealing at least one microfluidic channel.

In one embodiment, the microfluidic channels comprise adhesive sealable portions.

In one embodiment, the reaction chamber comprises an indicator and the method comprises the step of: providing an indication of a presence of a target agent within the test sample.

In one embodiment, the indicator comprises a window and the step of providing an indication comprises: detecting the presence of the target agent through the window.

In one embodiment, the window is transparent and the step of detecting comprises: optically detecting the presence of the target agent.

In one embodiment, the indicator comprises an electrochemical detector operable to electrochemically detect the presence of the target agent, the electrochemical detector being coupled with a metallic coupling formed on the assay card to provide an electrical connection between the electrochemical detector and the assay card controller.

In one embodiment, the reaction chamber comprises a predetermined reagent.

In one embodiment, the predetermined reagent is provided on substrate within the reaction chamber.

In one embodiment, the assay card is extruded and thermoformed with the aid of a vacuum.

In one embodiment, the assay card comprises a plurality of sheets arranged to form a laminate, at least one of the sheets being a thermoplastic having the plurality of compressible reagent reservoirs, the reaction chamber and the microfluidic channels formed thereon.

In one embodiment, the assay card comprises a heating element provided on one of the sheets operable to heat the assay card.

In one embodiment, the heating element comprises an electrical heating element and the method comprises the step of powering the heating element.

In one embodiment, the method comprises the step of controlling the heating element achieve a predetermined assay card temperature.

In one embodiment, the step of compressing comprises: moving a roller relative to the assay card, along the actuation path.

In one embodiment, the step of compressing comprises: moving a pair of rollers operable to receive the assay card therebetween relative to the assay card, along the actuation path.

In one embodiment, the step of compressing comprises: moving a secondary roller relative to the roller to compress predetermined areas of the assay card.

In one embodiment, the assay card comprises microfluidic channels routed to the predetermined areas to enable selective compression of the microfluidic channels.

In one embodiment, the pair of rollers are coupled by gears.

In one embodiment, the step of compressing comprises: varying a speed of the compressing each of the plurality of compressible reagent reservoirs to cause each associated reagent to be injected into the reaction chamber to react with the test sample with predetermined timings.

In one embodiment, the method comprises the step of detecting an indexing mechanism provided on the assay card to produce at least one control signal.

In one embodiment, the method comprises the step of detecting a plurality of indexing mechanisms provided on the assay card to produce a plurality of control signals.

In one embodiment, the method comprises the step of: detecting the presence of the target agent.

In one embodiment, the step of detecting comprises: optically detecting the presence of the target agent.

In one embodiment, the step of detecting comprises: amplifying a signal provided from the electrochemical detector to electrochemically detect the presence of the target agent.

In one embodiment, the method comprises the step of: indicating the presence of the test agent in response to an indication provided by the step of detecting.

According to a third aspect, there is provided an assay card, comprising: a substrate having at least one compressible reagent reservoir, each compressible reagent reservoir containing an associated reagent and each compressible reagent reservoir being coupled with a reaction chamber operable to receive a test sample.

In one embodiment, each of a plurality of compressible reagent reservoirs is located at predetermined locations along a predetermined actuation path along the assay card.

In one embodiment, the actuation path is linear.

In one embodiment, the actuation path extends along an axis of the assay card.

In one embodiment, the reaction chamber is located along the actuation path and those of a plurality of compressible reagent reservoirs located furthest away from the reaction chamber along the actuation path are compressible to enable injection of associated reagents prior to those of the plurality of compressible reagent reservoirs located closest to the reaction chamber.

In one embodiment, at least two compressible reagent reservoirs are located at the same predetermined location along the actuation path to enable simultaneous injection of associated reagents into the reaction chamber.

In one embodiment, each reservoir chamber comprises a reagent sack which operable to receive the associated reagent.

In one embodiment, the method comprises the step of forming the reagent sack by stamping a metal foil to form a protrusion, placing the reagent within the protrusion and sealing a polymer backing thereon.

In one embodiment, the step of sealing comprises applying a pressure using a conductive coil to the metal foil and polymer backing and applying a high frequency rotating field to the conductive coil.

In one embodiment, the assay card comprises: a waste chamber operable to receive excess from the reaction chamber.

In one embodiment, the waste chamber comprises: material operable to retain the excess.

In one embodiment, the assay card comprises: a sealable inlet port coupled with the reaction chamber and operable to receive the test sample.

In one embodiment, the inlet port is sealable.

In one embodiment, the sealable inlet port comprises an adhesive operable to seal the inlet port when compressed.

In one embodiment, the assay card comprises a vacuum device operable to generate a negative pressure at the inlet port to assist receiving the test sample.

In one embodiment, the vacuum device comprises a resiliently compressible reservoir in fluid communication with the inlet port.

In one embodiment, the resiliently compressible reservoir comprises a reforming device operable, following compression of the resiliently compressible chamber, to assist decompression of the resiliently compressible reservoir.

In one embodiment, the reforming device comprises a sponge operable to receive the test sample.

In one embodiment, the sponge comprises at least one reagent retained therein.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber.

In one embodiment, the resiliently compressible reservoir is in fluid communication with the reaction chamber via at least one reagent retained within a substrate.

In one embodiment, the assay card comprises a flow preventer operable to selectively fluidly decouple the reaction chamber from at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the flow preventer is removable to fluidly couple the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the flow preventer is operable to compress microfluidic channels coupling the reaction chamber with the at least one of the compressible reagent reservoirs and the resiliently compressible reservoir.

In one embodiment, the assay card comprises: at least one microfluidic channel operable to couple the inlet port with the reaction chamber.

In one embodiment, the assay card comprises: a plurality of microfluidic channels operable to couple at each of the plurality of compressible reagent reservoirs with the reaction chamber.

In one embodiment, the microfluidic channels are selectively sealable.

In one embodiment, the microfluidic channels comprise adhesive on sealable portions.

In one embodiment, the reaction chamber comprises: an indicator operable to provide an indication of a presence of a target agent within the test sample.

In one embodiment, the indicator comprises: a window through which the presence of the target agent may be detected.

In one embodiment, the window is transparent to enable optical detection of the presence of the target agent.

In one embodiment, the reaction chamber comprises: a predetermined reagent.

In one embodiment, the predetermined reagent is provided on substrate within the reaction chamber.

In one embodiment, the assay card comprises: an electrochemical detector operable to electrochemically detect the presence of the target agent, the electrochemical detector being coupled with a metallic coupling to provide an electrical connection between the electrochemical detector and the assay card controller.

In one embodiment, the assay card is extruded and thermoformed with the aid of a vacuum.

In one embodiment, the assay card comprises: a plurality of sheets arranged to form a laminate, at least one of the sheets being a thermoplastic having the plurality of compressible reagent reservoirs, the reaction chamber and the microfluidic channels formed thereon.

In one embodiment, the assay card comprises a heating element provided on one of the sheets operable to heat the assay card.

In one embodiment, the heating element comprises an electrical heating element.

In one embodiment, the assay card comprises a thermometer operable to control the heating element achieve a predetermined assay card temperature.

In one embodiment, the assay card comprises microfluidic channels routed to predetermined areas to enable selective compression of the microfluidic channels.

In one embodiment, the assay card comprises comprising an indexing mechanism provided on the assay card.

In one embodiment, the assay card comprises a plurality of indexing mechanisms provided on the assay card.

According to a fourth embodiment, there is provided an assay card controller comprising: a compression mechanism operable to compress at least one compressible reagent reservoirs containing an associated reagent provided on an assay card to cause each associated reagent to be injected in a predetermined order into a reaction chamber provided on the assay card to react with a test sample.

In one embodiment, the assay card controller comprises a prevention mechanism operable to prevent actuation of the compression mechanism on detection of a flow preventer on the assay card.

In one embodiment, the compression mechanism is operable to follow a predetermined actuation path along the assay card to compress each of the plurality of compressible reagent reservoirs.

In one embodiment, the compression mechanism comprises: a roller operable to move relative to the assay card, along the actuation path.

In one embodiment, the compression mechanism comprises: a pair of rollers operable to receive the assay card therebetween and operable to move relative to the assay card, along the actuation path.

In one embodiment, the assay card controller comprises: gears operable to couple the pair of rollers.

In one embodiment, the compression mechanism comprises: a secondary pair of rollers operable to move relative to the pair of rollers to compress predetermined areas of the assay card.

In one embodiment, the assay card controller comprises: a controller operable to vary speed of movement the compression mechanism along the actuation path.

In one embodiment, the controller is operable to change direction of movement of the compression mechanism the along the actuation path.

In one embodiment, the assay card controller comprises: electrical contacts operable to power a heating element on the assay card.

In one embodiment, the assay card controller comprises: a thermometer operable to control the heating element achieve a predetermined assay card temperature.

In one embodiment, the assay card controller comprises: an index detector operable to detect an indexing mechanism provided on the assay card to produce at least one control signal to be provided to the controller.

In one embodiment, the index detector is operable to detect a plurality of indexing mechanisms provided on the assay card to produce a plurality of control signals to be provided to the controller.

In one embodiment, the assay card controller comprises: a detector operable to detect the presence of the target agent.

In one embodiment, the detector comprises: an optical detector operable to optically detect the presence of the target agent.

In one embodiment, the detector comprises: an amplifier operable to amplify a signal provided from the electrochemical detector.

In one embodiment, the assay card controller comprises: an indicator operable to indicate the presence of the test agent in response to an indication provided by the detector.

Further particular and preferred aspects of the present invention are set out in the accompanying independent and dependent claims. Features of the dependent claims may be combined with the features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA* Methods in Enzymology, Academic Press; Using Antibodies: A Laboratory Manual: Portable Protocol NO. I by Edward Harlow, David Lane, Ed Harlow (1999, Cold Spring Harbor Laboratory Press, ISBN 0-87969-544-7); Antibodies : A Laboratory Manual by Ed

Harlow (Editor), David Lane (Editor) (1988, Cold Spring Harbor Laboratory Press, ISBN 0-87969-314-2), 1855. Handbook of Drug Screening, edited by Ramakrishna Seethala, Prabhavathi B. Fernandes (2001, New York, NY, Marcel Dekker, ISBN 0-8247-0562-9); and Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Edited Jane Roskams and Linda Rodgers, 2002, Cold Spring Harbor Laboratory, ISBN 0-87969-630-3. Each of these general texts is herein incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

Embodiments of the present invention will now be described further, with reference to the accompanying drawings, in which:

Figure 1 illustrates components of the assay apparatus according to one embodiment;

Figure 2 illustrates an assay card according to one embodiment;

Figure 3 illustrates a section through the assay card of Figure 2;

Figure 4 illustrates an example process for performing a multi-stage reaction according to one embodiment;

Figures 5A to 5C illustrate an example operation for drawing a test sample according to one embodiment;

Figure 6 illustrates the assay card being presented to the assay apparatus;

Figures 7A to 7D illustrate relative movement of the assay card with respect to rollers of the assay apparatus along a compression path;

Figure 8 illustrates optical changes in reaction material due to varying concentrations of a target agent;

Figure 9 illustrates a fluid diagram showing the equivalent function of the assay card of Figure 2;

Figures 10 and 11 illustrate desired example reactions to be performed by reagents within the reaction chamber of Figure 2;

Figure 12 illustrates a sensor disk utilised in the reaction chamber in one embodiment;

Figure 13 illustrates processing steps for the preparation of the sensor disk of Figure 12;

Figure 14 illustrates an arrangement of a test sample loading device according to one embodiment;

Figure 15 illustrates an arrangement of a sealable channel according to one embodiment;

Figure 16 illustrates an example operation of the test sample loading device of Figure 14 utilising the sealable channel of Figure 15;

Figure 17 illustrates a double roller arrangement according to one embodiment;

Figures 18A to 18C illustrate a double roller arrangement according to another embodiment;

Figure 19 illustrates an arrangement of a channel having sealable and non-sealable portions according to one embodiment;

Figures 20A to 20D illustrates a schematic representation of an assay card and an example silver enhancement process performed by the assay apparatus using that assay card according to one embodiment;

Figure 21 illustrates a schematic representation of an assay card and an example silver enhancement process performed by the assay apparatus using that assay card according to one embodiment;

Figures 22A to 22D illustrates a schematic representation of an assay card and an example heavy metal detection process performed by the assay apparatus using that assay card according to one embodiment;

Figure 23 illustrates a schematic representation of an assay card and an example one step blood test process performed by the assay apparatus using that assay card according to one embodiment;

Figure 24 illustrates a schematic representation of an assay card and an example sequential injection process performed by the assay apparatus using that assay card according to one embodiment;

Figure 25 illustrates a schematic representation of an assay card and an example polymerase chain reaction (PCR) Lab-on-a-chip (LOC) process performed by the assay apparatus using that assay card according to one embodiment;

Figure 26 illustrates a schematic representation of an assay card and an example creatinine LOC process performed by the assay apparatus using that assay card according to one embodiment;

Figure 27 illustrates a schematic representation of an assay card and an example DNA extraction Lab-on-a-chip process performed by the assay apparatus using that assay card according to one embodiment;

Figure 28 illustrates a schematic representation of an assay card and an example adenosine triphosphate (ATP) Lab-on-a-Chip (LOC) process performed by the assay apparatus using that assay card according to one embodiment;

Figure 29 illustrates an assay apparatus according to one embodiment;

Figure 30 illustrates the formation of a reagent blister according to one embodiment;

Figure 31 illustrates sealing the reagent blister of Figure 30;

Figure 32 is a perspective view of the reagent blister of Figure 30;

Figure 33 illustrates an assay card according to one embodiment;

Figure 34 is a flow chart showing interactions between an assay apparatus and mobile phone according to one embodiment; and

Figure 35 illustrates an absorptive joint arrangement.

DETAILED DESCRIPTION

ASSAY APPARATUS

Figure 1 illustrates an arrangement of an assay apparatus, generally 10, according to one embodiment. The assay apparatus 10 provides a simple, reliable and effective arrangement for determining the presence of a target agent in a test sample.

The main components of the assay apparatus 10 are shown with its cover removed to help improve clarity. The assay apparatus 10 comprises an assay card 20 which, as will be

explained in more detail below, contains a number of reagent reservoirs, each of which contains a reagent which reacts with the test sample introduced into the assay card 20 via an inlet port 180 in order to determine the presence of the target agent, as will also be described in more detail below.

The assay card 20 includes an aperture 30 in its reaction chamber to enable a detection of the characteristics of the reaction occurring within the reaction chamber to be made in order to determine the presence of the target agent.

DETECTORS

In this example, the assay apparatus 10 comprises an optical source 40 and an optical detector 50, although other types of source and detector may be provided dependent upon the characteristics of the particular reactions that are intended to occur.

Accordingly, the optical properties of the fluid resulting from the reaction occurring within the reaction chamber can be determined when the aperture 30 is aligned with the optical source 40 and the optical detector 50. Typically, the concentration of the target agent can be determined from these optical characteristics and an indication of the presence of the target agent can be provided on the display 60.

However, it will be appreciated that other characteristics could be measured to determine the presence of a target agent. For example, an electrochemical detector may be provided which couples with metallic strips on the assay card. The electrochemical detector then measures electrochemical characteristics of the fluid in the reaction chamber, the metallic strips convey any signal to an amplifier which amplifies these characteristics, if required, and an indication of the presence of the target agent can be provided on the display 60.

ROLLERS

In overview, the operation of the assay apparatus 10 is as follows. The assay card 20 having a test sample provided therein is introduced into the apparatus between a pair of polymer-coated rollers 70. A switch 80 is activated which sends a signal to a roller controller 90 to drive the pair of rollers 70 using a motor within the roller controller 90 via the gear train 100.

The motor may be a stepper motor or may be provided with a position indicator to enable the roller controller 90 to provide accurate control. The presence of the gear train 100

causes each roller within the pair 70 to counter rotate and slowly draw the assay card between the rollers in the direction A. As will be explained in more detail below, this causes the reservoirs on the assay card to be compressed sequentially to cause reagents therein to be released into the reaction chamber simultaneously and/or in series.

Figure 17 illustrates an example double roller arrangement which may be used in place of the single pair of rollers 70 mentioned above. In the double roller arrangement a pump roller 500 is provided which is offset by a predetermined distance from a valve roller 510. The pump roller 500 operates to compress the reservoirs and displace the fluids contained therein to cause them to move throughout the microfluidic channels on the assay cards 20 as mentioned above. The valve roller 510 operates to selectively seal the microfluidic channels either temporarily or permanently in order to prevent mixing of fluids and to assist the reaction, as will be described in more detail below in Figures 18A to 18D.

As can be seen in Figures 18A to 18D, the pump roller 500 and the valve roller 510 are offset by a predetermined distance in the direction A and, in the example, have different lengths. In this example, the valve roller 510 has a shorter length than the pump roller 500. However, the exact dimensions and relative locations of the two rollers can be varied dependent on the layout of the assay card 20.

Each roller may even be formed of a number of separate rollers having gaps in between. The pump roller 500 is dimensioned and located to displace any reservoirs. The valve roller 510 is dimensioned and located to be able to interact with microfluidic channels routed on the assay card 20 to enable those microfluidic channels to be selectively sealed. For example, the valve roller 510 in this example will seal any microfluidic channels within the region 540 whilst leaving any microfluidic channels outside of this region 540 unaffected.

Hence, as shown in Figure 18A, the rollers 500, 510 have a fixed distance between them in the direction A. As the pump roller 500 compresses the reservoir 520, the valve roller 510 compresses the microfluidic channel 535 to prevent any flow of fluid along this microfluidic channel 535 into the reservoir 530. Although the dimensioning of this Figure is not exactly to scale, the layout of the microfluidic channel 535 is such that the valve roller 510 keeps the microfluidic channel 535 closed for the whole time that the pump roller 500 compresses the reservoir 520.

Thereafter, as shown in Figure 18B, following any required delay to allow any reaction to proceed, the pump roller 500 compresses the reservoir 530. Although the pump roller 500 will also compress and seal the microfluidic channel 525, the valve roller 510 is also now in a location to seal the microfluidic channel 525 and the microfluidic channel 535 is no longer sealed. Accordingly, fluid will be emitted from the reservoir 530 along the microfluidic channel 535 but the microfluidic channel 525 will be sealed to prevent any backflow towards the reservoir 520.

As shown in Figure 18C, the compression of both the reservoirs 520 and 530 by the pump roller 500 has completed and the valve roller 510 retains the microfluidic channels 525 and 535 in their closed configurations.

It will be appreciated that, if required, a self sealing region 480 may be provided in the vicinity of the location of the valve roller 510 in the position shown in Figure 18C in order to permanently seal the microfluidic channels 525 and 535.

LOCATION SENSING

One or more sensors (not shown) are provided on the assay apparatus 10 which interact with indexing on the assay card 20 to determine its location and to provide control signals to control the operation of the rollers.

For example, one or more photodetectors may be provided, each of which generates a signal when an aperture in the assay card 20 is sensed. The different photodetectors may be used to generate different signals such as pausing for different amounts of time, causing the assay card 20 to be heated, vibrated or subjected to other stimulus. Placing apertures in the assay card 20 at appropriate locations will enable signals to be produced by the photodetectors to enable the processing of the assay card 20 to be paused for appropriate amounts of time to enable, for example, reactions within the assay card 20 to take place, or to be heated or vibrated at the appropriate time to facilitate a reaction.

Although photodetectors and apertures are described, it will be appreciated that any appropriate indexing mechanism may be provided which provides for a mechanical, optical, electrical, magnetic or other means of providing control signals from the assay card 20 based on its relative location within the assay apparatus 10.

HEATING

Furthermore, electrical contacts (not shown) may be provided which contact with heating elements placed on the assay card 20. These heating elements may be configured to contact with the electrical contacts when the assay card 20 reaches a desired point during processing.

These heating elements heat the assay card 20 to improve the efficacy of reactions within the assay card 20. A detector such as a contact thermometer or an infra-red detector (not shown) may be used to provide feedback to control the temperature of the assay card 20. This temperature monitoring could be performed by the optical detector 50 mentioned above. Although electrical heating elements are described, it will be appreciated that any suitable means of heating the assay card 20 may be provided such as, for example, heating the void within the assay apparatus 10 which receives the assay card 20.

In addition, a detector, such as a photodetector, is provided (not shown) which detects for the presence of a flow preventer which is used to decouple the reaction chamber from the inlet port 180 to facilitate loading of the test sample, as will be described in more detail below. Upon detecting the presence of this flow preventer, the pair of rollers 70 may be prevented from being actuated to prevent damage such as bursting of the reservoirs, chambers or microfluidic channels.

In this example, the reagents are selected to cause a change in the optical properties of the resultant composition in the reaction chamber, with the optical properties varying in dependence on the concentration of target agent within the test sample. When the aperture 30 is aligned with the optical source 40 and the optical detector 50, the optical properties of the composition can be detected and processed to provide an indication of the presence of the target agent using the display 60.

ASSAY CARD

Figure 2 illustrates an assay card, generally 20', according to one embodiment. The assay card 20' comprises a substrate 110 which is a laminate having an extruded thermoplastic layer into which reagent reservoirs 120 to 160, microfluidic channels 185, a reaction chamber 170 and a waste chamber 190 are provided, collectively referred to as an assay arrangement, as illustrated in more detail in Figure 3 below.

The extruded thermoplastic may be thermoformed with the aid of a vacuum. These extruded components are compressible by the pair of rollers 70. In this example, there are provided five reagent reservoirs, each of which contains a different reagent. As can be seen, the reagent reservoirs 120 to 160 may be different sizes, dependent upon the volume of reagent to be contained therein, and are located at different locations on the surface of the assay card 20'.

Typically, the reagent reservoirs 120 to 160 are dome-shaped or cylindrical. Each reservoir chamber may contain a reagent sack (not shown) which contains each associated reagent. The reagent sack provides protection for each reagent from humidity, light and/or oxygen. The reagent sack also enables convenient manufacture of the assay card since no loose fluids are present. Also, by retaining the reagents in the sack, inadvertent premixing is avoided. Each sack is small and thin. The sack is punctured upon the action of the rollers to enable injection of the reagent into the reaction chamber.

The position of the reagent reservoirs 120 to 160 on the assay card 20' is determined by the order in which the reagents are intended to be delivered into the reaction chamber 170. In this example, the assay card 20' is intended to be drawn into the assay apparatus 10 in the direction marked by the arrow A. In other words, the assay card 20' moves relative to the rollers in the direction A. Accordingly, the reagents are delivered into the reaction chamber 170 via the associated microfluidic channels 185 as the reagent reservoirs are compressed in order which pumps and seals at the same time, withstanding any backpressure.

First, reagent reservoir 120 is compressed, followed by reagent reservoir 130, then reagent reservoir 140, then reagent reservoir 150 and finally reagent reservoir 160. If the assay card 20' is drawn into the assay apparatus at constant speed, then reagent reservoir 120 is compressed at time t1, reagent reservoir 130 at time t2, reagent reservoir 140 at time t3, reagent reservoir 150 at time t4, and reagent reservoir 160 at time t5. In that example, different time differences occur between the times at which each reaction reservoir is compressed. These delivery times can be varied by locating the reservoirs at different positions on the card in the direction A, based on the speed of the rollers.

Alternatively, and typically in practice, it will be appreciated that the delivery times are varied by varying the speed of the rollers or even stopping the rollers for periods of time. Such an arrangement provides for a much more compact assay card. Also, when stopping the

rollers provides a stationary hydrodynamic phase which is useful for providing time for biochemical reactions, particularly those which are slow such as, for example, antigen-antibody binding. As will also be explained in more detail below, as each reagent is introduced into the reaction chamber 170 to react with the test sample and any surplus fluid is displaced into the waste chamber 190 where it is retained typically by an absorbent material such as a pulped material.

It will be appreciated that embodiments may provide for two or more reagent reservoirs being located at exactly the same location so that the associated reagents are simultaneously injected. Furthermore, it will be appreciated that the length of one of the reagent chambers may extend further towards the reaction chamber 170 than the other in order that one associated reagent is continued to be injected after the injection of another has completed.

The assay card 20' may be provided with an inlet port 180 and aperture 30 as shown in Figure 1. In this embodiment, the aperture 30 enables the optical properties of the fluid within the reaction chamber to be measured. However, in another embodiment, a metallic coupling may be provided to enable an electrical connection between an electrochemical detector provided in the reaction chamber 170 and an amplifier of the assay apparatus to be made.

The inlet port 180 is coupled with the reaction chamber 170 via a microfluidic channel 185 as shown in Figure 5A. As also shown in Figure 5A, a narrow gate 210 is formed in the microfluidic channel 185, which may be sealed using a heating technique to decouple the reaction chamber 170 from the inlet port 180 once the test sample has been introduced. Alternatively, a further roller or other compression device may be provided to squeeze the test sample into the reaction chamber and then retain its position to seal the inlet port 180.

Although the assay cards 20; 20' are shown as having one assay arrangement on one side of the substrate, it will be appreciated that more than one assay arrangement may be provided on each assay card and that these assay arrangements may be provided on more than one side of the assay card. This would enable, for example, a single assay card to perform multiple tests on the same test sample (assuming the inlet port was coupled with multiple reaction chambers) or the same test to be performed on multiple test samples, or a combination of both. Of course, it will be appreciated that the assay apparatus may need additional optical sources and the optical detectors.

LOCATION INDEXING

The assay cards 20; 20' may be provided with an index hole (not shown) to mark a starting position of the assay card when entering the rollers. It will be appreciated that a starting position of the assay card may also be determined by the card being detected when entering the rollers by activating a sensor on the assay apparatus 10.

Likewise, the assay cards may be provided with apertures or markings such as a linear encoding pattern (not shown) which are sensed by a detector on the assay apparatus 10 and used to feedback position information to the roller controller 90. Typically, in order to stop the rollers, apertures may be provided in the card having positions in the direction A relative to the positions of the reservoirs. For example, the apertures may be located along the dotted lines shown in Figure 2 or a predetermined offset from those lines along the axis A, depending on the particular location of the photodetectors.

Furthermore, the apertures may be provided at different locations along an axis generally orthogonal to the axis A to provide for actuation of different photodetectors, each of which may provide a different control signal. For example, three photodetectors may be provided. Actuation of one photodetector causes a 5 minute pause, a second causes a 15 minute pause and a third causes a 30 minute pause.

Equally, the photodetectors may be used to cause other actions to occur such as, for example, actuation of electrical contacts, vibration of the assay card, irradiating the assay card, etc. It will be appreciated that the control signals produced may be programmable and selected based on the particular type of the assay card inserted and may be selected either by the operator or by automatic detection from an indicator on the assay card read by the assay apparatus 10.

Figure 3 illustrates a section through the assay card 20; 20'. The assay card 20; 20' is a laminate comprising a substrate layer 200, to which is bonded a thermoplastic layer 215. The thermoplastic layer 215 is formed from a suitable thermoplastic material which is compressible, but not necessarily elastic such as, for example, polyvinyl chloride, polypropylene, polyethylene, polyethylene terephthalate, poly(methyl methacrylate), and the like. The thermoplastic layer 215 is formed using vacuum-assisted hot embossing to form parts of the reagent reservoirs 120 to 160, the reaction chamber 170, the microfluidic channels 185 and the waste chamber 190.

A hole may be punched through the substrate 200 and the thermoplastic sheet 215 at the appropriate location to locate the aperture 30. Suitable windows may then be sealed into the resultant holes, optionally with a sensor disc located therebetween, as will be explained in more detail below.

SAMPLE LOADING

Also, it will be appreciated that reservoirs could be provided which are compressed to form a negative pressure which enables fluid to be draw into a particular region, as will now be described in more detail.

Figure 14 illustrates an example arrangement of a reservoir 400 provided on an assay card which is operable to generate a negative pressure. Such an arrangement will typically be provided at an inlet port 420 for loading a sample into the assay card, such as the inlet port 180 mentioned above. The reservoir 400 includes a resilient compression member operable, following a compression of the reservoir 400, to expand to substantially reverse the compression. For example, the reservoir 400 may be provided with a sponge-like structure therein which may deform upon compression but then expand back to its original state. The reservoir 400 is coupled with the inlet port 420 via a conduit, such as a microfluidic channel 410.

Operation of the reservoir 400 to facilitate loading of the test sample will now be explained.

At (i), the reservoir 400 is compressed and any contents, such as air, are expelled through the inlet port 420 via the microfluidic channel 410.

At (ii), a liquid such as, for example, a test sample 430 is brought into contact with the inlet port 420.

At (iii) to (v), the reservoir 400 expands drawing the test sample 430 through the channel 410 and filling the reservoir 400.

Figure 16 illustrates an example arrangement and operation of the inlet port 420 for receiving the test sample 430 according to one embodiment. The inlet port 420 may be an example of the inlet port 180 mentioned above. The inlet port 420 is coupled via the microfluidic conduit 410 with the reservoir 400. A microfluidic conduit 435 couples the microfluidic conduit 410 with, for example, the reaction chamber 170. A removable sealing

device 440 is provided to intermittently seal the microfluidic conduit 435 to prevent flow of fluid to the reaction chamber 170. The removable sealing device 440 may comprise a clamp which is held onto the surface of or passes through the assay card 20.

As shown in (i), the clip 440 remains in place whilst the sample 430 is drawn into the reservoir 400 in the manner mentioned above.

Thereafter, at (ii), the microfluidic conduit 410 is sealed either by heat-sealing, mechanical sealing or by the adhesive sealing technique mentioned in Figure 15 below. Once the microfluidic conduit 410 has been sealed, the removable sealing device 440 is removed. This enables the reservoir 400 to be coupled with the reaction chamber 170.

At (iii), the reservoir 400 is compressed, the contents flow through the microfluidic conduit 410 but are prevented from escaping through the inlet port 410 by the sealed microfluidic conduit. However, the absence of the clip 440 enables the fluid to flow towards the reaction chamber 170.

It will be appreciated that the compression of the reservoir 400 may occur either by operation of a user prior to placing the assay card into the assay apparatus 10 or may occur by activation of the rollers once received within the assay apparatus 10.

CHANNEL SEALING

In order to provide additional control of the flow of fluids within the assay card 20 to prevent, for example, pre-mixing or back-flow into previously-emptied areas, various mechanisms are provided. These include providing self-sealable microfluidic conduits and microfluidic conduits which are routed on the assay card to interact with the rollers to be selectively sealed and unsealed at particular times during the processing of the assay card 20.

Figure 15 illustrates a sealable conduit. In this example, the sealable conduit may comprise any of the microfluidic channels 185 formed on the assay card. An adhesive may be provided on either of the opposing surfaces of the microfluidic channels 180. In this example, an adhesive layer in the form of a double-sided adhesive tape (not shown) is provided on the surface of the substrate layer 200, prior to the thermoplastic layer 215 being placed thereon. When the microfluidic channel 180 is compressed, in this example by the movement of the roller 70 (although it will be appreciated that the channel could be pinched between the fingers

of a user or by using a hand-held roller or a pliers-type device), the two surfaces contact and effectively seal the microfluidic channel 180.

Figure 19 illustrates an arrangement of a sealable channel in more detail. An adhesive, in this example double-sided adhesive tape 450, is placed over either the entire surface of the substrate layer 200 or just in required locations. The tape 450 has slitted holes 460 formed therein, typically by laser beam ablation. When the thermoplastic layer 215 is placed thereon, regions 470 are created in the microfluidic channel where no adhesive is present and regions 480 are created in the microfluidic channel where the microfluidic channel can be sealed.

EXAMPLE OPERATION – IMMUNOASSAY

Figure 4 illustrates an example process for performing a multi-stage reaction that may be undertaken when processing the assay card 20 with the assay apparatus 10. At step S10, the test sample is drawn into the card, as illustrated with reference to Figures 5A to 5C. As illustrated in Figures 5A and 5B, the test sample 200 (such as a blood sample) is drawn into the inlet port 180 and flows to the aperture 30 in the reaction chamber 170 by capillary force. Any excess of the test sample 200 goes to the waste chamber 190. After the test sample 200 is drawn, a gate 210 of microfluidic channel 180 is sealed off by means of hot wire sealer provided separately with the sampling kit.

Returning to Figure 4, once the test sample 200 has been sealed into the assay card 20, then the assay card 20 is placed into the assay apparatus 10 as shown in Figures 6 and 7A. The reaction chamber 170 is preconfigured to contain an immobilized antibody, typically provided on a substrate as will be described in more detail below. The assay card 20 is drawn through the pair of rollers 70 in the direction A until, at step S20, the pair of rollers 70 compress the reservoir 120 and the test sample 200 is washed by the reagent contained in the reservoir 120 being injected into the reaction chamber 170 to flush the test sample and any unbound target agent, such as an antigen, as illustrated in Figure 7B. At this stage, the speed of the pair of rollers 70 may reduce or movement stop completely to enable the washing to complete.

The assay card 20 continues to move relative to the rollers 70 until, at step S30, the enzyme labelled antibody is injected from the reagent reservoir 130, as illustrated in Figure 7C. The enzyme labelled antibody binds to the antigen which is already adhered to the immobilized antibody on the substrate creating a sandwich structure: antibody-antigen-

antibody. Again, at this stage, the speed of the pair of rollers 70 may reduce or movement stop completely to enable the reaction to complete.

The assay card 20 continues to move relative to the pair of rollers 70 until, at step S40, the reservoir 140 releases its reagent to wash any unbound antibodies, as shown in Figure 7D. Again, at this stage, the speed of the pair of rollers 70 may reduce or movement stop completely to enable the reaction to complete.

The assay card 20 continues to move relative to the pair of rollers 70 until, at step S50, a reagent is released from the reagent reservoir 150, as also illustrated in Figure 7D. Again, at this stage, the speed of the pair of rollers 70 may reduce or movement stop completely to enable the reaction to complete. The reagent molecules react with the enzyme to generate luminescence, fluorescence or a colour change.

Accordingly, it can be seen that the reagent reservoir 140 engages with the pair of rollers 70 slightly earlier than the reagent reservoir 150. However, it will be appreciated that there will be a period when the associated reagents will be simultaneously released into the reaction chamber 170.

The assay card 20 continues to move relative to the pair of rollers 70 until, at step S60, a washing reagent is released from the reagent reservoir 160. Again, at this stage, the speed of the pair of rollers 70 may reduce or movement stop completely to enable the reaction to complete.

The assay card 20 continues to move relative to the pair of rollers 70 until, at step S70, the window 30 aligns with the optical transmitter 40 and the optical detector 50 whereupon a colour change in the reaction materials is detected to provide an indication of the concentration of the presence of the target agent within the test sample, as illustrated in more detail in Figure 8.

Figure 8 shows an example antibody immobilised spot in the aperture 30 which is stained with, for example, silver. As the concentration of any target agent or antigen in the test sample increases, the grey level becomes darker. The grey level can be detected by the optical emitter 40 and detector 50. The emitter 40 may be a LED, the detector 50 may be a photodiode or may be a sensitive black and white charge-coupled device (CCD) camera. This

grey level may be detected and a corresponding concentration of target agent displayed on the display 60.

Hence, as can be seen, the processing operation is sequential. Although the timing is important, this can be easily controlled by carefully locating the reservoirs 120 to 160 at the appropriate distance along the assay card 20 in the direction A and by controlling the speed of the movement relative to the rollers 70 by changing the rotation speed or even switching the rollers 70 on and off. The flow rate may also affect performance and this can also be readily controlled by varying the rotation speed of the rollers 70, adjusting the size of the microfluidic channels 185 and/or the cross-sectional area of the reagent reservoirs 120 to 160 presented to the rollers 70.

In one embodiment the reagents are a washing solution, a gold nanoparticle labelled antibody solution, a silver enhancement solution A and solution B, a washing solution.

Figure 9 illustrates a fluid diagram showing the equivalent function of the assay card 20;20' and shows the five equivalent pumps for pumping the associated reagents into the reaction chamber 170. The quantity of reagent is controlled by the volume of the associated reagent reservoir 120 to 160. The flow rate is a function of the cross-sectional area of the associated reagent reservoir 120 to 160 presented to the pair of rollers 70 and the speed at which the assay card 20 moves relative to the rollers 70. The operation time is a function of the location of each reaction reservoir along the actuation path A and the speed with which the assay card moves relative to the pair of rollers 70, which is controlled by the controller 90.

EXAMPLE REACTIONS

Figures 10 and 11 illustrate desired example reactions to be performed by reagents within the reaction chamber 170.

Figure 10 illustrates an antibody 300 immobilised on a sensor card 310 provided within the aperture 30 of the reaction chamber 170. The target agent or antigen 330 is contained in the test sample 205. The enzyme labelled antibody 320 is supplied from a reagent reservoir.

As shown in Figure 11, embodiments may utilise a silver enhancement of gold in a nano-particles technique. Typically, gold nano–particles are used as an antibody label because of the gold nano-particle's deep red colour and the ease of conjugation with the antibody

[*Immunochemistry, 8, 1081(1071)*]. However, colour detection of gold nano-particles requires expensive optical filters and a sensitive detector. Also, the signal is often not sensitive enough for some applications. To amplify the signal, therefore, a silver enhancement technique is utilised (as described in US patent 6602669). Silver nitrate and silver acetate are good sources of silver ions, while hydroquinone, n-propyl galate, p-penylenediamine and formaldehyde are commonly used as reducing agents.

SENSOR DISK

As shown in Figure 12, an embodiment uses a separate sensor disk 250 made of a piece of indium-tin-oxide (ITO) film, which is a conductive and transparent film widely used in the touch-screen industry. The ITO film provides well defined and stable surface for antibody immobilization. The sensor disk 250 is inserted in to the reaction chamber 170 just before the aperture 30 is covered with a cover film.

As shown in Figure 13, an embodiment uses (3-glycidoxypropyl)trimethoxysilane (GPTES) 211 as a cross linker between the ITO surface hydroxyl group and the antibody's amine group. The ITO film of the sensor disk 250 is cleaned and treated with acid to activate the surface hydroxyl group. Then, the cross linking agent is applied on the activated ITO surface by dipping the ITO film in 1% solution of GPTES for overnight at room temperature. The antibody 213 solution is dropped onto the surface and incubated for overnight at 4°C. Additional rinsing and blocking with bovine serum albumin (BSA) steps finalize the antibody deposition.

It will be appreciated that the sensor disk 250 may be provided with a layer of biochemical agent such as an enzyme or an antibody that reacts specifically to certain molecules to be detected in the test sample.

EXAMPLE OPERATION – SILVER ENHANCEMENT – ARRANGEMENT 1

Figure 20A illustrates schematically the layout of an example assay card 20A and illustrates the operation of the assay apparatus 10 when processing such an assay card 20A. The assay card 20A comprises the inlet arrangement mentioned in Figure 16 above. An overview of the assay components is provided in Figure 20B, the timing parameters are shown in Figure 20C and the assembly process is described in Figure 20D.

Coupled between the inlet port 420 and the detection zone of the reaction chamber (not shown, but located between the printed control antibody C and the absorbent pad AP) are a number of antibody providers, I, T, C. Two reagents contained within separate reservoirs R1 and R2 are also provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20A will readily be apparent to the skilled person.

At time T1, the reservoir 400 is depressed with the clip 440 in place to seal the channel 435. Accordingly, any air is then expelled through the inlet port 420. A sample is offered to the inlet 420 and at time T2 the reservoir 400 is released thereby drawing the sample into the reservoir 400.

At time T3, the microfluidic conduit 410 is sealed.

At time T4, processing is paused to enable any reagent within the reservoir 400 (such as an anti-coagulant) to react with the sample.

At time T5, the clip 440 is removed.

At time T6, the reservoir 400 is compressed and its contents flow through the microfluidic channel 435 towards the detection zone of the reaction chamber. The sample flows through a dried indicator antibody I, a printed capture antibody T and a printed control antibody C prior to arriving at the detection zone of the reaction chamber.

At time T7, processing pauses to allow a reaction to take place.

At time T8, the assay apparatus 10 detects the absence of the clip 440 and the reservoir R2 is compressed by the pump roller 500, the valve roller 510 is located at position p1. The microfluidic channel coupling with reservoir R1 is sealed by the valve roller 510. The microfluidic channel coupling with reservoir R2 is not sealed by the valve roller 510. This causes the contents of the reservoir R2 to flow towards the detection zone of the reaction chamber.

At time T9, the rollers 500, 510 continue to advance.

At time T10, the pump roller 500 compresses the reservoir R1, the valve roller 510 is located at position p2. The valve roller 510 seals the microfluidic channel coupled with the

reservoir R2 but does not seal the microfluidic channel coupling with reservoir R1. The contents of R1 then mix with the test sample in the detection zone of the reaction chamber.

At time T11, the apparatus 10 detects indexing means on the assay card 20A and a signal is generated to cause further processing to pause.

At time T12, after the required time, the rollers 500, 510 activate.

At time T13, the valve roller 510 moves to position P3 and has sealed both microfluidic channels coupling with the reservoirs R1 and R2.

At time T14, a result is detected by reading the reaction chamber.

EXAMPLE OPERATION – SILVER ENHANCEMENT – ARRANGEMENT 2

Figure 21 illustrates and immunoassay technique using silver enhancement. It will be understood that silver enhancement is a commonly-used system that is used to amplify immunogold labelled samples in lateral flow assay and blotting applications. It occurs through the reduction of silver from one solution (for example, an enhancer) by another solution (for example, an initiator) in a presence of gold particles. The reduction reaction causes silver to build up preferentially over a 10 to 15 minute period on the surface of the gold particles which are conjugated (for example, attached) to antibodies bound to the target analyte. Typically, this results in the amplification of the gold by 10 to 100 fold which has the effect of reducing the assay's limit of detection (LOD). Typically, this is performed as a manual intervention where the enhancer and initiator solutions are mixed by a technician who then washes the sample to remove any salts before pipeting the silver solution onto the sample. However, Figure 21 illustrates a schematic arrangement and operation of any assay card 20B operable with the assay apparatus 10 to automatically perform such an operation.

The assay card 20B comprises the inlet arrangement mentioned in Figure 16 above. Coupled between the inlet port 420 and the detection zone of the reaction chamber (not shown, but located between the printed control antibody C and the absorbent pad AP) are a number of antibody providers, I, T, C. Two reagents contained within separate reservoirs R1 and R2 are also provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20B will readily be apparent to the skilled person.

At time T1, the reservoir 400 is depressed and the blood or urine sample is applied to the inlet port 420.

At time T2, the reservoir 400 is released and the sample is drawn into the assay card 20B.

At time T3, the inlet port 420 is pinched to seal the channel to prevent sample backflow or exit from the assay card 20B.

At time T4, the clip 440 is then removed to allow entry of the sample into the reaction chamber.

At time T5, the reservoir 400 is depressed which pushes the sample through a dried gold conjugate indicator antibody I, re-suspending it and simultaneously allowing the binding of the antibody with the analyte/silver. The liquid passes through to the printed capture antibody T as well as passing through the positive control antibody C and into the detection zone of the reaction chamber. Any excess volume passes through to the waste reservoir and is held in the absorbent pad AP.

At time T6, the assay card 20B is then placed into the assay apparatus 10 and then the programme initiated.

At T7, after a 10 minute pause, the pump roller 500 bursts the balloon within the reservoir R3 releasing the distilled water into the system, whilst the valve roller 510 is in position p1, blocking the channel coupling with reservoirs R1 and R2 to prevent any backflow, whilst allowing flow through the conduit coupled with reservoir R3.

At time T8, the pump roller 500 bursts the balloons within reservoirs R1 and R2 releasing silver nitrate signal enhancement chemicals into the system whilst the valve roller 510 is in position p2, sealing the microfluidic conduit to the reservoir R3 to prevent any backflow, whilst allowing flow through the conduit coupled with reservoirs R1 and R2.

At time T9, the valve roller 510 moves forward to position p3 to block all channels while the signal develops over 10 minutes.

At time T10, the signal is read from both the positive control signal provided by the printed control antibody and the test signal from the reaction chamber and a result is displayed.

EXAMPLE OPERATION – HEAVY METAL DETECTION

Figure 22A illustrates schematically the layout of an example assay card 20C and illustrates the operation of the assay apparatus 10 when processing such an assay card 20C. The assay card 20C comprises the inlet arrangement mentioned in Figure 16 above.

A detection electrode D is provided within the reaction chamber. Figure 22B shows the fabrication process for the screen printing of mercury and silver electrodes on a substrate such as a PVC card which can be used as an arrangement of an example detection electrode D. Two reagents contained within separate reservoirs R1 and R2 are also provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20A will readily be apparent to the skilled person.

As will be mentioned below, a mercury plated detection electrode is formed for use in an anodic stripping analysis. Anodic stripping analysis is a very sensitive method to analyze heavy metal in water, blood or other samples. With a mercury working electrode, silver reference electrode and simple electronic amplifier, many heavy metals can be detected in this way. However, such an approach is typically tedious since mercury film creation is required contemporaneously for every analysis because the mercury is easily oxidized. However, this approach enables a fresh mercury film to be created automatically prior to processing the test sample. As just discussed, to make a sensitive electrode, a fresh mercury film must be created just before analysis.

Accordingly, as will be discussed in more detail below, and described in Figures 22C and 22D (from Analytical electrochemistry, Joseph Wang, Wiley-VCH, 3rd ed, 2006), a reagent containing mercury ions is moved to the electrode and a reduction potential is applied on the electrode. Then, the mercury ions are reduced on the electrode creating a fresh mercury film. The sample is injected to the electrode while applying an accumulation potential. Positive ions, such as lead, are attracted to the electrode by the applied potential and accumulates on the mercury film. The potential is the reversed. Then the lead atoms are stripped from the electrode leaving electrons. The electrons cause a current that can be detected by an amplifier.

At time T-4 to time T0, the mercury film is created. In this example, the assay apparatus 10 detects the presence of the clip 440 and the reservoir R2 is compressed by the

pump roller 500, the valve roller 510 is located at position p1. The microfluidic channel coupling with reservoir R1 is sealed by the valve roller 510. The microfluidic channel coupling with reservoir R2 is not sealed by the valve roller 510. This causes the contents of the reservoir R2 to flow towards the detection zone of the reaction chamber. This reagent contains mercury ions for forming the mercury film on the detection electrode in the detection zone.

At time T-3, the rollers 500, 510 continue to advance.

At time T-2, the pump roller 500 compresses the reservoir R1, the valve roller 510 is located at position p2. The valve roller 510 seals the microfluidic channel coupled with the reservoir R2 but does not seal the microfluidic channel coupling with reservoir R1.

At time T-1 an electroplating potential is applied to bond the mercury ions onto the detection electrode.

At time T0, the apparatus 10 detects indexing means on the assay card 20C and a signal is generated to cause further processing to pause.

At time T1 to T4, the sample is loaded into the assay card 20C.

At time T1, the reservoir 400 is depressed with the clip 440 in place to seal the channel 435. Accordingly, any air is then expelled through the inlet port 420. A sample is offered to the inlet 420 and at time T2 the reservoir 400 is released thereby drawing the sample into the reservoir 400.

At time T3, the microfluidic conduit 410 is sealed.

At time T4, processing is paused to enable any reagent within the reservoir 400 (such as an anti-coagulant) to react with the sample.

At time T5, the clip 440 is removed.

At time T6, the reservoir 400 is compressed and its contents flow through the microfluidic channel 435 towards the detection zone of the reaction chamber.

At time T7, processing pauses to allow a reaction to take place.

At time T8, an accumulation potential is applied and positive ions, such as lead, are attracted to the electrode by the applied potential and accumulate on the mercury film.

At time T9 to 11, the valve roller 510 moves from position P2 and the contents of R1 then move to the detection zone of the reaction chamber for washing. The valve roller is now in position P3 and has sealed both microfluidic channels coupling with the reservoirs R1 and R2.

At time T12, processing pauses.

At time T13 and T14 a stripping potential is applied. This reverse potential causes the lead atoms to be stripped from the electrode leaving electrons.

The electrons cause a current that can be detected by an amplifier at time T15 to provide an indication of the amount of heavy metal present in the sample.

EXAMPLE OPERATION - ONE STEP BLOOD TEST PLATFORM

Figure 23 illustrates schematically the layout of an example assay card 20D. The assay card 20D comprises the inlet arrangement mentioned in Figure 16 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20D will readily be apparent to the skilled person

Coupled between the inlet port 420 and the detection zone D of the reaction chamber is a filter F. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20D will readily be apparent to the skilled person.

The reservoir 400 is depressed with the clip 440 in place to seal the channel 435. Accordingly, any air is then expelled through the inlet port 420. A sample is offered to the inlet 420 and the reservoir 400 is released thereby drawing the sample into the reservoir 400.

The microfluidic conduit 410 is sealed.

Processing is paused to enable any reagent within the reservoir 400 (such as a blood pre-treatment reagent) to react with the sample.

The clip 440 is removed.

The reservoir 400 is compressed and its contents flow through the microfluidic channel 435 towards the detection zone D of the reaction chamber. The sample flows through the filter prior to arriving at the detection zone D of the reaction chamber. Any excess is retained within the absorbent pad AP.

The detection zone D is then examined to determine the result.

EXAMPLE OPERATION - SEQUENTIAL INJECTION

Figure 24 illustrates schematically the layout of an example assay card 20E. The assay card 20E comprises the inlet arrangement mentioned in Figure 16 above.

Coupled between the inlet port 420 and the detection zone D of the reaction chamber is a filter F. Two reagents contained within separate reservoirs R1 and R2 are also provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20E will readily be apparent to the skilled person.

The reservoir 400 is depressed with the clip 440 in place to seal the channel 435. Accordingly, any air is then expelled through the inlet port 420. A sample is offered to the inlet 420 and the reservoir 400 is released thereby drawing the sample into the reservoir 400.

The microfluidic conduit 410 is sealed.

Processing is paused to enable any reagent within the reservoir 400 (such as a blood pre-treatment reagent) to react with the sample.

The clip 440 is removed.

The reservoir 400 is compressed and its contents flow through the microfluidic channel 435 towards the detection zone D of the reaction chamber. The sample flows through the filter prior to arriving at the detection zone D of the reaction chamber. Any excess is retained within the absorbent pad AP.

The reservoir R1 is compressed by the pump roller 500. The microfluidic channel coupling with reservoir R2 is sealed by the valve roller 510. The microfluidic channel coupling with reservoir R1 is not sealed by the valve roller 510. This causes the contents of the reservoir R2 to flow towards the detection zone D of the reaction chamber.

The rollers 500, 510 continue to advance.

The pump roller 500 compresses the reservoir R2. The valve roller 510 seals the microfluidic channel coupled with the reservoir R1 but does not seal the microfluidic channel coupling with reservoir R2. The contents of R2 then mix with the test sample in the detection zone D of the reaction chamber.

A result is detected by reading the detection zone D of the reaction chamber.

EXAMPLE OPERATION - POLYMERASE CHAIN REACTION (PCR) LAB-ON-A-CHIP (LOC) UTILISING ROLLER BLISTER SYSTEM FOR REAGENT DELIVERY TO A COUPLED DEVICE

Figure 25 illustrates schematically the layout of an example assay card 20F. Two reagents contained within separate reservoirs A and B are also provided which are activated by a double roller arrangement mentioned in Figure 18 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20F will readily be apparent to the skilled person.

PCR is used to amplify a specific region of a Deoxyribonucleic acid (DNA) strand. Typically, PCR methods amplify DNA fragments of up to approximately 10 kilo base pairs (kb). PCR typically requires the following reagents:

- *DNA template* that contains the DNA region (target) to be amplified
- Two *primers* that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target
- DNA polymerase with a temperature optimum at around 70 °C.
- *Deoxynucleotide triphosphates* (dNTPs) from which the DNA polymerases synthesizes a new DNA strand
- *Buffer solution,* providing a suitable chemical environment for optimum activity and stability of the DNA polymerase
- *Divalent and monovalent cations,* i.e. magnesium, generally Mg^{2+} and potassium

The PCR is performed in a vessel that can be thermally cycled, heating and cooling the reaction from 20-40 times. Usually there are 3 main temperature steps:

- *Denaturation*: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template. The primers are unable to bind to the template at this temperature.

- *Annealing*: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the melting temperature (Tm) of the primers used. The polymerase binds to the primer-template hybrid and begins DNA synthesis.

- *Extension*: The DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends primarily on the length of the DNA fragment intended to be amplified (~ 1000 bp per minute).

A final single step is performed after the last cycle is completed by holding the reaction at 70–74 °C for 10 minutes ensuring that any remaining single-stranded DNA is fully extended. The reaction can then be cooled to 4°C for an indefinite time storage of the reaction.

The presence of DNA can be detected through the use of nucleic acid stains such as Ethidium Bromide or monomeric cyanine dyes through ultra-violet (UV) illumination or through the use of a fluorescent stain such as SYBR Green (trade mark) which will give greater sensitivity and potentially require less thermocycling saving time.

In this example, the assay card 20F can be coupled with another device (not shown) and used as a reagent delivery system for PCR. Dispensed reagents will flow through an aperture into the separate device which will be thermocycled.

The assay card 20F includes a sample application well S into which the test sample is dispensed; a sponge blister SB used to draw the sample into the card by pressing and releasing (press when adding sample, release to draw sample into card in a similar manner to that described with reference to Figure 16 above); a pinch valve (not shown, but arranged in the manner of the sealing inlet port as indicated above) to close the sample well port; a card integrity pin P for removal to open up exit microfluidic path; a blister or reservoir A which

contains 100ul of reaction buffer, ions and primers and DNA polymerase; and a blister B which contains 50ul of the nucleic acid stain, such as Ethidium Bromide or SYBR green depending on the detection system used within the reader. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20F will readily be apparent to the skilled person

In operation, the sample blister SB is depressed and the sample containing DNA template is applied to the assay card 20F.

The sample blister SB is released and the sample is drawn into the assay card 20F.

The sampling channel S is pinched closed.

The pin P is removed.

The sample blister SB is depressed and the sample flows into the reaction chamber (not shown).

The assay card 20F is placed into the assay apparatus 10.

The blister B channel is depressed and the blister A is crushed which causes a reagent flow to the exit.

Once the thermocycled reaction is complete, the assay card 20F is returned to the assay apparatus 10.

The blister B channel is released, the blister A channel is depressed and blister B reagents flow to exit.

EXAMPLE OPERATION - CREATININE LOC TESTING DEVICE

Figure 26 illustrates schematically the layout of an example assay card 20G. Four reagents contained within separate reservoirs A to D are also provided which are activated by a double roller arrangement mentioned in Figure 18 above. The assay card 20G comprises the inlet arrangement mentioned in Figure 16 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20G will readily be apparent to the skilled person.

Creatinine is a waste product formed in muscle from a high-energy storage compound, creatine phosphate. Creatine phosphate can be stored in muscle at approximately four times

the concentration of adenosine triphosphate. In muscles it spontaneously undergoes degradation to form a cyclic anhyride-creatinine. The blood concentration of creatinine and its excretion in urine are remarkably constant in normal individuals. Therefore serum creatinine level is used as an indicator for assessing kidney function. Creatinine that is present in serum or plasma directly reacts with alkaline picrate resulting in the formation of a red colour, the intensity of which is measured at a wavelength of 505nm. Protein interference is eliminated using sodium lauryl sulphate. A second absorbance reading after acidifying with 30% acetic acid corrects for non-specific chromogens in the samples.

Reservoir A contains reagent 1: NaOH, Trisodium phosphate ($Na_3PO412H_2O$), sodium tetraborate [$Na_2B_4O_710H_2O$]. Reservoir B contains reagent 2: Sodium lauryl sulphate. Reservoir C contains reagent 3: Anhydrous picric acid. A working reagent is formed from a mix of equal volumes of the three reagents before use. Reservoir D contains reagent 4: 30% (v/v) Ethanoic Acid

A 200ul test sample is dispensed into a sample application well S.

The sample is drawn into the assay card 20G by pressing and releasing the sponge blister SB (press when adding sample, release to draw sample into assay card 20G in a similar manner to that described with reference to Figure 16 above).

A pinch valve is activated to close the sample well S.

A card integrity pin P is removed to open up the reaction chamber microfluidic path.

Reservoir A contains 100ul of the sodium salts/hydroxide. Reservoir B contains 100ul of the Sodium lauryl sulphate. Reservoir C contains 100ul of the Anhydrous picric acid.

A mixing chamber MC is coupled with reservoirs A to C.

Reservoir D contains 200ul of the Ethanoic Acid.

A reaction chamber RC is provided, coupled to which is a waste reservoir AP containing a cellulose based wadding or pad.

In operation, the sponge blister SB is depressed and the urine sample is applied to the assay card 20G.

The sponge blister SB is released and the sample is drawn into the assay card 20G.

The pinch valve is closed.

The card integrity pin P is removed.

The sponge blister SB is depressed and the sample flows into the reaction chamber RC.

The assay card 20G is placed into the assay apparatus 10.

Reservoirs A, B and C are depressed simultaneously by the pump roller 500, the reagents flow into the mixing chamber MC whilst the microfluidic channel for reservoir D is pinched shut by the valve roller 510.

The mixing chamber MC is depressed by the roller with the contents flowing into the reaction chamber RC. The microfluidic channel for reservoir D remains pinched shut.

The assay card 20G is incubated at ambient temperature for 30 minutes.

The signal is detected using the assay apparatus 10 optical device 50 at 505nm.

Reservoir D is depressed by the pump roller 500 while its microfluidic channel is released by the valve roller 510 allowing the ethanoic acid to flood the reaction chamber RC. Any waste overflows into absorbent pad AP.

The assay card 20G is incubated at ambient temperature for 30 minutes.

The signal is detected using the assay apparatus 10 optical device 50 at 505nm. This absorbance value is deducted from the previous absorbance reading to provide an indication of the creatinine level.

EXAMPLE OPERATION - DNA EXTRACTION LAB-ON-A-CHIP (LOC) UTILISING ROLLER BLISTER SYSTEM

Figure 27 illustrates schematically the layout of an example assay card 20H. Three reagents contained within separate reservoirs A to C are also provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. The assay card 20H comprises the inlet arrangement mentioned in Figure 16 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20H will readily be apparent to the skilled person.

DNA extraction is performed by binding of charged DNA onto SiO_2. The SiO_2 is charged using GuHCl. The sample is added and the DNA binds to the SiO_2. Unbound proteins are washed away using ethanol and the bound DNA is eluted by neutralising the DNA's charge with water.

A sample application well S is provided onto which the test sample is dispensed.

A sponge blister SB is provided to facilitate drawing of the sample into the assay card 20H by pressing and releasing (press when adding sample, release to draw sample into assay card 20H).

A pinch valve is used to close the sample application well S.

A card integrity pin P is provided which is removed to open up the extraction chamber EC microfluidic path.

Reservoir A contains 100ul of guanidine hydrochloride (GuHCl) salt in tris(hydroxymethyl)aminomethane ethylenediaminetetraacetic acid (TE) buffer. Reservoir B contains 100ul of Ethanol. Reservoir C contains 100ul of water.

A waste chamber WC is provided containing void volume and wadding.

A harvest chamber HC is provided.

In operation, the sponge blister SB is depressed and a protease treated sample is applied to the assay card 20H.

The sponge blister SB is released and the sample is drawn into the assay card 20H.

The sampling channel S is pinched closed.

The card integrity pin P is removed.

The assay card 20G is placed into the assay apparatus 10.

The reservoir A is depressed by the pump roller 500 and the reagent flows into the extraction chamber EC.

The harvest chamber channel and reservoir B & C channel's are pinched shut by the valve roller 510'.

The sponge blister SB is depressed by the pump roller 500 and the sample flows into the extraction chamber EC.

Reservoir B is depressed by the pump roller 500 and the reagent flows into the extraction chamber EC.

Reservoir C is depressed by the pump roller 500 and the reagent flows into the extraction chamber EC.

The waste chamber channel is pinched closed by the valve roller 510' and the harvest chamber channel is opened to enable the extracted DNA to be harvested.

EXAMPLE OPERATION - ADENOSINE TRIPHOSPHATE (ATP) LAB-ON-A-CHIP (LOC) TESTING DEVICE

Obtaining microbial counts for food, food preparation areas or water sources using conventional microbiological techniques can be slow and typically requires the use of central laboratory facilities. A number of rapid POC devices that utilise bioluminescence allow for much faster readouts that provide results often in minutes. Bioluminescence can be used to detect ATP (adenosine triphosphate), a ubiquitous compound used in the biological metabolism found within all living cells.

An enzyme, luciferase, together with its substrate, luciferin, can be used to assay ATP content. ATP is used to help catalyse the conversation of luciferin (substrate) to oxyluciferin (product). The amount produced, which results in the production of the short but stable production of light, is detected and quantified and is proportional to the amount of ATP that has been extracted from any bacteria present.

Figure 28 illustrates schematically the layout of an example assay card 20I. Two reagents contained within separate reservoirs A and B are provided which are activated by a double roller arrangement 500, 510 mentioned in Figure 18 above. The assay card 20I comprises the inlet arrangement mentioned in Figure 16 above. Again, this is a schematic representation showing the general layout and is not to scale; however, the detailed layout of the assay card 20I will readily be apparent to the skilled person.

A sample application well S is provided into which a 100ul test sample is dispensed.

A sponge blister SB is provided provided to facilitate drawing of the sample into the assay card 20I by pressing and releasing (press when adding sample, release to draw sample into assay card 20I).

A pinch valve is provided to close the sample application well.

A card integrity pin P is provided which is removed to open up the reaction chamber RC microfluidic path.

A reservoir A is provided which contains 50ul of a lysis solution (Triton N-101 (4g/L), 100mM NaOH).

A reservoir B is provided which contains 50ul of a working reagent solution Sodium lauryl sulphate (350mM HEPES (pH 7.4), 4mM EDTA, 4mM MgCl2, 150mM NaH2PO4, 3.0g/L BSA, 10g/L, 10g/L Trehalose, 0.4 mM D-Luciferin, 12 mg/L Luciferase).

A reaction chamber RC is provided.

A waste blister AP is provided containing cellulose-based wadding or pad.

In operation, the sponge blister SB is depressed and the sample, such as a urine sample, is applied to the assay card 20I.

The sponge blister SB released and the sample is drawn into the assay card 20I.

The sampling channel S is pinched closed.

The pin P is removed to couple the sponge blister SB with the reaction chamber RC.

The sponge blister SB is depressed and the sample flows into the reaction chamber RC.

The assay card 20I is placed into the assay apparatus 10.

The reservoir A is depressed by the pump roller 500 and the lysis solution flows into the reaction chamber RC while reservoir B's microfluidic channel is pinched shut by the valve roller 510.

The assay card 20I is incubated at ambient temperature for 2 minutes. It will be appreciated that this may be signaled to the assay apparatus 10 by indexing provided on the assay card 20I.

Reservoir B is then depressed by the pump roller 500, the pump roller 500 closes the microfluidic channel coupled with reservoir A, and the sodium lauryl sulphate solution flows into the reaction chamber RC.

The assay card 20I is incubated at ambient temperature for 30 minutes. It will be appreciated that this may be signaled to the assay apparatus 10 by indexing provided on the assay card 20I.

The signal from the reaction chamber RC is then detected using the assay apparatus 10 luminescent detection device 50.

Any waste overflows into absorbent pad in the waste blister AP.

ALTERNATIVE ASSAY APPARATUS

Figure 29 illustrates an assay apparatus, generally 10A, according to one embodiment. This arrangement utilises a single roller pump mechanism on a flat tray for the immunocard. Accordingly, a base tray 600 is provided into which the assay card 20, for example, is placed. A compression mechanism is provided having a single roller 70A on a gear track 620 which squeezes the reservoirs. The compression mechanism may be provided on the base tray 600. The compression mechanism may be sprung to press the single roller 70A onto the assay card 20 to apply a constant pressure.

This arrangement is similar to that of a CD-ROM push-in mechanism found on computers and provides for increased control on the mechanism, on the applied pressure and on the positioning of the assay card 20 within the assay apparatus 10A. Using a single roller 70A reduces the likelihood of slip which may occur in a double roller arrangement.

In addition, a charge coupled device (CCD) camera (not shown) may be mounted directly on the reaction chamber to provide for real-time monitoring and verification that a reagent is being transferred into the reaction chamber.

The arrangement of the assay card 20 in the base tray 200 enables the exact location of the assay card 20 to be determined and separate pins (not shown) can be provided that push onto the base tray 600/assay card 20 for sealing conduits.

Such an arrangement provides the benefits of: having fixed reagent window points; having the CCD camera always above the reaction chamber facilitates real-time monitoring; the pressure on the assay card 20 and the roller 70A is easier to control due to having one moving part which enables increased control; no slippage; and knowing exactly where the roller 70A is.

Accordingly, it can be seen that embodiments provide a versatile diagnostic platform for detecting chemical or biological agents in biological samples like blood or urine, as well as water, soil and other environmental samples.

Embodiments provide a means of sequential injection of reagent liquids into a reaction chamber in a pre-programmed manner by means of very simple and reliable squeezing mechanism. In one embodiment, a name-card sized, thin diagnostic device is provided on which one or more reagent reservoirs and one waste chamber are extruded, while those reservoirs and chambers are interconnected with microfluidic channels. The location of each reservoir is carefully determined to make sure the contained liquid is squeezed out sequentially.

For example, a typical sandwich immunoassay requires following steps: the antigen in a sample is bound with the antibody immobilized on the reaction zone. A washing solution is pumped to the reaction zone to flush the sample and unbound antigen. An enzyme labelled antibody solution is pumped to the reaction zone. The antibody is bound to the antigen which is already adhered to the immobilized antibody creating a sandwich structure: antibody-antigen-antibody.

The washing solution is pumped to the reaction zone again to flush the unbound antibody. The last step is to pump the substrate solution to the reaction zone. The substrate molecules react with the enzyme generating luminescence, fluorescence or colour change. The light signal is detected by an appropriate electro-optical detector module.

REAGENT SACK

In order to provide for long term storage of a reagent in a reservoir or blister, a reagent sack is provided as mentioned above. This approach prevents the reagent from migrating along the microfluidic channels; prevents the reagent reacting with any adhesive chemicals and losing activity; and prevents the reagent from being denatured by light and humidity.

The reagent sack is an aluminium foil sack sealed to a polypropylene backing with highly localized induction heating. The reagent sack is made from aluminium foil which is nominally 15μm thick and typically may be up to 50μm thick. The aluminium foil is coated with an inert surface coating material such as lacquer paint.

A polished circular plastic disk former 1010 is provided. In this example the polished circular plastic disk former 1010 has a diameter of 50mm, a thickness of 3mm and is formed from polyacetal or polycarbonate. A rectangular hole 1015 having dimensions of 10mm x 4mm is created by CNC milling. Of course, it will be appreciated that the dimensions of the polished circular plastic disk former 1010 and hole 1015 will need to match the dimensions of the reservoirs within which the reagent sack is to be placed.

The aluminium foil 1020 is placed on the polished circular plastic disk former 1010 and a stamp 1030 made of an elastic silicon rubber is pressed over the rectangular hole 1015.

The aluminium foil 1020 is deformed which creates a container by the force of the stamp 1030.

The reagent is then injected onto the aluminium container.

A piece of polypropylene film 1040 is placed over the aluminium foil 1020.

As shown in Figure 31, a tube shaped copper coil 1050 is aligned over the aluminium foil 1020 and polypropylene film 1040.

Pressure is applied to the tube and a high frequency (typically between 0.1 MHz and 1 MHz) rotating magnetic field is applied to the tube shaped copper coil 1050 for a very short time (typically between 0.1s and 0.01s). Electrons within the aluminium foil 1020 vibrate creating intense heat. Accordingly, a highly localized, short lived heat pulse is generated along the edge of the rectangular hole 1015. This causes the polypropylene film 1040 to be sealed to

the aluminium foil 1020 along the edge by the heat and pressure. The reagent is unaffected by the heat or the magnetic field.

A punch (not shown) is pressed onto the foil and the reagent sack 1060 containing the reagent is obtained, as shown in Figure 32.

The reagent sack 1060 may then be inserted into the blister or reservoir. When the rollers squeeze the reagent sack 1060, the aluminium foil 1020 ruptures and the reagent is released into the blister or reservoir and flows into the microfluidic channel.

QR CODES AND PHONE READER

Figure 33 illustrates an assay card 1100 according to one embodiment. This embodiment has three reagent reservoirs 1120, 1130, 1140 each containing an associated reagent, although more reservoirs may be provided. A sample may be loaded into the assay card 1100 through an inlet and retained in a chamber 1150. A lateral assay strip 1160 is provided such as a lateral immunoassay strip. A split roller arrangement 1170 is provided which moves relative to the assay card 1100 in the direction A and displaces reagents from each reservoir in sequence to react with the sample and seals each microfluidic channel associated with each reservoir, as will be described in more detail below. The split roller arrangement 1170 has a gap to prevent compression of the lateral assay strip 1160. The split roller arrangement 1170 has a comparatively large diameter (in this example 12mm) with thick elastic silicon rubber. This enables the rollers to move back and forth whilst squeezing the reservoirs and squashing the channels. The thickness is influenced by the amount of bare assay card area, the reservoir zone and the fluidic channel zone can be absorbed by the highly elastic silicon rubber.

Each reagent reservoir 1120, 1130, 1140 has a microfluidic channel associated therewith coupled with the chamber 1150. The microfluidic channels associated with the reagent reservoirs 1120, 1130, 1140 are located away from the split roller arrangement 1170 at the bottom or tail of each reservoir such that the microfluidic channels are only contacted by the split roller arrangement 1170 after the associated reagent reservoir 1120, 1130, 1140 has been compressed.

To enable the microfluidic channels associated with the reagent reservoirs 1120, 1130, 1140 to be routed to the chamber 1150 requires routing space adjacent the reagent reservoirs 1120, 1130, 1140. To increase the reagent reservoirs density, the reagent reservoirs 1120,

1130, 1140 are staggered in two or more columns. This provides the space required for routing the microfluidic channels. The split roller arrangement 1170 has an associated roller part which compresses reagent reservoirs within those columns.

A QR code 1110 is provided which provides location and timing information for use by the assay apparatus. The QR code 1110 may be read by the assay apparatus or, in this example, is read by a mobile phone and communicated to the assay apparatus via, for example, a Bluetooth transceiver. In this embodiment, the mobile phone is a LG Gt540 which runs an open source QR code image processing library known as 'zxing'. Zxing is a library that contains a kernel supporting QR code detection. Additional functionality is provided such as a command interpreter, QR code result transmission to the assay apparatus procedures, error handling procedures, lighting control procedures and communication procedures to control the positioning of the assay card to enable, for example, the assay card to be positioned in a location that can be imaged by the mobile phone. The assay apparatus contains a microprocessor running software to receive the information from the mobile phone, to control the operation of the assay apparatus and to send information to the mobile phone using, in this example, a Bluetooth transceiver.

The QR code encodes five items of position information (P1 to P5) and five items of timing information (T1 to T5). In addition, the QR code may encode the length of each or all reservoirs and/or the speed S1 to S3 at which each or all reservoirs should be compressed (although the length and/or speed information may be pre-programmed within the assay apparatus). In addition, the QR code may encode information relating to the width, volume or contents of each reservoir. Of course, it will be appreciated that more or less information may be encoded. In this example, P1 is the distance from the assay card 110 edge to the centre of the QR code 1110; P2 is the distance to edge of the first reservoir 1120; P3 is the distance to edge of the second reservoir 1140; P4 is the distance to edge of the third reservoir 1130; and P5 is the distance from the far edge of the third reservoir 1130 to the detection zone 1160. In the example, {P1,P2,P3,P4,P5} ={22,10,15,15,15} in mm.

The length of the reservoirs will typically be fixed and this length will typically be stored in the assay apparatus. The volume of the reservoir may then be changed by varying the width of the reservoir. However, the assumed length of the reservoirs may be changed by length information encoded in the QR code.

The operation of the assay apparatus and mobile phone will now be described in more detail with reference to Figure 34.

At step S100, the assay card 1100 is inserted into the assay apparatus (denoted as reader in Figure 34).

At step S110, the assay card 1100 is advanced by the distance P1 rapidly to reduce processing time using the rollers 1170. An offset routine applies an offset to this distance which displaces the card by a pre-programmed amount to prevent the QR code 1110 from being obscured by the split roller arrangement 1170.

At step S120, the QR code is read by the mobile phone. After a delay time of T1 to allow mobile phone to interpret the QR code, a message is displayed to the user at step S120 and the position and timing information and any other encoded information such as speed is transmitted to the assay apparatus at step S130. The offset applied by the offset routine at step S110 is then reversed.

At step S140 the assay card 1100 is advanced by P2 rapidly, then advanced slowly at speed S1 for the length L of the reservoir 1120 to squeeze reservoir 1120 and stopped. A signal is then sent to the mobile phone and a message is displayed to the user. The assay apparatus then waits for delay time T2.

At step S150 the assay card 1100 is advanced by P3-L rapidly (which closes the microfluidic channel coupling reservoir 1120 with the chamber 1150), then advanced slowly at speed S2 for the length L of the reservoir 1140 to squeeze reservoir 1140 and stopped. A signal is then sent to the mobile phone and a message is displayed to the user. The assay apparatus then waits for delay time T3.

At step S160 the assay card 1100 is advanced by P4-L rapidly (which closes the microfluidic channel coupling reservoir 1140 with the chamber 1150), then advanced slowly at speed S3 for the length L of the reservoir 1130 to squeeze reservoir 1130 and stopped. A signal is then sent to the mobile phone and a message is displayed to the user. The assay apparatus then waits for delay time T4.

At step S170, the assay card 1100 is ejected by P5 rapidly and stopped. The apparatus then waits for delay time T5 to allow an immunoreaction to occur. A signal is then sent to the mobile phone and a message is displayed to the user.

The user then takes a photo of the detection zone and the mobile phone analyzes the image. Using internal calibration data, the colour signal from the image is converted into a concentration of a target substance in the test sample.

The assay apparatus then ejects the assay card 1100 completely until an optical card sensor (such as a photosensor) detects the assay card edge.

In this example, {T1,T2,T3,T4,T5}={10,15,15,15,300} in seconds.

Although the timing in this embodiment does not take account of the speed of advancing and squeezing the reservoirs, for other embodiments, the speed of advancing and the distance advanced may be taken into account to adjust timings for more time-critical reactions.

The P1 and T1 values may be pre-stored as 22mm and 10s in the firmware or software of the mobile phone. If the QR code is printed in the same way at the same location each time, then these information items may remain constant. To register a new P1 and T1 value, the user will scan the QR code manually and the mobile phone will send this information to the assay apparatus as an update. This P1 and T1 information is then effective for next assay card of the same kind.

It will be appreciated that the QR code technique could equally be applied to the other embodiments mentioned above to provide position information, timing information or other processing information such as when to heat or perform some other operation on the assay card.

ABSORPTIVE JOINT

Figure 35 illustrates an absorptive joint arrangement. Whenever multiple fluidic channels meet, there is a risk of back flow between the fluidic channels which can result in mixing or loss of fluid. Although the sealable channels and double roller arrangements mentioned above can address this problem, these approaches can increase complexity and cost.

Accordingly, an absorptive pad 1200 is provided (which may be provided in the chamber 1150 mentioned above). The absorptive pad 1200 such as a glass wool disk is provided with effectively an infinite flow sink such as a nitrocellulose membrane provided as

a lateral immunoassay strip 1240 in combination with an absorption pad 1250. The absorptive pad 1200 is highly hydrophilic.

In this example, three reservoirs 1210, 1220, 1230 are provided which are coupled with the absorptive pad 1200 via microfluidic channels. The absorptive pad 1200 is provided at the joint of the microfluidic channels coupled to the reservoirs 1210, 1220, 1230.

The absorptive pad 1200 has a flow rate f0. The flow rate f1, f2, f3 provided by the reservoirs 1210, 1220, 1230 is managed by controlling the size and configuration of the reservoirs 1210, 1220, 1230, the microfluidic channels and the speed of the rollers to be less than the absorptive flow rate f0. Hence, the absorptive pad 1200 has the strongest absorption speed, quickly absorbs fluid and slowly releases it to the nitrocellulose membrane that has a lower capillary flow rate. In this way, the absorptive pad 1200 acts as a buffering zone.

Accordingly, the flow that reaches the absorptive pad 1200 is absorbed by the glass wool pad which prevents back flow.

This arrangement is simple and robust, low cost and obviates the need for sealable channels or double rollers.

It will be appreciated that the features of the different embodiments mentioned above can readily be combined together by the skilled person, as appropriate, in other combinations than those explicitly disclosed.

Each of the applications and patents mentioned in this document, and each document cited or referenced in each of the above applications and patents, including during the prosecution of each of the applications and patents ("application cited documents") and any manufacturer's instructions or catalogues for any products cited or mentioned in each of the applications and patents and in any of the application cited documents, are hereby incorporated herein by reference. Furthermore, all documents cited in this text, and all documents cited or referenced in documents cited in this text, and any manufacturer's instructions or catalogues for any products cited or mentioned in this text, are hereby incorporated herein by reference.

Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific

preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

CLAIMS

1. An assay apparatus, comprising:
an assay card comprising a substrate having at least one compressible reagent reservoir thereon, each compressible reagent reservoir containing an associated reagent and each compressible reagent reservoir being coupled with a reaction chamber and a sealable inlet port coupled with said reaction chamber and operable to receive a test sample to be conveyed to said reaction chamber;
an assay card controller comprising a compression mechanism operable to compress each compressible reagent reservoir to cause each associated reagent to be injected in a predetermined order into said reaction chamber to react with said test sample; and
a vacuum device operable to generate a negative pressure at said inlet port to assist receiving said test sample;
wherein said vacuum device comprises a resiliently compressible reservoir containing a sponge in fluid communication with said inlet port operable to receive said test sample.

2. The assay apparatus of Claim 1, wherein each of a plurality of compressible reagent reservoirs is located at predetermined locations along a predetermined actuation path along said assay card to be followed by said compression mechanism.

3. The assay apparatus of Claim 2, wherein said reaction chamber is located along said actuation path and those of a plurality of compressible reagent reservoirs located furthest away from said reaction chamber along said actuation path are compressible to enable injection of associated reagents prior to those of said plurality of compressible reagent reservoirs located closest to said reaction chamber.

4. The assay apparatus of Claim 2 or 3, wherein at least two compressible reagent reservoirs are located at the same predetermined location along said actuation path to enable simultaneous injection of associated reagents into said reaction chamber.

5. The assay apparatus of any one of Claims 2 to 4, wherein said compression mechanism comprises:

a roller operable to move relative to said assay card, along said actuation path.

6. The assay apparatus of Claim 5, comprising a secondary roller operable to move relative to said roller to compress predetermined areas of said assay card.

7. The assay apparatus of Claim 6, wherein said assay card comprises microfluidic channels routed to said predetermined areas to enable selective compression of said microfluidic channels.

8. The assay apparatus of Claim 7, wherein said microfluidic channels are selectively sealable.

9. The assay apparatus of Claim 7 or 8, wherein said assay card comprises:

a plurality of sheets arranged to form a laminate, at least one of said sheets being a thermoplastic having said plurality of compressible reagent reservoirs, said reaction chamber and said microfluidic channels formed thereon.

10. The assay apparatus of Claim 9, comprising a heating element provided on one of said sheets operable to heat said assay card.

11. The assay apparatus of Claim 10, wherein said heating element comprises an electrical heating element and said assay apparatus comprises electrical contacts operable to power said heating element.

12. The assay apparatus of any one of Claims 2 to 11, wherein said assay card controller comprises:

a controller operable to vary speed of movement said compression mechanism along said actuation path.

13. The assay apparatus of any preceding claim, comprising an index detector operable to detect an indexing mechanism provided on said assay card to produce at least one control signal to be provided to said controller.

14. The assay apparatus of Claim 13, wherein said index detector is operable to detect a plurality of indexing mechanisms provided on said assay card to produce a plurality of control signals to be provided to said controller.