

ASSAY

FIELD

The present invention relates to an assay and method.

BACKGROUND

5 Assays for the detection of target substances in a sample are known. For example, a dipstick assay is also known. A dipstick assay is typically a simple one-layer membrane having dried reagents. The dipstick assay is very low cost, and is very easy to make in a narrow and long format which is beneficial for direct urine sampling. However, such dipstick assays provide simple colorimetric biochemical testing for qualitative screening, which means only relatively
10 simple testing can be performed.

 A lateral flow immunoassay (LFI) is widely used in clinical laboratories because of its simplicity, low cost, speed, reliability and ease of use. Such assays do not require electrical power or mechanical drive. Typically, an LFI is used to detect a specific analyte in a liquid sample. An LFI is typically composed of a test strip and a cassette. The core of the test strip is typically a
15 nitrocellulose membrane that is microporous to create lateral capillary flow. In addition, there is a conjugate (typically an antibody and gold nanoparticle complex) release pad and a sample filter on one end, and an absorbent pad on the other end. The arrangement is placed into a cassette, which is typically formed in two parts from plastic pieces. The test strip is typically placed on a first part of the cassette and the second part of the cassette is added and joined to the first part by
20 a friction fit. Lateral capillary flow is a slow process, typically taking 5 to 15 minutes. The provision of the cassette enables the device to be handled during sampling. In some devices, a sampling cup is provided to collect a urine sample. The sample cup requires an additional plastic cup, causing additional cost and an additional sampling step. A long cassette may be provided to enable the urine sample to be collected directly. However, the long cassette is costly because it
25 consumes more plastic material and is wasted after use.

 Although each of these assays enables target substances to be detected, each have their drawbacks. Accordingly, it is desired to provide an improved assay.

SUMMARY

According to a first aspect there is provided a laminated assay card, comprising: a support substrate; an assay strip assembly provided on the support substrate operable to test a sample; and a retaining substrate provided overlying the support substrate and the assay strip assembly to form a laminated structure retaining the assay strip assembly therein.

The first aspect recognises that a problem with the dipstick assay is that the reactions that can be performed are relatively simplistic and, whilst LFI devices can provide more complex reactions, these need to be housed in a complex structure which needs to be mechanically robust and manually assembled.

Accordingly, a laminated assay card is provided. The laminated assay card may comprise a support substrate. An assay strip assembly which test the sample may be provided on the support substrate. A retaining substrate may also be provided. The retaining substrate may overlie both the support substrate and the assay strip assembly to form a laminated structure which retains the assay strip assembly. The substrates and strip assembly may comprise laminar structures to form a laminated card, rather than being provided within a housing. In this way, it can be seen that an assay strip assembly which may perform more complex reactions than a single layer dipstick can be provided without the complex and costly arrangement of a multi-component plastic housing to retain the assay strip assembly therein. Instead, a simple laminated structure may be provided, which may be formed around the assay strip assembly to provide a simple and robust assay. This provides for a simpler mechanical arrangement which is less susceptible to mechanical damage, which reduces the complexity of manufacture and lowers the cost of production since the need for a complex housing is obviated.

In one embodiment, the retaining substrate provided overlying the support substrate and the assay strip assembly encapsulates the assay strip assembly. Accordingly, the retaining substrate and the support substrate together may form a laminate, which encapsulates the assay strip assembly. Encapsulating the assay strip assembly within a laminate provides a simple arrangement having high mechanical integrity.

In one embodiment, the support substrate and the retaining substrate are bonded together. Accordingly, the surfaces of the support substrate and the retaining substrate may be bonded or

adhered together to form the laminated structure. Such bonding facilitates the assembly of the assay and improves its mechanical robustness.

In one embodiment, the laminated assay card comprises an adhesive layer operable to bond the support substrate to the retaining substrate. Accordingly, an adhesive may be provided between the support substrate and the retaining substrate to enable the two substrates to be laminated together.

In one embodiment, the support substrate comprises one of a polymer and cellulose.

In one embodiment, the support substrate comprises paper.

In one embodiment, the support substrate comprises polyvinylchloride.

In one embodiment, the support substrate is planar. Accordingly, the support substrate may comprise a sheet. It will be appreciated that such a configuration provides for simple and convenient automated assembly. The support substrate may be rigid.

In one embodiment, the retaining substrate is generally planar for contact with the support substrate. Accordingly, the retaining substrate may also be a sheet. The retaining substrate may then overlie the support substrate. Providing a retaining substrate in a generally sheet form again simplifies the manufacturing process.

In one embodiment, the laminated assay card comprises a barrier layer provided between the assay strip assembly and the support substrate to isolate the assay strip assembly from the support substrate. Accordingly, the assay strip assembly may be isolated from the support substrate to prevent any contamination from the surrounding structures or any adhesives.

In one embodiment, the assay strip assembly comprises a plurality of components. Accordingly, the assay strip assembly may be formed from a number of separate components. The separate component may comprise a number of individual strips which together form the assay strip assembly. Even though the assay strip assembly may be formed from separate components, placing these within a laminated structure ensures that the components maintain a desired positional relationship and the lamination improves the robustness of the assay card.

In one embodiment, the assay strip assembly comprises at least one reagent layer operable to react with the sample. Accordingly, one or more layers may be provided which may have at least one reagent therein which may react with the test sample. This ensures that the appropriate reagents are provided for the assay at the appropriate locations within the assay card to support the required reaction.

In one embodiment, each reagent layer has reagents therein operable to react with the sample. Accordingly, each reagent layer may have one or more reagents immobilized therein in order to provide the required combination of reagents at the required locations within the assay card.

In one embodiment, the assay strip assembly comprises at least one reservoir. Accordingly, one or more reservoirs may be provided by the assay card for storing fluids therein.

In one embodiment, each reservoir is in fluid communication with at least one reagent layer. Accordingly, fluids may be transferred between the reagent layers or other components of the assay card. It will be appreciated that this provides the flexibility to enable transfer of fluids between areas of the assay card to, for example, enable control over the application of an ordered sequence of reagents, to control the application of the test sample, to remove any intermediate products or to remove any waste fluid.

In one embodiment, the reservoir contacts a surface of at least one reagent layer. Accordingly, a reservoir may be provided in close proximity and contacting to a reagent layer.

In one embodiment, the reservoir overlaps the surface and end at least one reagent layer. Accordingly, the reservoir may contact a large surface area of a reagent layer to maximise fluid transfer.

In one embodiment, the laminated assay card comprises a plurality of compressible reagent reservoirs located at a predetermined locations along a predetermined actuation path along the laminated assay card to be followed by a compression mechanism. Accordingly, the assay card may have a number of compressible, squeezable or squashable reservoirs. Each of the reservoirs may contain an associated reagent and each reservoir may be coupled with the assay strip assembly. Each of the reservoirs may contain an associated reagent. The reservoirs may be

compressed manually or placed into a compression mechanism for automatic compression. The reagents contained therein may then be displaced and provided to the assay strip assembly in a predetermined order. For example, such ordering may enable one reagent to be provided prior to another, one reagent to be provided whilst another one is already being provided, or more than one reagent to be provided substantially simultaneously. It will be appreciated that such an arrangement enables sequential compression of the reservoirs to enable sequential injection of reagents in a predetermined manner using a very simple and reliable squeezing mechanism. The location of each reservoir 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 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. If the reservoirs are to be compressed manually, then a simple indication of the order of compression may be provided to the user. It will be appreciated that this may provide for a simple, convenient and reliable arrangement which supports potentially complicated, multi-stage and time-critical reactions.

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 laminated assay card comprises: a plurality of microfluidic channels operable to couple each of the plurality of compressible reagent reservoirs with the assay strip assembly. The provision of microfluidic channels between each of the reservoirs and the assay strip assembly may help to minimise any inadvertent premixing of 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 assay strip assembly. In one embodiment, the microfluidic channels may couple with components of the assay card other than with the assay strip assembly.

5 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 an assay apparatus 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.

10 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.

15 In one embodiment, the adhesive is a selectively printed adhesive printed in areas on the support substrate. Hence, these selective sealable channels might be the result of printing or not printing adhesive onto the supporting substrate. By using a printed substrate with varying surface properties, selectively sealable and functional channels may be created.

 In one embodiment, the reservoir comprises an inlet reservoir operable to receive the sample. Accordingly, an inlet reservoir may be provided into which the test sample is received.

20 In one embodiment, the inlet reservoir is operable to filter the sample. Accordingly, the inlet reservoir may also filter the test sample to remove any large particles, coagulants or impurities.

 In one embodiment, the inlet reservoir comprises a multiple stage filter operable to filter the sample. Hence, a multiple stage filter may be provided to improve the filtration process.

25 In one embodiment, the reservoir comprises an outlet reservoir operable to receive waste from the assay strip assembly. Accordingly, an outlet reservoir may be provided which receives waste or excess fluid from the assay strip assembly.

In one embodiment, the laminated assay card comprises at least one reagent reservoir operable to receive a reagent. Hence, reservoirs containing predetermined reagents may be provided to enable more complex reactions to take place.

5 In one embodiment, the laminated assay card comprises fluid conduits operable to couple each reagent reservoir with assay strip assembly. Hence, the reagent reservoirs may be coupled with the assay strip assembly to facilitate delivery of the reagents at the appropriate times to appropriate locations.

10 In one embodiment, the retaining substrate comprises a shaped primary recess operable to receive assay strip assembly. Accordingly, to assist in the lamination process, the retaining substrate may be pre-formed into a shape within which the assay strip assembly may be received. This provides for more reliable lamination and improves the mechanical arrangement of the assay strip assembly.

15 In one embodiment, the shaped primary recess is dimensioned to compress assay strip assembly onto the support substrate. Accordingly, as well as the laminated structure retaining the assay strip assembly, the recess may be dimensioned to provide a particular predetermined compression force onto the assay strip assembly to provide for reliable contact between the component parts of the assay strip assembly and to provide for predictable capillary action. It will be appreciated that providing too little compressive force may prevent adequate contact and inhibit capillary action, whilst providing too much compressive force may inhibit the flow of the
20 fluid.

In one embodiment, the retaining substrate comprises shaped secondary recesses operable to define at least one of the reagent reservoirs and the fluid conduits. Accordingly, the reservoirs and/or the conduits may also be formed by the retaining substrate. Such an approach significantly simplifies manufacture by reducing the component count and providing a predictable spatial
25 arrangement between the conduits, reservoirs and assay strip assembly.

In one embodiment, the retaining substrate is vacuum moulded. It will be appreciated that vacuum moulding is a relatively simple and accurate technique for forming the retaining substrate.

In one embodiment, the retaining substrate comprises polypropylene. It will be appreciated that any suitable polymer, paper or laminar may be used.

In one embodiment, the substrate comprises at least one of printed and pre-etched structures formed thereon.

5 In one embodiment, the retaining substrate comprises an inlet hole through which the sample is received. Accordingly, an aperture may be formed to act as an inlet through which the sample may be applied to the assay card.

 In one embodiment, the retaining substrate comprises an outlet hole through which air is vented. Accordingly, an aperture in the retaining substrate may be provided through which air or
10 other fluids may be expelled to facilitate the transfer of fluids through the assay card.

 In one embodiment, the retaining substrate comprises an elongate aperture arranged coincident with the at least one reagent layer. Providing an elongate aperture overlying the assay strip assembly removes a fluid path along which the test sample may track without passing through the assay strip assembly. Such bypassing of the assay strip assembly may occur if the
15 retaining structure fails to completely contact the assay strip assembly. Bypassing the assay strip assembly reduces the effectiveness of the assay card. However, removing at least a portion of the retaining substrate in the vicinity of the assay strip assembly removes this bypass fluid path and increases the amount of fluid passing through the assay strip assembly.

 In one embodiment, the laminated assay card comprises a retaining structure at least
20 partially surrounding the inlet hole operable to retain the sample in a vicinity of the inlet hole. Accordingly, a retaining or barrier structure may be provided in the vicinity of the inlet hole to retain the test sample. Such an arrangement may enable a volume of test sample to be applied to the card and retained in what is effectively a well, which stores the test sample until it is transferred into the assay card. This enables the complete test sample to be administered in one go
25 and helps to avoid any spillage.

 In one embodiment, the retaining structure comprises a protrusion forming a cavity at least partially surrounding the inlet hole. It will be appreciated that forming a protrusion conveniently provides a cavity or well which may retain the test sample.

In one embodiment, the retaining structure comprises an absorbent material operable to absorb excess of the sample. Providing an absorbent material facilitates the retention of any excess test sample that may be provided and avoids spillage.

5 In one embodiment, the laminated assay card comprises a vacuum device operable to generate a negative pressure at the inlet hole to assist receiving the test sample. Accordingly, rather than simply relying on capillary action to administer the test sample to the assay card, the assay card itself may facilitate its application by generating a negative relative pressure at the inlet to draw the test sample into the assay card.

10 In one embodiment, the vacuum device comprises a resiliently compressible reservoir in fluid communication with the inlet hole. 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 test sample.

15 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 test sample therein.

20 In one embodiment, the sponge comprises at least one reagent retained therein. Accordingly, the test sample may be pre-treated with a reagent prior to being provided to the assay strip assembly. Such a pre-treatment reagent may comprise an anticoagulant when testing blood samples.

25 In one embodiment, the resiliently compressible reservoir is in fluid communication with the at least one reagent layer. Hence, the test sample may be provided to the assay strip assembly from the reservoir. This may occur following sealing of the inlet hole, upon further compression of the resiliently compressible reservoir.

In one embodiment, the laminated assay card comprises a flow preventer operable to selectively fluidly decouple the at least one reagent layer from at least one of the 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 initial loading of the test sample. This helps to create the required pressure differential to facilitate loading of the test sample and to prevent any uncontrolled mixing with reagents within the card.

In one embodiment, the flow preventer is removable to fluidly couple the at least one reagent layer with the at least one of the reagent reservoirs and the resiliently compressible reservoir. The flow preventer may be removed once the inlet has been sealed to enable the test 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 channels coupling the at least one reagent layer with the at least one of the reagent reservoirs and the resiliently compressible reservoir. Compressing the channel may effectively block those channels.

In one embodiment, the laminated assay card comprises an indicator encoded on the laminated assay card operable to provide an indication of at least one of a configuration of the laminated assay card, instructions for using the laminated assay card, user identification, physician identification and a date of dispensing. Accordingly, one or more indicators may be provided on the assay card. The indicators may indicate the assay card type, its contents, the intended user of the card, the physician who dispensed the card, a dispensing date and/or a use-by date.

In one embodiment, the indicator comprises at least one of a barcode, a QR-code, and a radio frequency identifier tag. It will be appreciated that any suitable indication means may be provided in order to impart the necessary information either optically, electrically or magnetically, thereby facilitating data collection.

In one embodiment, the laminated 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.

According to a second aspect, there is provided a method of manufacturing a laminated assay card, the method comprising the steps of: (i) providing a support substrate; (ii) positioning
5 an assay strip assembly operable to test a sample on the supporting substrate; and (iii) overlying a retaining substrate on the support substrate and the assay strip assembly to form a laminated structure retaining the assay strip assembly therein.

In one embodiment, the method of manufacture comprises a continuous roll-on-roll process.

10 In one embodiment, the step (i) comprises providing the support substrate from a support substrate roll and the step (iii) comprises providing the retaining substrate from a retaining substrate roll.

In one embodiment, the method comprises the step of assembling the assay strip assembly from a number of layers provided by a corresponding number of rolls.

15 In one embodiment, the method comprises the step of cutting said assay strip assembly into individual strips for positioning at the step (ii).

In one embodiment, the step (iii) comprises encapsulating the assay strip assembly with the retaining substrate and the support substrate.

20 In one embodiment, the step (iii) comprises bonding the support substrate to the retaining substrate.

In one embodiment, the step (iii) comprises bonding the support substrate to the retaining substrate with an adhesive layer.

In one embodiment, the support substrate comprises one of a polymer and cellulose.

In one embodiment the support substrate comprises paper.

25 In one embodiment, the support substrate comprises polyvinylchloride.

In one embodiment, the support substrate is planar.

In one embodiment, the method comprises the step of: placing a barrier layer between the assay strip assembly and the support substrate to isolate the assay strip assembly from the support substrate.

5 In one embodiment, the method comprises the step of: assembling the assay strip assembly from a plurality of components.

In one embodiment, the assay strip assembly comprises at least one reagent layer operable to react with the sample.

10 In one embodiment, each reagent layer has reagents therein operable to react with the sample.

In one embodiment, the step of assembling comprises providing at least one reservoir.

In one embodiment, each reservoir comprises a reagent sack operable to receive an associated reagent.

15 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.

20 In one embodiment, the step of assembling comprises positioning the reservoir in fluid communication with at least one reagent layer.

In one embodiment, the step of assembling comprises contacting the reservoir with a surface of at least one reagent layer.

25 In one embodiment, the step of assembling comprises overlapping the reservoir with the surface and an end at least one reagent layer.

In one embodiment, the method comprises providing a plurality of compressible reagent reservoirs located at a predetermined locations along a predetermined actuation path along the laminated assay card to be followed by a compression mechanism.

5 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 assay strip assembly.

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.

10 In one embodiment, the method comprises the step of: selectively printing adhesive on the support substrate to create said adhesive sealable portions. Hence, these selective sealable channels might be the result of printing or not printing adhesive onto the supporting substrate. By using a printed substrate with varying surface properties, selectively sealable and functional channels may be created.

15 In one embodiment, the step of assembling comprises providing an inlet reservoir operable to receive the sample.

In one embodiment, the inlet reservoir is operable to filter the sample.

In one embodiment, the step of assembling comprises providing the inlet reservoir as a multiple stage filter operable to filter the sample.

20 In one embodiment, the step of assembling comprises providing an outlet reservoir operable to receive waste from the assay strip assembly.

In one embodiment, the step of assembling comprises providing at least one reagent reservoir operable to receive a reagent.

In one embodiment, the method comprises the step of: forming a shaped primary recess operable to receive assay strip assembly in the retaining substrate.

In one embodiment, the step of forming comprises forming the shaped primary recess dimensioned to compress assay strip assembly onto the support substrate.

5 In one embodiment, the method comprises the step of: forming shaped secondary recesses operable to define at least one of the reagent reservoirs and the fluid conduits in the retaining substrate.

In one embodiment, the step of forming comprising the step of: vacuum moulding the retaining substrate.

In one embodiment, the retaining structure comprises polypropylene.

10 In one embodiment, the method comprises the step of forming at least one of printed and pre-etched structures on the substrate.

In one embodiment, the method comprises the step of: forming an inlet hole in the retaining substrate through which the sample is received.

In one embodiment, the method comprises the step of: forming an outlet hole through which air is vented.

15 In one embodiment, the method comprises the step of: forming an elongate aperture arranged coincident with the at least one reagent layer.

In one embodiment, the method comprises the step of: forming a retaining structure at least partially surrounding the inlet hole operable to retain the sample in a vicinity of the inlet hole.

20 In one embodiment, the step of forming a retaining structure comprises forming a protrusion forming a cavity at least partially surrounding the inlet hole.

In one embodiment, the method comprises the step of: placing an absorbent material operable to absorb excess of the sample in the retaining structure.

25 In one embodiment, the method comprises the step of: forming a vacuum device operable to generate a negative pressure at the inlet hole to assist receiving the sample.

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

5 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 method comprises the step of: placing a sponge operable to receive the test sample in the reforming device.

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

10 In one embodiment, the resiliently compressible reservoir is in fluid communication with the at least one reagent layer.

In one embodiment, the method comprises the step of: forming a flow preventer operable to selectively fluidly decouple the at least one reagent layer from at least one of the reagent reservoirs and the resiliently compressible reservoir.

15 In one embodiment, the method comprises the step of: placing an indicator encoded on the laminated assay card operable to provide an indication of at least one of a configuration of the laminated assay card, instructions for using the laminated assay card, user identification, physician identification and a date of dispensing.

In one embodiment, the indicator comprises at least one of a barcode, QR-code, and a radio frequency identifier tag.

20 In one embodiment, the method comprises the step of: corona treating the laminated assay card prior to the step of placing the indicator.

25 In one embodiment, the method comprises the step of: placing a fluid absorber 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.

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.

5 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
10 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
15 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,
20 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
25 incorporated by reference.

 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.
30 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 a laminated assay card according to one embodiment;
- Figure 2 is a cross-sectional view of the laminated assay card shown in Figure 1;
- Figure 3 is a view of the laminated assay card shown in Figure 1 held by a user;
- Figure 4 illustrate manufacturing of the test strip assembly;
- Figure 5 illustrate manufacturing of the retaining substrate;
- Figure 6 illustrate manufacturing of the laminated assay card shown in Figure 1;
- Figure 7 illustrates a laminated assay card according to one embodiment;

Figure 8 illustrate manufacturing of the retaining substrate of the laminated assay card shown in Figure 7;

Figure 9 illustrates a laminated assay card according to one embodiment;

Figure 10 illustrates an arrangement of a test sample loading device according to one
5 embodiment;

Figure 11 illustrates an arrangement of a sealable channel according to one embodiment;

Figure 12 illustrates an example operation of the test sample loading device of Figure 10 utilising the sealable channel of Figure 11;

Figure 13 illustrates the formation of a reagent blister according to one embodiment;

10 Figure 14 illustrates sealing the reagent blister of Figure 13;

Figure 15 is a perspective view of the reagent blister of Figure 13;

Figure 16 illustrates an assay card according to one embodiment;

Figure 17 is a flow chart showing interactions between an assay apparatus and mobile phone according to one embodiment;

15 Figure 18 illustrates an absorptive joint arrangement;

Figure 19 illustrates an assay apparatus;

Figure 20 illustrates an example double roller arrangement;

Figures 21A to 21C illustrates an example operation of the double roller arrangement;

20 Figure 22 illustrates an arrangement of a channel having sealable and non-sealable portions according to one embodiment; and

Figure 23 illustrates an assay card according to one embodiment.

DETAILED DESCRIPTION

EXAMPLE 1

Figure 1 illustrates a laminated assay card, generally 10, according to one embodiment. The laminated assay card comprises a Polyvinyl chloride (PVC) support substrate 20 on which a test strip assembly 30 is disposed, overlying which is a polypropylene film retaining substrate 40. In other embodiments, the support substrate may comprise a cellulose substrate such as paper. The retaining substrate 40 overlies the test strip assembly 30 and bonds with the support substrate 20 to form a laminated structure, as will be described in more detail below.

The major components of the test strip assembly 30 are a cellulose filter 50, a glass wool pad 60, a nitrocellulose membrane 70, a cellulose absorbent pad 80, provided on a PVC backing layer (not shown).

As also shown in Figure 1, an indexing hole 90 is also provided to provide positional information to a reading or actuating apparatus.

A QR-code 100 is provided which may provide a variety of information to a reader.

Alternatively, or additionally, a barcode or other printed material may be provided to provide a variety of information to the reader. The QR-code 100 may indicate to the reader the type of assay card so that the reader may control environmental conditions to facilitate any reactions expected to occur on the laminated assay card 10. The information may provide details of how and where, in relation to the index hole 90 or a card edge, to read the test result from the nitrocellulose membrane 70. The information may also provide details of how and where, in relation to the index hole 90 or a card edge, reagent reservoirs are located as well as squeezing speed information, reaction timing information and/or other information used to control an assay apparatus which processes the assay card. In addition, the QR-code 100 may provide details of the patient so that the result may be automatically associated with the patient's record. Furthermore, the QR-code 100 may provide details of the patient's physician or the entity which dispensed the laminated assay card 10, in order that the results may be automatically provided to those entities. The QR-code 100 may include a "best before" indicator which may be checked by the reader to either reject or raise an alert, should an out of date assay card be used.

Figure 2 is a cross-sectional view of the laminated assay card 10 shown in Figure 1. As can be seen, the support substrate 20 has an overlying adhesive layer 110. The assay strip assembly 30 is provided on top of the adhesive layer 110.

The assay strip assembly 30 comprises a 200µm PVC isolation layer 120 on which is provided a 50µm adhesive layer 130. Overlying the adhesive layer 130 is the nitrocellulose membrane 70. In this embodiment, the nitrocellulose membrane 70 has a thickness of 50µm. The glass wool pad 60 is in fluid contact with the nitrocellulose membrane 70 and is disposed towards a first end thereof. In this embodiment, the glass wool pad 60 has a thickness of 200µm and is supported by the PVC isolation layer 120. The glass wool pad 60 abuts one end of the nitrocellulose membrane 70 and, in this embodiment, extends over a major surface thereof in order to increase the contact area between the glass wool pad 60 and the nitrocellulose membrane 70. The cellulose filter 50 has a thickness of 500µm and is also supported by the PVC isolation layer 120. The cellulose filter 50 (in this example, a cellulose blood filter) abuts one end of the glass wool pad 60 and, in this embodiment, extends over a major surface thereof in order to increase the contact area between the cellulose filter 50 and the glass wool pad 60. The nitrocellulose membrane 70 is an elongate structure. Disposed towards a second end of the nitrocellulose membrane 70 is the cellulose absorbent pad 80. The cellulose absorbent pad 80 abuts the second end of the nitrocellulose membrane 70 and, in this example, extends over a major surface thereof in order to increase the contact area between the cellulose absorbent pad 80 and the nitrocellulose membrane 70.

Overlying the support substrate 20 and the assay strip assembly 30 is the polypropylene retaining substrate 40. In this example, the polypropylene retaining substrate 40 has a thickness of 30µm. The polypropylene retaining substrate 40 may be either applied over the surface and then heated to mould over the assay strip assembly 30 or may be vacuum moulded, as will be explained in more detail below. The polypropylene retaining substrate 40 bonds with the adhesive layer 110 to form a laminated structure which retains the assay strip assembly 30 therein.

To enable a test sample to be applied to the laminated assay card 10, an aperture (not shown) is formed as an inlet in the polypropylene retaining substrate 40 in the region of the cellulose filter 50. Such an inlet may be formed by a laser beam scanner to selectively cut out the

required area. The laser source can be a CO₂ laser, a neodymium-doped yttrium aluminium garnet (ND-YAG) laser and, preferably, a diode-pumped solid-state (DPSS) laser. In addition, an aperture (not shown) may be formed as an outlet in the retaining substrate 40 in the vicinity of the cellulose absorbent pad 80. Such an outlet aperture may provide an outlet for fluids, such as any displaced air or excess fluid received at the cellulose absorbent pad 80 from the nitrocellulose membrane 70.

The nitrocellulose membrane 70 is intended to operate as an open flow capillary channel, which means that the channel has no covering layer and so an elongate aperture (not shown) is provided over the nitrocellulose membrane 70. This arrangement prevents an alternative flow path from the first end of the nitrocellulose membrane 70 to the second end of the nitrocellulose membrane 70 which may otherwise occur at an interface between the surface of the nitrocellulose membrane 70 and the polypropylene film 40 has the elongate aperture not been formed.

EXAMPLE OPERATION

In overview, a test sample is applied through the inlet in the polypropylene retaining substrate 40 and onto the cellulose filter 50. The test sample is absorbed into the cellulose filter 50. The cellulose filter 50 filters out any major coagulants or impurities and the filtered fluid flows under capillary action to the glass wool pad 60.

The glass wool pad 60 will typically contain a dried reagent which reacts with the filtered fluid. The filtered fluid and reagent combination flows under capillary action into the nitrocellulose membrane 70.

The filtered fluid and reagent combination flows from the first end of the nitrocellulose membrane 70 to the second end of the nitrocellulose membrane 70 under capillary action and is received in the cellulose absorbent pad 80. The reagent reacts with any target substances within the sample. Typically, a visual indication of whether a target substance was present or not within the sample can be deduced by examining the surface of the nitrocellulose membrane 70. For example, the surface of the nitrocellulose membrane 70 may undergo a measurable change in optical characteristics as a result of a reaction between the target substance and the reagent. A user, a reader or a trained individual may then examine the laminated assay card 10 to determine whether a positive or negative result is indicated.

EXAMPLE - PREGNANCY TEST

In an example arrangement of the laminated assay card 10 when used as a pregnancy test, the human chorionic gonadotrophin (hCG) hormone level in a urine sample is measured by the laminated assay card 10. An anti-hCG antibody is immobilised on the nitrocellulose membrane 70. Another anti-hCG antibody is conjugated with gold nanoparticles by ionic interaction to produce a conjugate solution. The conjugate solution is dropped over the glass wool pad 60 and dried. The dried glass wool pad 60 stores the dried antibody.

In this embodiment, the cellulose filter 50 is not necessarily required and so the urine sample may be placed on the glass wool pad 60. This hydrates the dried antibody which dissolves into the urine sample. The hCG hormone in the urine sample binds to the antibody that is labelled with gold nanoparticles yielding an antigen-antibody-gold complex. The complex flows along the nitrocellulose membrane 70 and eventually meets the immobilised antibody. The hCG hormone in the complex binds to the immobilised antibody and the resulting gold nanoparticles are captured on the immobilised antibody. The gold nanoparticles show a red colour because of their nanometre diameter. A zone or band of immobilised antibody area shows a red colour whenever the antibody binds to the antigen-antibody-gold complex. Accordingly, a positive pregnancy test result is shown by the presence of a red zone or band on the nitrocellulose membrane 70, whereas the absence of such a red zone indicates no pregnancy.

Anti-hCG from mouse and anti-mouse IgG can be sourced from AbCam, UK. Gold nanoparticles are synthesized by adding citric acid on boiling chloroauric acid. Bovine serum albumin, tween-20, trehalose, sucrose can be sourced from Sigma, USA.

As can be seen in Figure 3, the resultant laminated assay card 10 can be produced in a convenient size with sufficient space to provide any information which may be required thereon. In embodiments, an inkjet printer is used to print solvent-based ultraviolet-curing ink. Likewise, adhesives, structures or compounds may be printed onto the substrates. Alternatively, a label may be stuck onto the surface of the laminated assay card 10 or introduced during lamination to ensure that the label cannot be removed. The information printed on the card may be a mixture of bar codes or QR-codes, as well as textual or pictorial information relating to the laminated assay card 10, the user and/or the dispensing entity.

MANUFACTURING PROCESS

OVERVIEW

Figures 4 to 6 illustrate an example of manufacturing process for producing the laminated assay card 10. In this manufacturing process, the lateral immunoassay in the form of the test strip assembly 30 is produced using a conventional continuous roll-to-roll process. The assay strip assembly 30 is then placed on the support substrate 20 and adhered to the support substrate 20 by the adhesive layer 110. It will be appreciated that the adhesive layer 110 may be either a laminated sheet or a spray adhesive. To provide a covering, the retaining substrate 40 is used. The retaining substrate 40 is moulded and then laminated onto the adhesive layer 110 of the support substrate 20. The support substrate 20 provides mechanical support and the retaining film 40 provides a protective surface as well as apply a clamping force to the assay strip assembly 30.

Taking the hCG pregnancy example mentioned above, the production process is divided into an upstream process and a downstream process. In the upstream process the assay strip assembly 30 is fabricated, whilst the downstream process involves support substrate 20 preparation (printing, die cutting and adhesive deposition), retaining substrate 40 moulding, assembly, in-situ printing and packaging. The upstream roll-to-roll process for production of the assay strip assembly 30 is well known and extensive literature on these processes have been published by Millipore which is part of the Merck group.

UPSTREAM PROCESS

In this example, six rolls of raw material are provided: a PVC roll providing the isolation layer 120, a double-sided adhesive tape roll providing the adhesive layer 130, a 25mm wide nitrocellulose roll from Millipore, USA providing the nitrocellulose membrane 70, a glass wool roll from Kin Bio, China providing the glass wool (conjugate release) pad 60, a cellulose roll from Kin Bio, China providing the cellulose filter 50, and a cellulose roll from Kin Bio, China providing the cellulose absorbent pad 80.

The isolation layer 120 is laminated with the adhesive layer 130. Alternatively, the isolation layer 120 may be sprayed with an adhesive. Then the nitrocellulose membrane 70 is laminated on the adhesive layer 130. Then the glass wool pad 60 is laminated at one end of the nitrocellulose membrane 70. In this example there is a 2mm overlap with the nitrocellulose

membrane 70. The cellulose absorbent pad 80 is laminated in the same manner with the opposing end of the nitrocellulose membrane 70. The cellulose filter 50 is then laminated over the glass wool pad 60.

5 Then an antibody solution is printed along the nitrocellulose membrane 70 to provide test and control signal bands by means of fine spray or jetting dispensing valve (not shown). The assay strip assembly 30 can then be cut into strip form, or can be wound to make an uncut sheet for use in the downstream process.

DOWNSTREAM PROCESS

10 As mentioned above, the downstream process is composed of the preparation of the support substrate 20, the moulding of the retaining substrate 40, laser beam trimming, assembly, printing, die cutting and packaging.

Support Substrate Preparation

15 For this process, a roll laminator machine is used. A 200 μ m thick, 50mm wide, 50m long PVC roll from Daeyoung Chemical, Seoul, Korea providing the support substrate 20 is mounted on a feed roller. A 50mm wide, 50m long double-sided acrylic based adhesive tape roll from Soogwang Chemical, Korea providing the adhesive layer 110 is mounted on another feed roller. When the feed rollers turn in synchronization, the support substrate 20 and adhesive layer 110 are merged and fed to a hot roll laminator, producing an adhesive coated PVC sheet continuously 20, 110.

20 A robotic pick and place machine beside the hot roll laminator picks a test strip assembly 30 and places this on the adhesive coated PVC sheet 20, 110. The test strip assembly 30 is placed at a specific position which will be aligned with one or more apertures moulded into the retaining substrate 40, as will be described in more detail below. The supporting substrate 20 with test strip assembly 30 is now ready for the next stage in the production process.

25 RETAINING SUBSTRATE MOULDING

A vacuum-moulding machine is used to mould the retaining substrate 40. Although it would be possible to simply place the retaining substrate 40 over the support substrate 20 and test strip assembly 30 produced by the previous stage and heated to effectively be shrink wrapped,

reliability and consistency of the laminated assay card 10 can be improved by moulding the retaining substrate 40. Such moulding enables a predetermined compressive force to be applied to the test strip assembly 30 and enables other structures to be formed on the laminated assay card 10, as will be described in more detail below. This removes the need for an external casing or clamping force that is common in prior arrangements.

In this example, as shown in figure 5, a 30 μ m thick, 490m long, 100mm wide polypropylene film from Daeyoung Chemical, Seoul, Korea is provided as the retaining substrate 40 and is mounted on a feed roller. The retaining substrate 40 is fed to a jig mounted on a vacuum mould 210. A hot air blower 200 is mounted over the jig and a strong vacuum pump 220 is coupled with the vacuum mould 210.

The retaining substrate 40 is fed to the jig, hot air is blown by the hot air blower 200 and vacuum suction is applied to the vacuum mould 210 by the vacuum pump 220. The retaining substrate 40 deforms, yielding an engraved pocket structure. Any shape of pocket structure can be constructed by changing the vacuum mould 210. The length and width of the pocket structure is dimensioned to fit the test strip assembly 30. The depth of the pocket structure will vary because the height of the test strip assembly 30 is not uniform across its complete length. The most important depth has been found to be the joint section where the glass wool pad 60 and the nitrocellulose membrane 70 overlap.

By increasing or decreasing the depth, the clamping force applied to the test strip assembly 30 can be adjusted. If tight clamping is necessary, then the depth can be decreased. The tension applied by the retaining substrate 40 creates a clamping force to secure capillary flow at, for example, an overlap between components of the test strip assembly 30. The overlap between the nitrocellulose membrane 70 and the absorbent pad 80 also requires clamping force. This clamping force can also be adjusted in the same manner.

In order to create various apertures in the retaining substrate 40, laser beam ablation using a laser beam scanner is performed. A hole is created in the retaining substrate 40 in the vicinity of the retaining substrate 40 which will overlie the cellulose absorbent pad 80. A wide opening is made in the retaining substrate 40 in the vicinity of the retaining substrate 40 which will overlie the filter 50. An area is also cut out from the retaining substrate 40 where this will overlie the

nitrocellulose membrane 70. Once these operations have been performed, then the retaining substrate 40 is ready to enter the assembly process.

ASSEMBLY

5 The assembly process uses two die-cutting presses, a robotic laminator and a non-contacting inkjet printer. As shown in Figure 6, whilst the retaining substrate 40 is still in the vacuum mould 210, the support substrate 20 with the test strip assembly 30 is positioned over the vacuum mould 210 in alignment with the pocket structure.

10 The test strip assembly 30 is placed into the pocket structure and the robotic laminator presses the adhesive layer 110 tightly against the retaining substrate 40. The robotic laminator squeezes the support substrate 20 with a roller to make a tight seal. The retaining structure 40 sticks to the adhesive layer 110 on the support substrate 20. Then the assembled laminated assay card 10 is removed from the mould 210 and turned 180° to face upwards.

PRINTING

15 An inkjet printer then prints typically a solvent-based UV-curing ink QR code on the laminated assay card 10. If necessary, other instructions or information can be printed as well.

DIE CUTTING

The laminated assay card 10 is then fed to the two die-cutting press which cuts out the laminated assay card 10. The cut out laminated assay card 10 is collected on a conveyor for packaging.

20 **PACKAGING**

The laminated assay card 10 is then inserted into a protective pouch and sealed with a hot sealer. Dry nitrogen gas can be inserted to improve reagent stability, if necessary.

EXAMPLE 2 - REAGENT BLISTERS

25 As mentioned above, other structures may be moulded into the retaining substrate 40 to improve the functionality of the laminated assay card.

Figure 7 illustrates a laminated assay card 10A according to one embodiment. This embodiment is similar to that mentioned above. However, in this embodiment, two reagent

reservoirs or blisters 300, 310 are provided which are coupled via fluidic channels 305, 315 with the nitrocellulose membrane 70 of the test strip assembly 30.

This arrangement enables additional reagents to be introduced, when required, to provide for more complicated reagent reactions. Although this embodiment shows two reagent reservoirs 300, 310, any number of reagent reservoirs may be provided. Each of the reagents may be placed within a balloon or similar burstable structure in order to retain the reagents within the reservoirs 300, 310 until required.

The reagent reservoirs 300, 310 may be located at different locations on the laminated assay card 10A in the direction A in order that the laminated assay card 10A may be placed into an apparatus (such as that illustrated in Figure 19) which automatically actuates the reservoirs 300, 310 by way of a compression mechanism such as one or more rollers which extend in the direction of the width of the laminated assay card 10A and which move relative to the laminated assay card 10A in the direction A. Alternatively, the reservoirs may be actuated by hand. As the reservoirs 300, 310 are compressed, the reagents contained within are expelled through the fluidic channels 305, 315 to the nitrocellulose membrane 70.

REAGENT DELIVERY

Although the laminated assay card 10A shown in Figure 7 and other embodiments shows both the reagents being delivered to the nitrocellulose membrane 70, it will be appreciated that this need not be the case. In particular, the arrangement of the reservoirs and the fluidic channels may be such that reagents may be delivered to other parts of the laminated assay card 10A. For example, one or more reagents may be delivered to the glass wool pad 60, cellulose filter 50, a calibration window or other parts of the laminated assay card 10A instead. Alternatively or additionally, one or more reagents may be delivered simultaneously to both the glass wool pad 60 and the nitrocellulose membrane 70, cellulose filter 50, a calibration window or other parts of the laminated assay card 10A by providing multiple fluidic channels from a single reservoir.

Figure 8 schematically illustrates an additional processing step required during production where the reservoirs 300, 310 are filled by a reagent-providing device 230 which occurs after moulding the reagent reservoirs 300, 310 into the retaining substrate 40.

EXAMPLE 3 - SPONGE BLISTER

As mentioned above, other structures may be moulded into the retaining substrate 40 to improve the functionality of the laminated assay card.

Figure 9 illustrates a laminated assay card 10B according to one embodiment. This embodiment is similar to that mentioned above. However, in this embodiment, two reagent reservoirs or blisters 300, 310 are provided which are coupled via fluidic channels 305, 315 with the nitrocellulose membrane 70' of the test strip assembly 30'. Although this embodiment shows two reagent reservoirs 300, 310, any number of reagent reservoirs may be provided. Each of the reagents may be placed within a balloon or similar burstable structure in order to retain the reagents within the reservoirs 300, 310 until required. As with Figure 7, the reagent reservoirs 300, 310 may be located at different locations on the laminated assay card 10B.

A reservoir 400 is provided on an assay card which is operable to generate a negative pressure, as illustrated in more detail in Figure 10. Such an arrangement will typically be provided at an inlet for loading a test sample 430 into the assay card, such as the inlet 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 an inlet port 420 via a conduit, such as a fluidic 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 fluidic channel 410.

At (ii), a liquid such as, for example, the 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 11 illustrates an example arrangement and operation of the inlet 420 for receiving the test sample 430 according to one embodiment. The inlet 420 may be an example of the inlet mentioned above. The inlet 420 is coupled via the fluidic conduit 410 with the reservoir 400. A fluidic conduit 435 couples the microfluidic conduit 410 with, for example, the glass wool pad 60'. A removable sealing device 440 is provided to intermittently seal the fluidic conduit 435 to prevent flow of fluid to the glass wool pad 60'. The removable sealing device 440 may comprise a clamp which is held onto the surface of or passes through the laminated assay card 10B.

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 fluidic conduit 410 is sealed either by heat-sealing, mechanical sealing or by the adhesive sealing technique mentioned in Figure 12 below. Once the fluidic conduit 410 has been sealed, the removable sealing device 440 is removed. This enables the reservoir 400 to be coupled with the glass wool pad 60'.

At (iii), the reservoir 400 is compressed, the contents flow through the fluidic conduit 410 but are prevented from escaping through the inlet port 410 by the sealed fluidic conduit. However, the absence of the clip 440 enables the fluid to flow towards the glass wool pad 60'.

It will be appreciated that the compression of the reservoir 400 may occur either by operation of a user or may occur by activation of a compression mechanism mentioned above.

SEALABLE CONDUITS

In order to provide additional control of the flow of fluids within the laminated assay card 10B to prevent, for example, pre-mixing or back-flow into previously-emptied areas, various mechanisms are provided. These include providing self-sealable fluidic conduits and fluidic 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 laminated assay card 10B or any of the laminated assay cards mentioned.

Figure 12 illustrates a sealable conduit. In this example, the sealable conduit may comprise any of the fluidic channels formed on the assay card. An adhesive may be provided on either of the opposing surfaces of the fluidic channels. In this example, an adhesive layer in the

form of a double-sided adhesive tape is provided on the surface of the support substrate 20, prior to the retaining substrate 40 being placed thereon. When the fluidic channel 180 is compressed, in this example by the movement of a 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 22 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 underlying layer or just in required locations. The tape 450 has slitted holes 460 formed therein, typically by laser beam ablation. When the overlying layer 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.

In another embodiment, the adhesive is a selectively printed adhesive printed in areas on the support substrate. These selective sealable channels are defined by printing or not printing adhesive onto the supporting substrate. By using a printed substrate with varying surface properties, selectively sealable and functional channels may be created.

INLET WELL

In order to accommodate excess biological sample being applied to the inlet, a U-shaped or horseshoe sample retention arrangement is provided in the vicinity of the inlet. This arrangement provides a raised area in the retaining substrate 40 in a U-shaped formation around the inlet aperture formed during vacuum moulding.

In one embodiment, an outer U-shaped formation of cotton wool or other absorbent material is provided. With this configuration, a quantity of sample can be applied to the inlet, and if too much has been applied, it flows over the dyke and gets absorbed by the outer U-shaped absorbent material.

In another embodiment, the outer U-shaped absorbent material is under an outer raised portion of the retaining substrate 40. This provides two U-shaped raised areas, one within the other, with the outer U-shaped area enclosing the absorbent material. Holes (pinholes, or rectangular shaped holes, etc) are cut into the retaining substrate 40 to allow the sample to be

absorbed by the absorbent material. Thus, if too much test sample is applied, it flows over the dyke. The excess test sample then flows into the holes cut into the top layer and gets absorbed by the absorbent material.

In another embodiment, only a single U-shaped formation is provided within a raised portion of the retaining substrate 40, within which is enclosed absorbent material. Here, the holes cut into the top layer are on the leeward side of the U-shape, so that any excess test sample flows over the dyke and gets absorbed by the absorbent material through the leeward cut holes.

Each of these embodiments avoids the need to precisely measure the amount of test sample that needs to be applied to the inlet. More than the required amount can be applied for the reaction to occur and any excess gets soaked up. This approach avoids mess and possible contamination through spillage.

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.

As shown in Figure 13, 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.

5 As shown in Figure 14, 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
10 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
15 reagent is obtained, as shown in Figure 15.

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

20 Figure 16 illustrates a laminated 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 laminated 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 and the laminated
25 assay card 1100 moves relative to split roller arrangement 1170 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 laminated assay card to enable, for example, the laminated 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 laminated assay card 1100 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.

It will be appreciated that the location of the reagent reservoirs, the chamber 1150 and the lateral assay strip 1160 may be changed to suit the needs of the reaction to be undertaken and the methodology of actuating the laminated assay card.

The operation of the assay apparatus and mobile phone will now be described in more detail with reference to Figure 17.

At step S100, the laminated assay card 1100 is inserted into an assay apparatus (denoted as reader in Figure 17).

At step S110, the laminated 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 laminated assay card 1100 is advanced by P2 rapidly, then advanced
5 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 laminated assay card 1100 is advanced by P3-L rapidly (which closes the
10 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 laminated assay card 1100 is advanced by P4-L rapidly (which closes the
15 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 laminated assay card 1100 is ejected by P5 rapidly and stopped. The
20 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 laminated assay card 1100 completely until an optical
25 card sensor (such as a photosensor) detects the laminated 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 18 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 f_0 . The flow rate f_1 , f_2 , f_3 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 f_0 . 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
5 pad which prevents back flow.

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

ASSAY APPARATUS

Figure 19 illustrates an arrangement of an assay apparatus according to one embodiment.
10 The assay apparatus 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 are shown with its cover removed to help improve clarity.

DETECTORS

In this example, the assay apparatus comprises an optical source 2040 and an optical
15 detector 2050, although other types of source and detector may be provided dependent upon the characteristics of the particular reactions that are intended to occur, such as the mobile phone mentioned above. Accordingly, the optical properties of the fluid resulting from the reaction occurring within the reaction chamber can be determined when the aperture 2030 is aligned with the optical source 2040 and the optical detector 2050. Typically, the concentration of the target
20 agent can be determined from these optical characteristics and an indication of the presence of the target agent can be provided on the display 2060. 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 laminated assay card. The electrochemical detector then measures electrochemical characteristics of the
25 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 2060.

ROLLERS

In overview, the operation of the assay apparatus is as follows. An assay card having a test sample provided therein is introduced into the apparatus between a pair of polymer-coated rollers 2070. A switch 2080 is activated which sends a signal to a roller controller 2090 to drive the pair of rollers 2070 using a motor within the roller controller 2090 via the gear train 2100. The motor may be a stepper motor or may be provided with a position indicator to enable the roller controller 2090 to provide accurate control. The presence of the gear train 2100 causes each roller within the pair 2070 to counter rotate and slowly draw the laminated assay card between the rollers in the direction A. As will be explained in more detail below, this causes the reservoirs on the laminated assay card to be compressed sequentially to cause reagents therein to be released into the reaction chamber simultaneously and/or in series.

Figure 20 illustrates an example double roller arrangement which may be used in place of the single pair of rollers 2070 mentioned above. In the double rollers 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 laminated assay cards 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 21A to 21C.

As can be seen in Figures 21A to 21C, 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 laminated assay card. Each roller may even be formed of a number of separate rollers having gaps inbetween. 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 laminated assay card 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 21A, 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 21B, 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 21C, 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 21C 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 which interact with indexing on the laminated assay card 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 laminated assay card is sensed. The different photodetectors may be used to generate different signals such as pausing for different amounts of time, causing the laminated assay card to be heated, vibrated or subjected to other stimulus. Placing apertures in the laminated assay card at appropriate locations will enable signals to be produced by the photodetectors to enable the processing of the laminated assay card to be paused for appropriate amounts of time to enable, for example, reactions within the laminated

assay card 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 laminated assay card based on its relative location within the assay apparatus.

HEATING

Furthermore, electrical contacts (not shown) are provided which contact with heating elements placed on the assay card. These heating elements are configured to contact with the electrical contacts when the assay card reaches a desired point during processing. These heating elements heat the assay card to improve the efficacy of reactions within the assay card. 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. This temperature monitoring could be performed by the optical detector 2050 mentioned above. Although electrical heating elements are described, it will be appreciated that any suitable means of heating the assay card may be provided such as, for example, heating the void within the assay apparatus which receives the assay card.

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 to facilitate loading of the test sample. Upon detecting the presence of this flow preventer, the pair of rollers 2070 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 2030 is aligned with the optical source 2040 and the optical detector 2050, 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 2060.

Accordingly it can be seen that the polypropylene film of the laminated assay card provides protection for the sensitive and fragile membrane from external contamination. The

polypropylene film also provides a sample loading opening. The user can dispense the sample to the sample loading opening by means of disposable plastic pipette or, preferably, by direct sampling by dipping into the sample.

The PVC backing provides a rigid support for the laminated assay card. Because the joint section of nitrocellulose membrane and conjugate release pad is very delicate, it should not be bent or deformed. If that happens, the surface property is changed and, as a result, the capillary flow pattern is changed. Then, the sensitivity and reproducibility could be affected. Therefore, it is important to provide a rigid support for the test strip to prevent such an undesirable changes

The PVC backing also provides a printing area to show operation instruction, a bar code (or a QR code) and warning or indication sign. The printing can be performed by screening printing, offset or flexo-printing, or preferably by inkjet printing.

The nitrocellulose membrane is designed to be an open flow capillary channel, which means the microfluidic channel has no cover layer. When a blood plasma is drawn to the channel, the plasma begins to fill the microscopic pores and the capillary flow begins. If an additional cover layer is placed on the surface of nitrocellulose membrane, the gap between the cover and membrane creates unnecessary flow. Then, a significant amount plasma liquid just flows through the flow channel without having an opportunity to meet the immobilized antibody.

To prevent such a loss, nitrocellulose membrane has an open channel. To achieve this, a burning laser beam scanner is used to selectively cut out the opening area. The laser source can be CO₂ laser, Nd-YAG laser and, preferably, DPSS laser. The scanning laser beam also used to cut out the sample loading opening.

ALTERNATIVE LAMINATED ASSAY CARD

Figure 23 illustrates a laminated assay card 1100' according to one embodiment. This embodiment includes a sample pad 1200', an enlarged waste chamber 1210' and microfluidic channels that couple directly with the lateral assay strip 1160', rather than via a chamber 1150'. The laminated assay card 1100' 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 laminated assay card 1100 through an inlet and retained in the sample pad 1200'. A lateral

assay strip 1160' is provided such as a lateral immunoassay strip. The sample flows from the sample pad 1200' via the chamber 1150' and into the lateral assay strip 1160' under capillary action.

5 A split roller arrangement 1170' is provided and the laminated assay card 1100' moves relative to split roller arrangement 1170' 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
10 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. The reagent reservoirs 1130', 1140' are coupled with different parts of the lateral assay
15 strip 1160' whilst the reagent reservoir 1120' is 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.

20 To enable the microfluidic channels associated with the reagent reservoirs 1120', 1130', 1140' to be routed to the lateral assay strip 1160' and 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
25 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 in a similar manner to that described above.

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 L1 to L3 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 laminated assay card 1100' 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 of the lateral assay strip 1160'. In the example, {P1,P2,P3,P4,P5} = {22,10,15,15,15} in mm.

The length of the reservoirs in this example varies, but they may instead be fixed in which case the volume of the reservoir may then be changed by varying the width of the reservoir.

It will be appreciated that the location of the reagent reservoirs 1120', 1130', 1140', the chamber 1150' and the lateral assay strip 1160' may be changed to suit the needs of the reaction to be undertaken and the methodology of actuating the laminated assay card.

The operation of the assay apparatus will now be described in more detail.

The laminated assay card 1100' is inserted into an assay apparatus.

The laminated 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'.

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 and the position and timing information and any other encoded information such as speed and reservoir length is transmitted to the assay apparatus. The offset applied by the offset routine is then reversed.

The laminated assay card 1100' is advanced by P2 rapidly, then advanced slowly at speed S1 for the length L1 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.

5 The laminated assay card 1100' is advanced by P3-L1 rapidly (which closes the microfluidic channel coupling reservoir 1120' with the chamber 1150'), then advanced slowly at speed S2 for the length L2 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.

10 The laminated assay card 1100' is advanced by P4-L2 rapidly (which closes the microfluidic channel coupling reservoir 1140' with the lateral assay strip 1160'), then advanced slowly at speed S3 for the length L3 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.

15 The laminated 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.

20 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 laminated assay card 1100 completely until an optical card sensor (such as a photosensor) detects the laminated assay card edge.

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

25 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.

10 REFERENCE CURVE PROVISION

In embodiments, structures and components to support a reference curve may be provided on the laminated assay card in parallel with the main assay reaction to provide a stand alone calibration curve. For example, two standards (a hi and a lo) may be provided for on the laminated assay card and may be injected simultaneously with the sample and processed using common reagents so that temperature and environmental effects that cause reduced or increased intensity signals are calibrated in real time against the standards.

Accordingly, an immunoassay card, and method for production are disclosed. The card has a backing sheet for a mechanical support, an assembly of a lateral flow test strip for an immunoassay and a cover film for providing a protection surface and a clamping force. On the backing sheet, there is a thin adhesive layer. The strip and the cover film adhere on the surface of adhesion layer. The film has vacuum moulded pocket fit to the strip. The clamping pressure to press the strip is controlled by adjusting the depth of the pocket. On the cover film, there are holes for venting air and for injecting the sample.

The production line is a roll-to-roll process. A backing sheet roll is laminated with an adhesive roll and a cover film roll. The cover film is previously moulded with vacuum moulding process using hot air and a vacuum to create a pocket structure. The test strip is a multilayer laminate structure composed of nitrocellulose membrane, a PVC backing film, an absorbent pad, a conjugate release sheet and a sample filter. The immunoassay card, a thin multilayer card

structure, can provide enough space to print barcode, label and instructions as well as finger grip space during sampling.

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.

REFERENCES

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. A laminated assay card, comprising:
a support substrate;
an assay strip assembly provided on said support substrate operable to test a sample; and
5 a retaining substrate provided overlying said support substrate and said assay strip assembly to form a laminated structure retaining said assay strip assembly therein.
2. The laminated assay card of Claim 1, wherein said support substrate and said retaining substrate are bonded together.
3. The laminated assay card of Claim 1 or Claim 2, wherein said assay strip assembly
10 comprises at least one reagent layer operable to react with said sample.
4. The laminated assay card of any preceding claim, wherein said assay strip assembly comprises at least one reservoir.
5. The laminated assay card of Claim 4, wherein each reservoir is in fluid communication with at least one reagent layer.
- 15 6. The laminated assay card of Claim 4 or Claim 5, wherein said reservoir comprises an inlet reservoir operable to receive said sample.
7. The laminated assay card of Claim 6, wherein said inlet reservoir is operable to filter said sample.
8. The laminated assay card of any preceding claim, comprising at least one reagent
20 reservoir operable to receive a reagent.
9. The laminated assay card of Claim 8, comprising fluid conduits operable to couple each reagent reservoir with assay strip assembly.
10. The laminated assay card of any preceding claim, wherein said retaining substrate comprises a shaped primary recess operable to receive assay strip assembly.

11. The laminated assay card of Claim 10, wherein said shaped primary recess is dimensioned to compress assay strip assembly onto said support substrate.
12. The laminated assay card of any one of Claims 4 to 11, wherein said retaining substrate comprises shaped secondary recesses operable to define at least one of said reservoirs and said
5 fluid conduits.
13. The laminated assay card of any preceding claim, wherein said retaining substrate comprises an elongate aperture arranged coincident with said at least one reagent layer.
14. The laminated assay card of any preceding claim, comprising a retaining structure at least partially surrounding an inlet hole operable to retain said sample in a vicinity of said inlet hole.
- 10 15. The laminated assay card of any preceding claim, comprising a vacuum device operable to generate a negative pressure at an inlet hole to assist receiving the sample.
- 16 The laminated assay card of any preceding claim, comprising an indicator encoded on said laminated assay card operable to provide an indication of at least one of a configuration of said laminated assay card, instructions for using said laminated assay card, user identification,
15 physician identification and a date of dispensing.
17. A method of manufacturing a laminated assay card, the method comprising the steps of:
- (i) providing a support substrate;
- (ii) positioning an assay strip assembly operable to test a sample on said supporting substrate; and
- 20 (iii) overlying a retaining substrate on said support substrate and said assay strip assembly to form a laminated structure retaining said assay strip assembly therein.