US 20210229090A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2021/0229090 A1

Doolan et al. (43) Pub. Date: Jul. 29, 2021

(54) **AN IMPROVED POINT-OF-CARE DIAGNOSTIC ASSAY CARTRIDGE**

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(21) Appl. No.: **17/050,635**

(22) PCT Filed: **Apr. 29, 2019**

(86) PCT No.: **PCT/EP2019/060885**

§ 371 (c)(1),
(2) Date: **Oct. 26, 2020**

(30) **Foreign Application Priority Data**

Apr. 27, 2018 (GB) 1806931.0

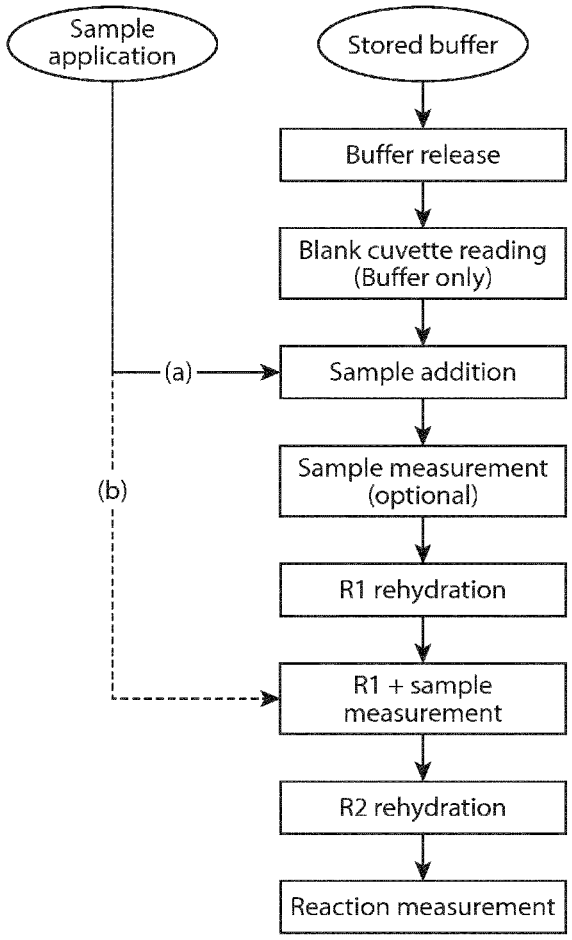
Publication Classification

(51) **Int. Cl.**
B01L 3/00 (2006.01)

(52) **U.S. Cl.**
CPC ... ***B01L 3/50273*** (2013.01); *B01L 2200/0621* (2013.01); *B01L 2200/16* (2013.01); *B01L 2400/0457* (2013.01); *B01L 2300/0867* (2013.01); *B01L 2400/0409* (2013.01); *B01L 2300/0654* (2013.01)

(57) **ABSTRACT**

The invention provides a microfluidic system comprising a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge. The cartridge is configured to rotate on an inclined plane, thus providing for a combination of centrifugal force and gravitational force to move the fluid sample within the cartridge. Such a configuration may facilitate the performance of a sequential assay by making it easier to move a fluid radially inwards within the cartridge. The cartridge provided comprises a reaction chamber (**15**) with at least three zones within the chamber: a first zone (Zone A) is positioned radially outward comprises a cuvette (**45**) for optical measurement; a second and third zones (Zones B and C) are positioned closer to a centre of rotation (**25**) and may comprise dried reagents spots (R**1**, R**2**).



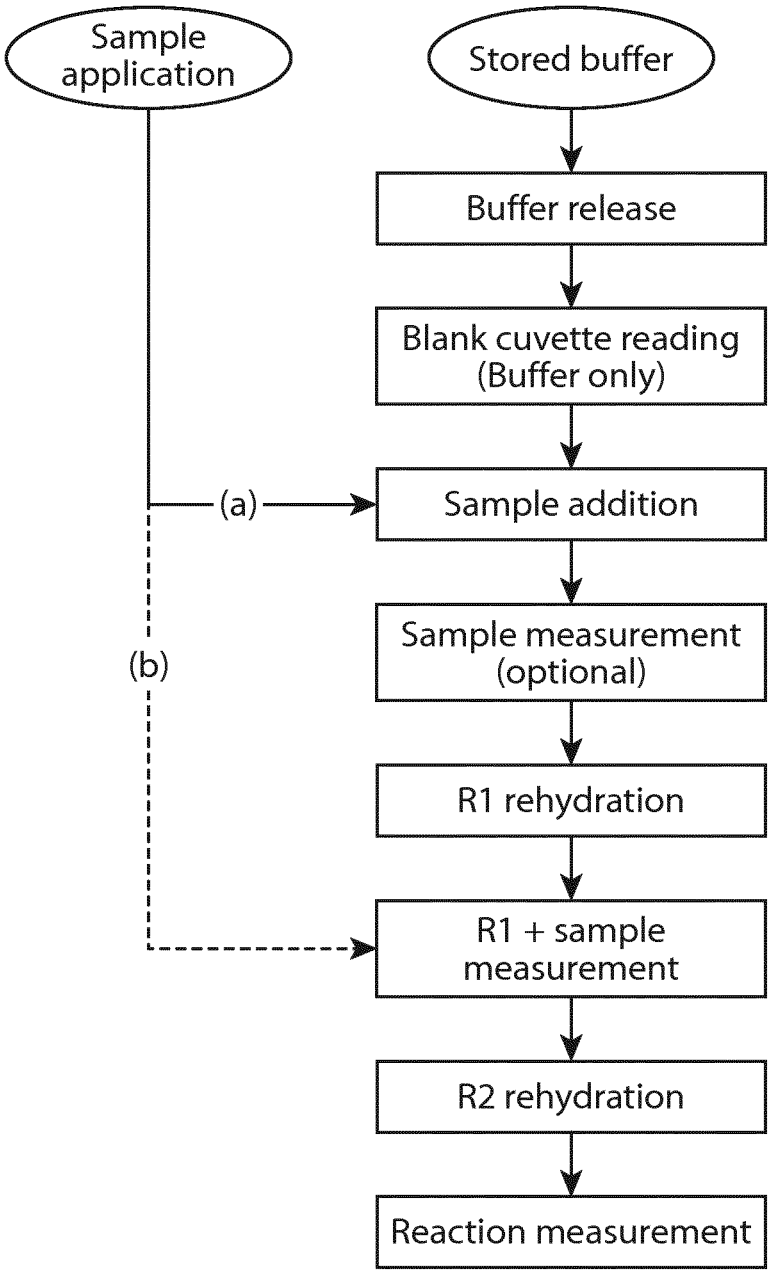

Sample application
Stored buffer
Buffer release
Blank cuvette reading (Buffer only)
(a)
Sample addition
Sample measurement (optional)
(b)
R1 rehydration
R1 + sample measurement
R2 rehydration
Reaction measurement

Figure 1

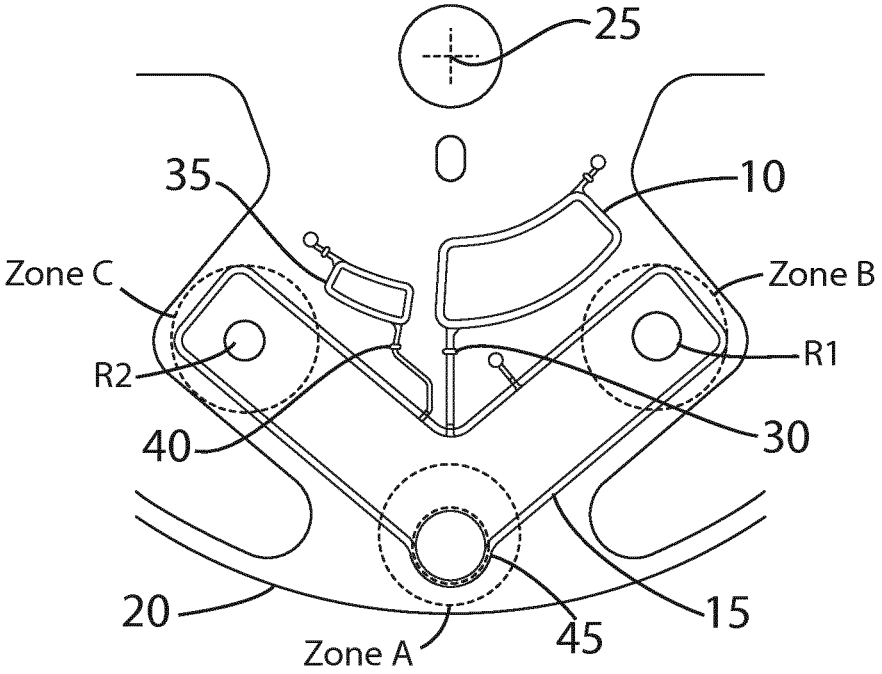


Figure 2

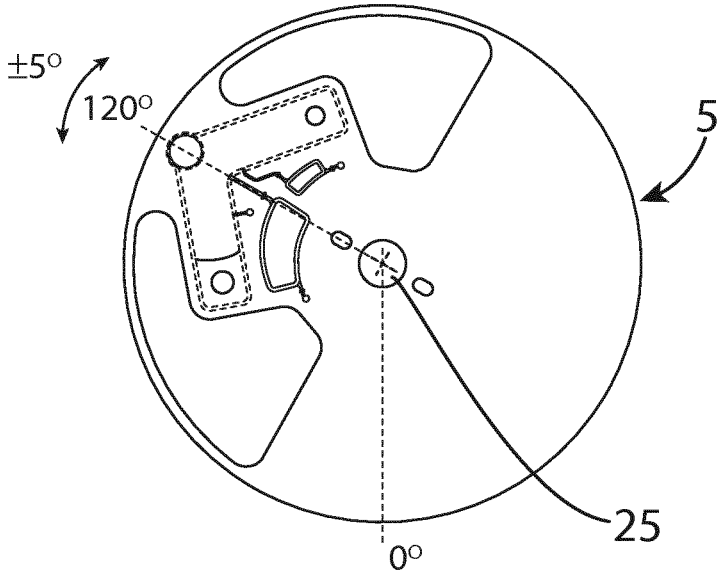


Figure 3

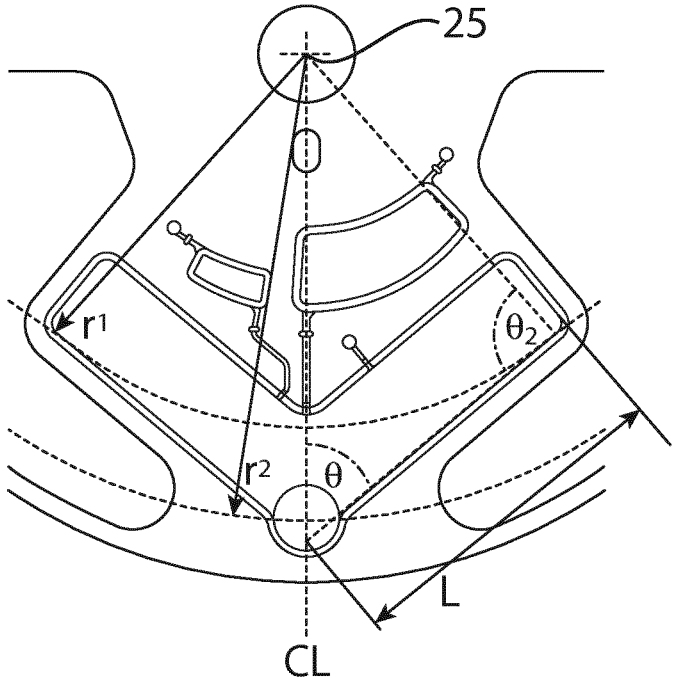


Figure 4

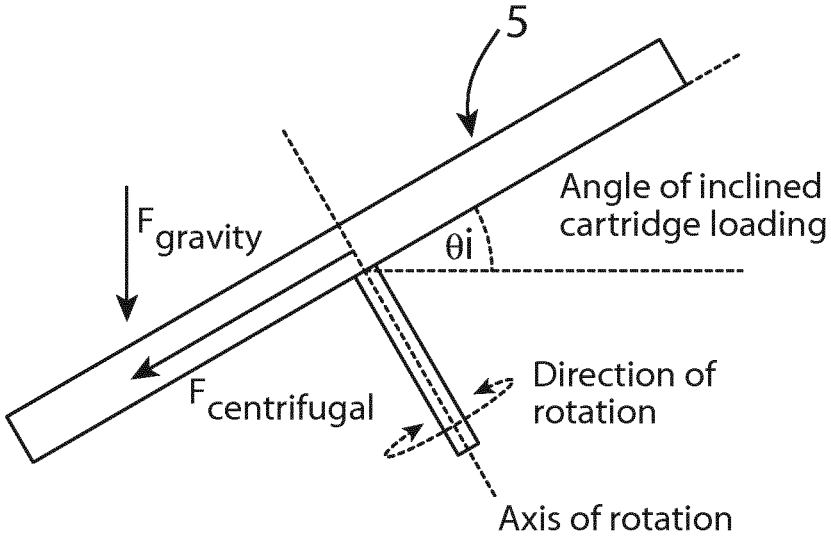


Figure 5

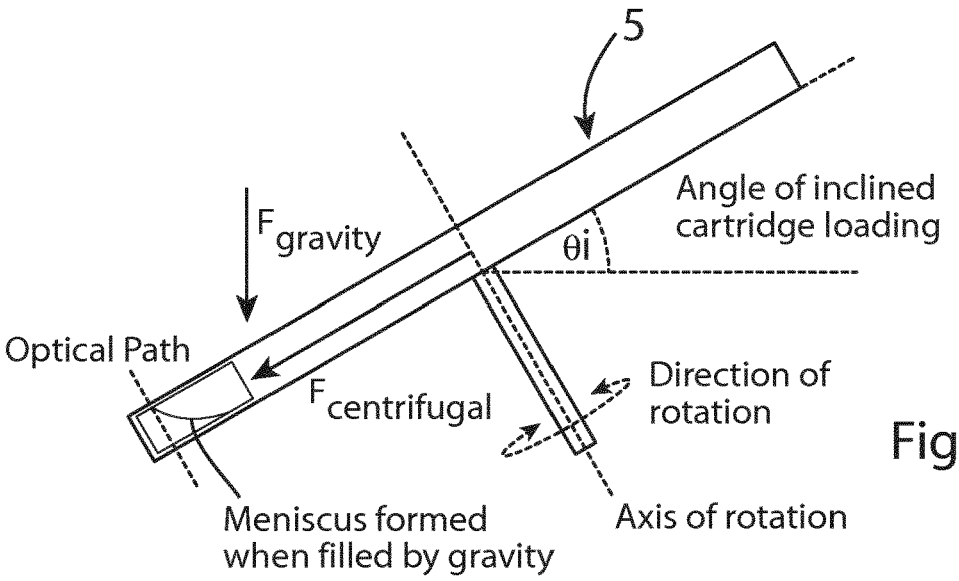


Figure 6

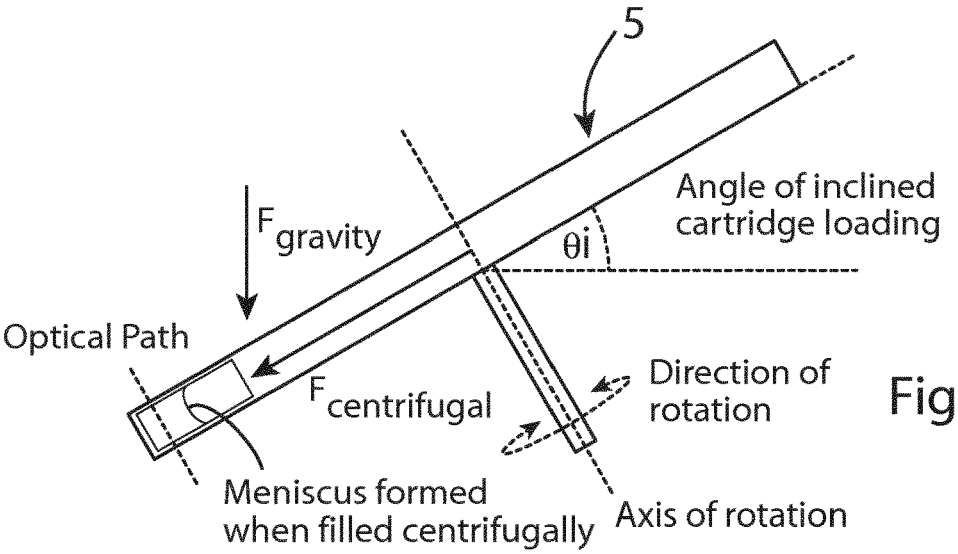
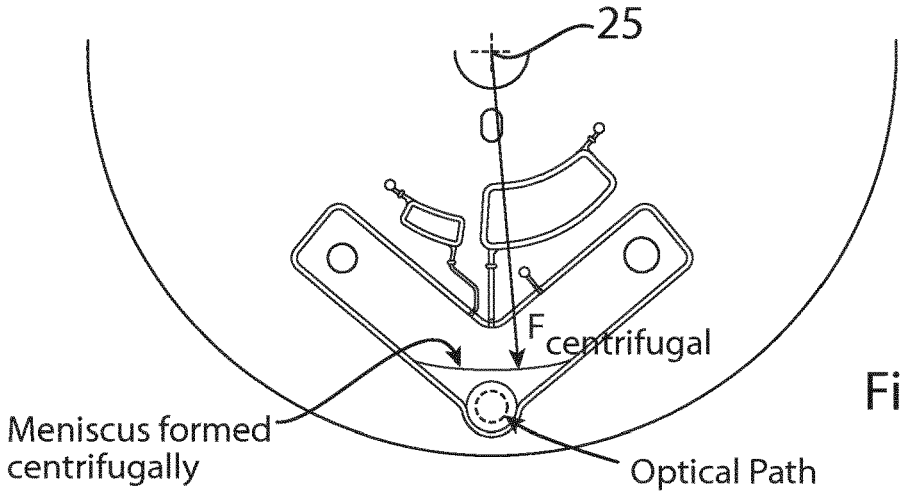


Figure 7



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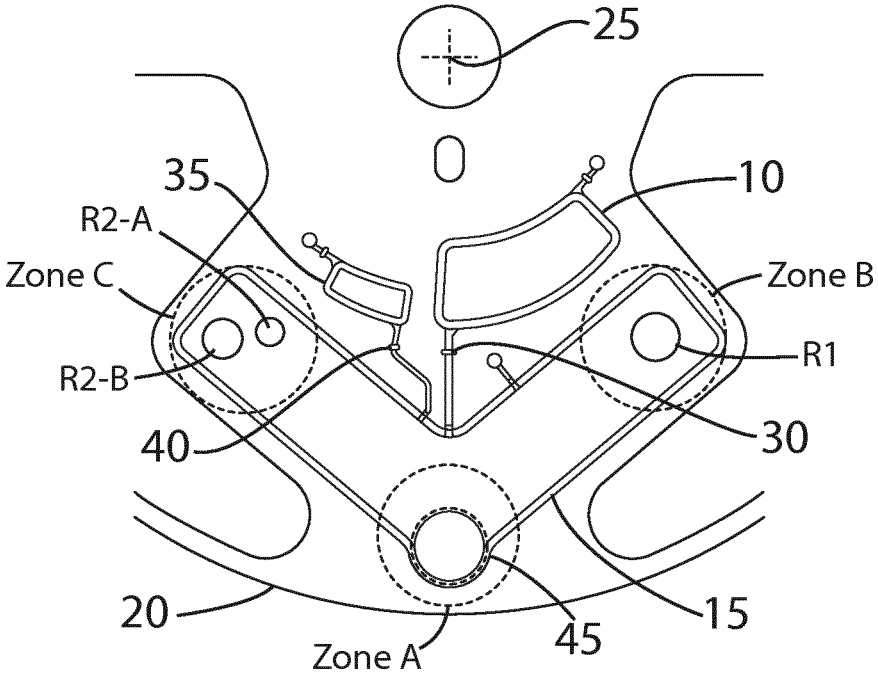

Figure 9

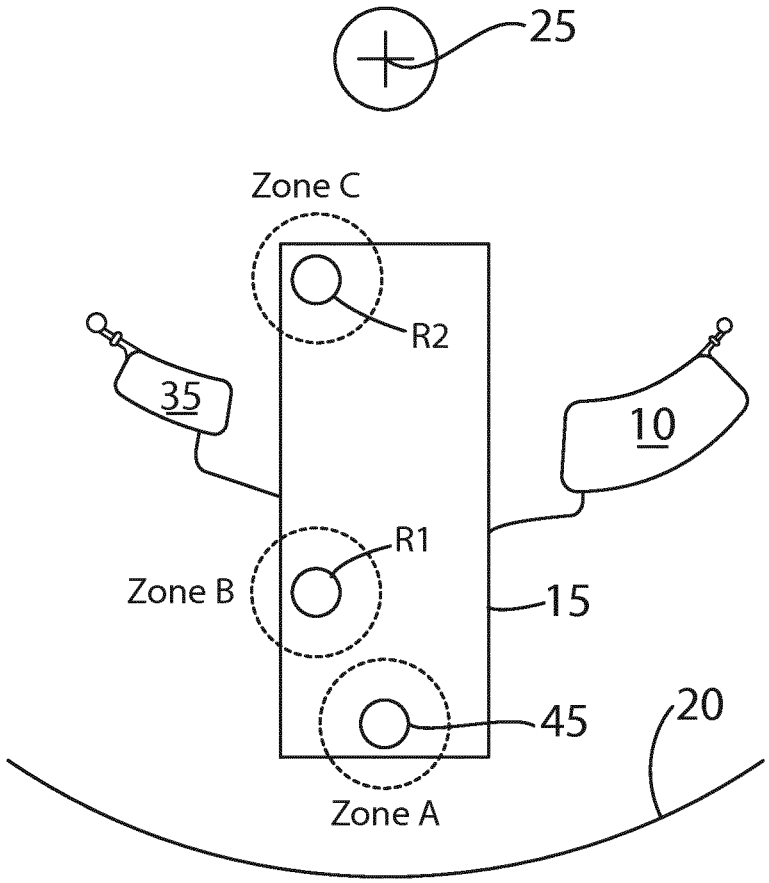

Figure 10

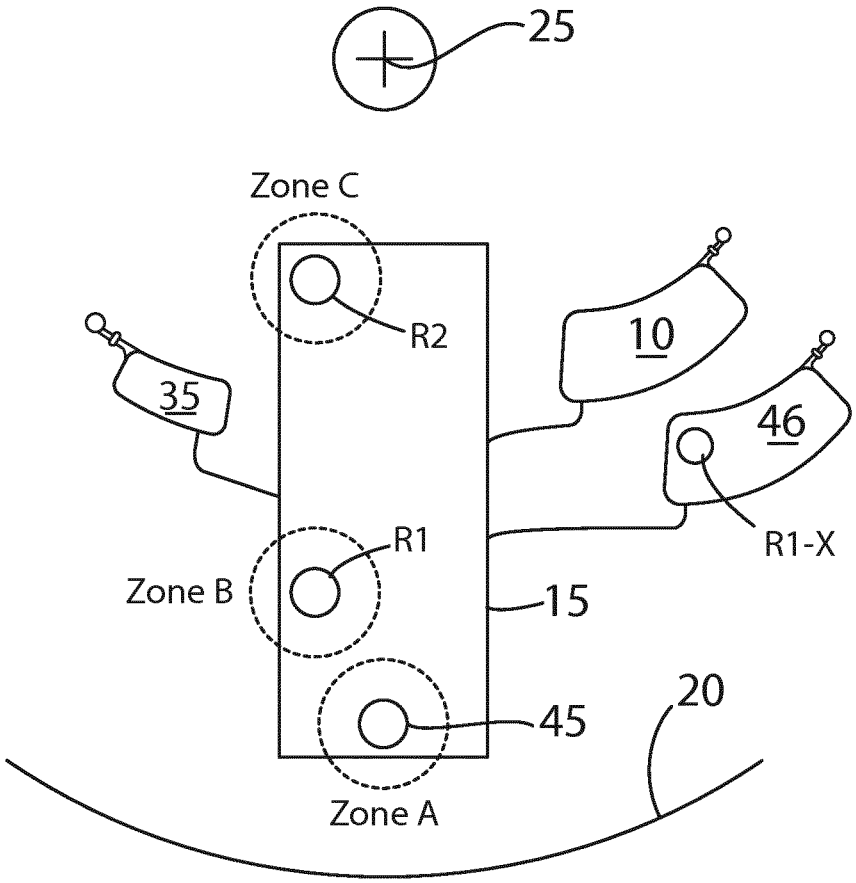

Figure 11(a)

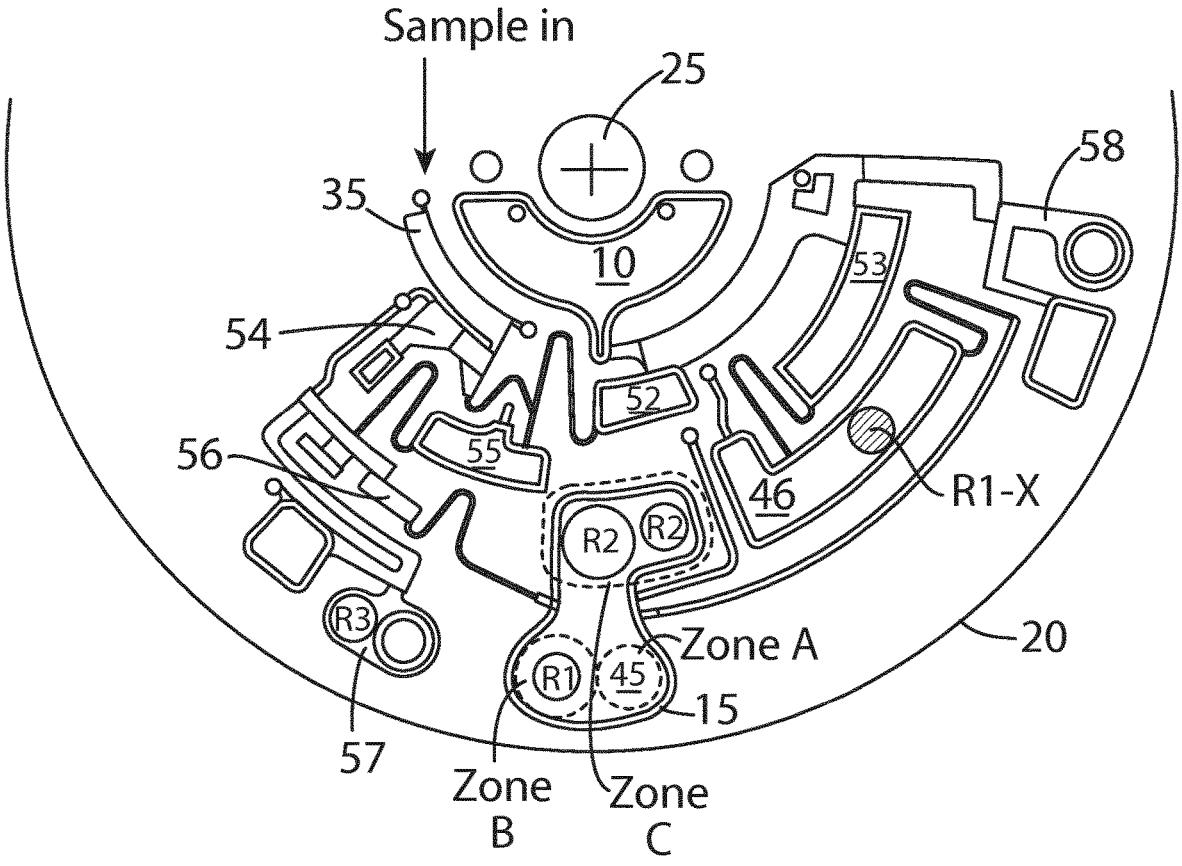
Sample in
25
58
35
10
53
54
52
56
55
46
R1-X
R2
R2
R3
Zone A
20
R1
45
15
57
Zone
B
Zone
C

Figure 11(b)

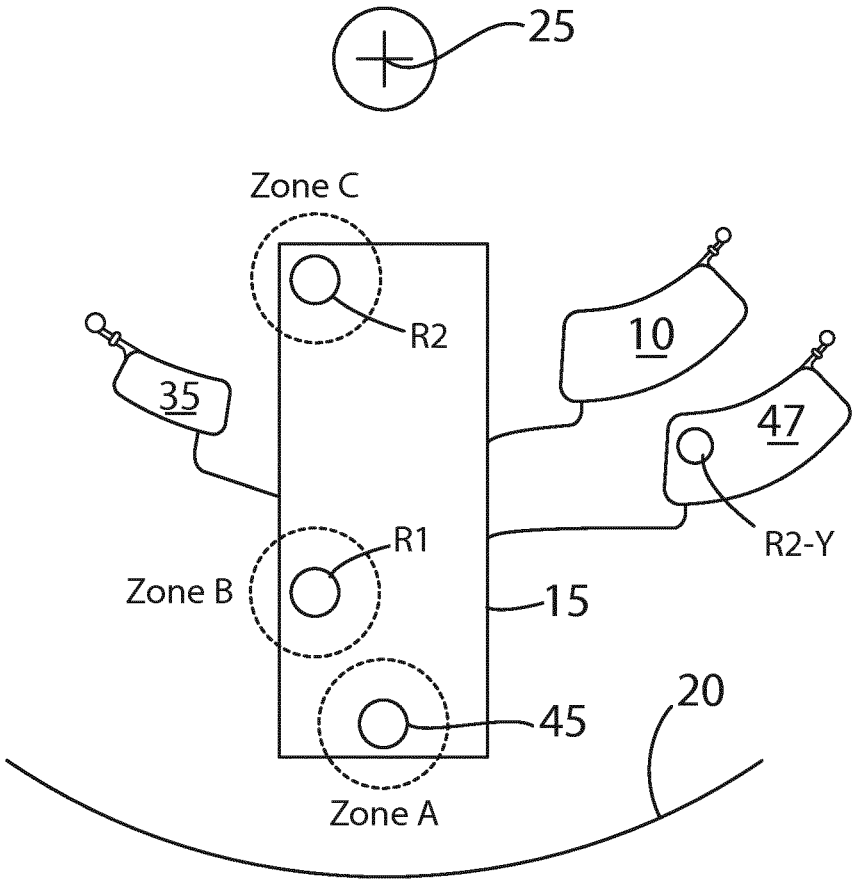

Figure 12

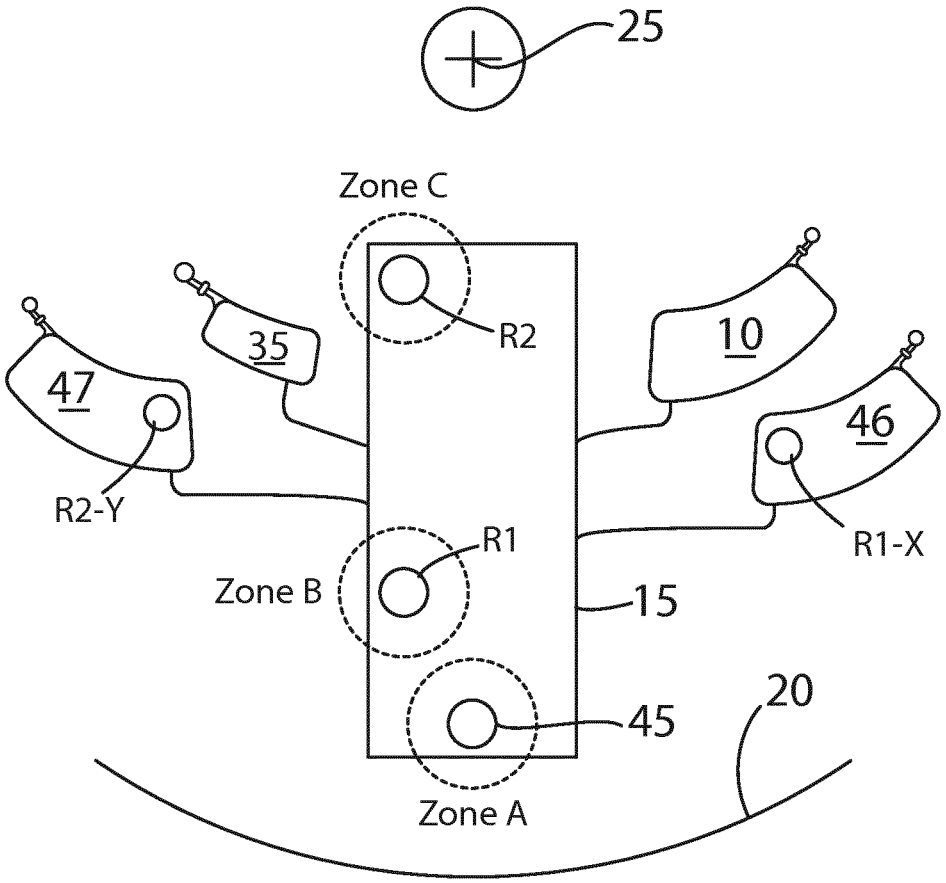

Figure 13

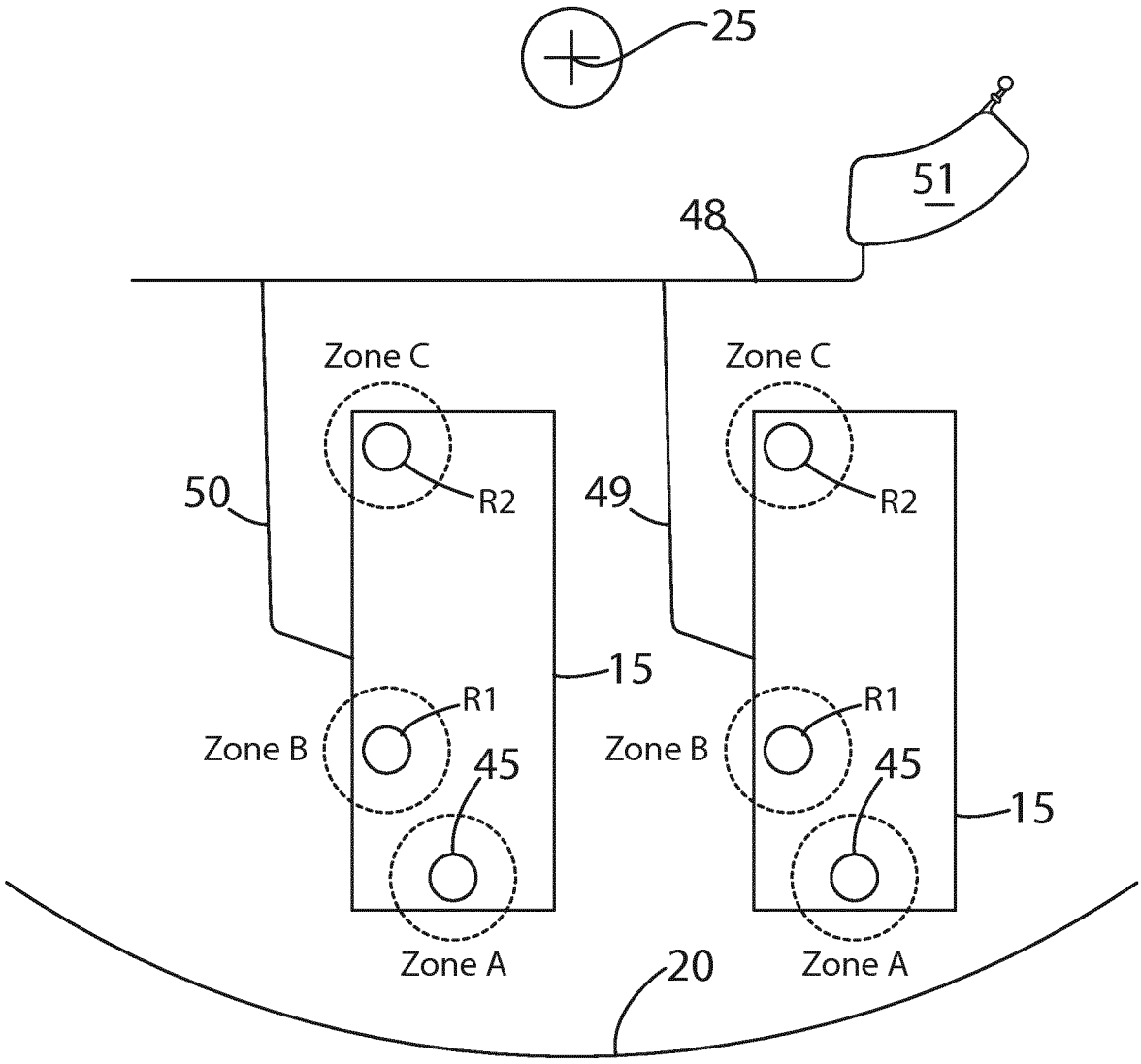
25
51
48
Zone C
Zone C
50
49
R2
R2
15
R1
R1
Zone B
Zone B
45
45
15
Zone A
Zone A
20

Figure 14(a)

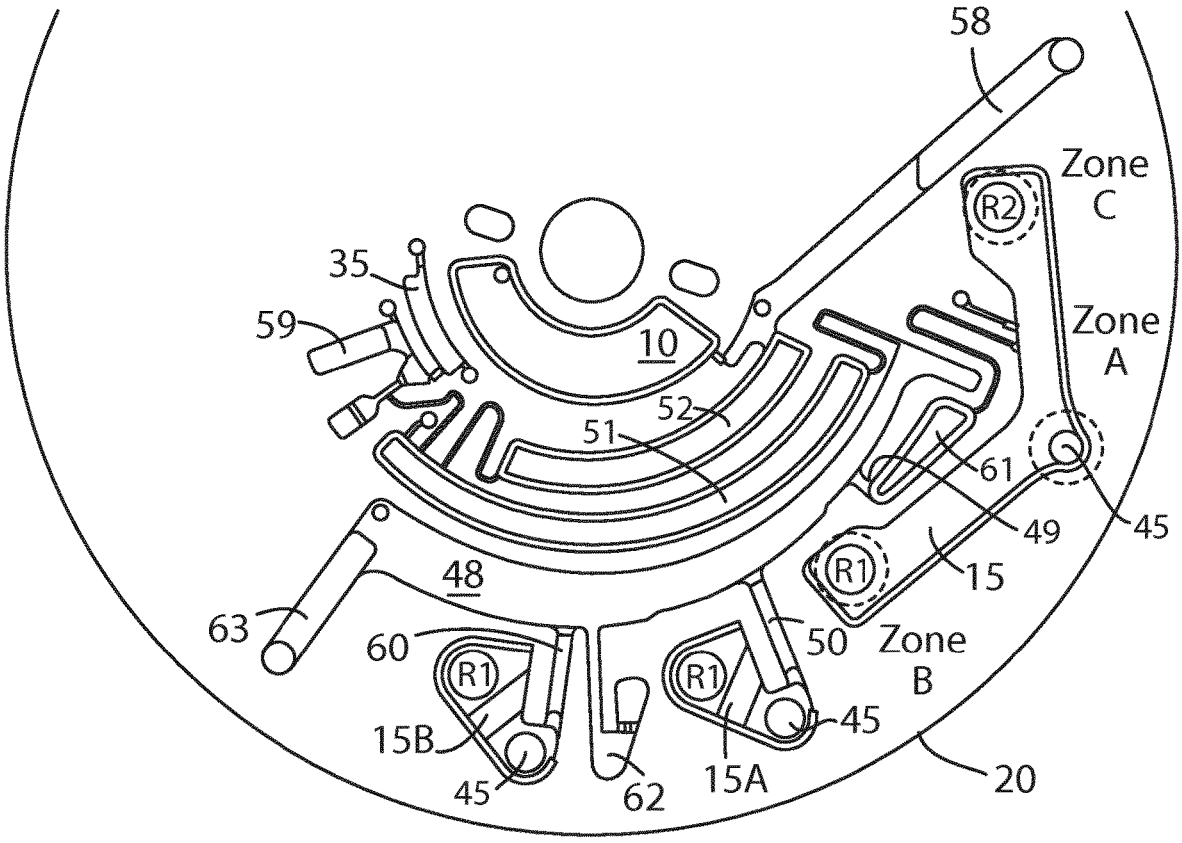
58
Zone C
R2
35
59
10
Zone A
52
51
61
49
45
48
R1
15
63
60
50
Zone B
R1
R1
45
15B
45
62
15A
20

Figure 14(b)

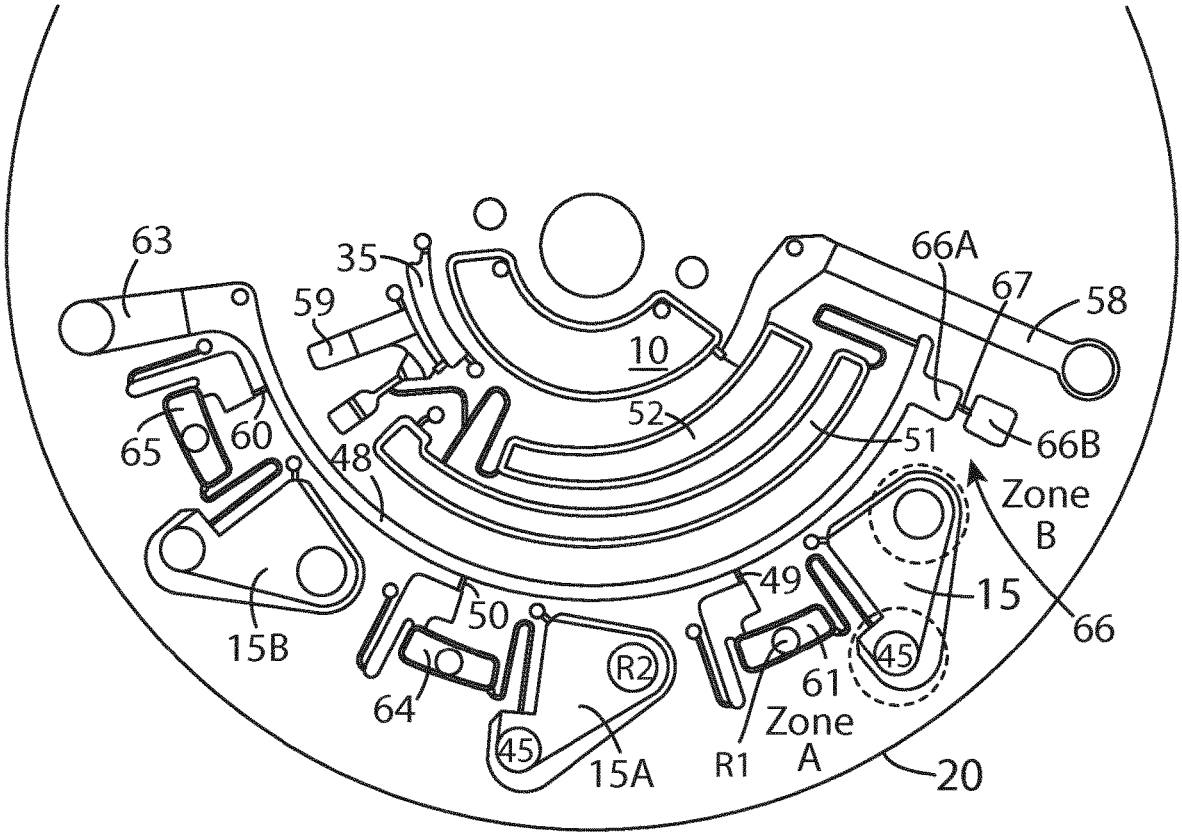
63
35
59
10
66A
67
58
65
60
52
51
66B
48
Zone
B
49
15
50
15B
66
R2
45
61
64
Zone
A
45
15A
R1
20

Figure 14(c)

AN IMPROVED POINT-OF-CARE DIAGNOSTIC ASSAY CARTRIDGE

FIELD

[0001] The invention relates to a point-of-care cartridge. In particular the invention relates to a point-of-care diagnostic assay system based on centrifugal microfluidic technology.

BACKGROUND

[0002] Manual processing to determine the biochemical content of various types of samples, is cost-prohibitive in many applications and is also prone to errors. Automation is also cost-prohibitive in many applications, and is inappropriate as currently practiced—using, for example, liquid handling robots—for applications such as point-of-care or doctor's office analysis. As a result, there is an unmet is need to provide sample processing for biochemical assays that is less expensive and less prone to error than current automation or manual processing.

[0003] Typically it is very difficult to move fluids radially inward using centrifugal microfluidics as the primary means of fluid movement. This can limit/restrict the options available to allow a sequential assay to be performed.

[0004] Certain point-of-care diagnostic assay systems based on centrifugal microfluidic technology are quite good at performing the necessary integrated sample preparation and assay measurement steps. Such a centrifugal microfluidic platform with optical detection allows for a variety of assay technologies to be implemented in parallel using a single instrument and disposable cartridges Examples of point-of-care diagnostic assay systems include U.S. Pat. No. 9,182,384B2 (Roche), U.S. Pat. No. 8,415,140B2 (Panasonic), U.S. Pat. No. 8,846,380 (Infopia), U.S. Pat. No. 5,591,643 (Abaxis), U.S. Pat. No. 5,409,665 (Abaxis).

[0005] US Patent Publication No. US 2010/074801 describes an analyser comprising a microchip coupled to a motor, where the microchip acquires a liquid sample by means of capillary action. The microchip overcomes the limitation of using capillary action to move a liquid sample by providing a structure which reduces capillary pressure. This is achieved by providing each channel with an adjoining cavity open to atmospheric pressure, which acts so as to prevent an increase in capillary pressure as the fluid length increases. Thus, in one embodiment of the invention, the microchip structure comprises an inlet for collecting a liquid sample, a capillary cavity for holding a predetermined amount of the liquid sample, a single holding chamber having an analytical reagent, a measuring chamber for measuring the mixture of the liquid sample and the reagent, a channel communicating with the holding chamber and the measuring chamber, and a channel connecting the measuring chamber with an atmospheric vent. In use, a liquid sample in the capillary cavity is transferred by centrifugal force into the holding chamber, where it is mixed with the analytical reagent. This mixture is then transferred out of the holding chamber to the inlet of the measuring chamber by capillary force, from where it is transferred into the measuring chamber itself by rotation of the analyser. At the measuring chamber, the concentration of a component of the liquid sample is measured. Accordingly, it will be understood that in this patent document, the microchip structure is configured such that once the holding chamber has delivered the mixture of the single reagent and the liquid sample to the measuring chamber, the mixture cannot be returned to the holding chamber.

[0006] US Patent Publication No. US 2015/104814 discloses a sample analysis apparatus for whole blood separation. It comprises a rotatable microfluidic apparatus which comprises a sample chamber for accommodating a sample, a channel that provides a path through which the sample flows, and a valve for opening the channel, which is coupled to a valve driver and a control unit. A separation chamber receives a sample flowing from the sample chamber due to centrifugal force, while a collection chamber for collecting target cells is connected to the separation chamber. In use, the apparatus is rotated to separate the sample into a plurality of layers in the separation chamber according to density gradients of materials in the sample, such as for example a DGM layer, an RBC layer, a WBC layer and a plasma layer. The target material located in the lowermost portion of the separation chamber along with the DGM is then transported to the collection chamber for recovery.

[0007] International Patent Publication No. WO 2009/016811 describes a device for analysing a liquid collected from an organism. The device comprises a plurality of individual cuvettes, wherein each cuvette measures a different phase of a reaction. US Patent Publication No. US2017138972 simply describes the use of gravity and centrifugal forces to transfer fluids between three reaction chambers in order to provide multiple washing steps to separate a composite from unbound or unreacted substances.

[0008] It is therefore an object to provide an improved point-of-care diagnostic assay systems based on centrifugal microfluidic technology.

SUMMARY

[0009] According to the invention, there is provided, as set out in the appended claims, a microfluidic system comprising:

[0010] a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge, wherein the cartridge is configured to rotate on an inclined plane with respect to a horizontal plane;

[0011] the cartridge comprises a reaction chamber having at least three zones, a first zone positioned near one end of the reaction chamber to define a detection zone, a second zone positioned proximal to the first zone and a third zone positioned near the other end of the reaction chamber, wherein each of the second zone and the third zone comprise a reagent zone; and the motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the at least three zones, wherein the first zone comprises a single cuvette positioned adjacent to the outer diameter of the cartridge and configured to allow for optical measurement of each phase of a reaction.

[0012] In one embodiment the first zone is positioned at a radial extent and at a furthest point from the centre of rotation of the reaction chamber.

[0013] In one embodiment the second zone is positioned radially inward with respect to the first zone and comprises first reagent spot location R1.

[0014] In one embodiment wherein the third zone is positioned between the most radially inward end of the

reaction chamber and the radial inward position of the second zone and the third zone comprises a second reagent spot location R2.

[0015] In one embodiment there is provided a first separate rehydration chamber to rehydrate an R1 reagent (R1-X) or a different reagent.

[0016] In one embodiment there is provided a buffer metering chamber coupled to the first separate rehydration chamber configured to meter a pre-defined volume of buffer solution for transfer to the first separate rehydration chamber to rehydrate the R1 reagent (R1-X) or a different reagent in the rehydration chamber.

[0017] In one embodiment there is provided a second separate rehydration chamber to rehydrate an R2 reagent (R2-Y) or a different reagent.

[0018] In one embodiment there is provided two or more reaction chambers.

[0019] In one embodiment the reaction chamber comprises at least one of an oblong shape; a circular shape, a square shape; a zigzag shape or a cross shape.

[0020] In one embodiment the first zone comprises a cuvette and is positioned adjacent to the outer diameter of the cartridge.

[0021] It will be appreciated the cartridge of the invention provides a number of advantages over the prior art:

- **[0022]** Overall cartridge concept uses gravitational and centrifugal microfluidic methods
- **[0023]** Single volume reaction, i.e. removes the need for any or all of the steps including: dilution, aliquoting or metering of reagents which simplifies operation and potentially improves test precision
- **[0024]** Sequential optical measurements in a single cuvette for each assay phase to improve precision
- **[0025]** Location of R1 and R2 reagents for sequential rehydration
- **[0026]** Homogenous mixing of sample and buffer
- **[0027]** Ability to carry out an optical measurement on buffer and/or sample
- **[0028]** Cuvette filling using centrifugal force to provide an even liquid meniscus for consistent optical interrogation
- **[0029]** Optical measurement of the assay reaction using static or dynamic (while rotating) methods

[0030] In one embodiment the first detection zone comprises a cuvette and positioned at the radial extent of the V shaped reaction chamber.

[0031] In one embodiment the V shaped chamber extends radially inward on two sides to create two zones that can be independently filled with fluid to define the second zone and third zone.

[0032] In one embodiment the second and/or third zone comprises a reagent storage and/or rehydration zones.

[0033] In one embodiment the second and/or third zone comprises a region adapted to be optically interrogated.

[0034] In one embodiment the cartridge is positioned and configured to rotate at a velocity such that a combination of centrifugal force and gravity moves the fluid sample radially outward and inward respectively.

[0035] In one embodiment the cartridge rotates at a velocity such that the relative centrifugal force (RCF) is greater than gravity, and the fluid sample can be moved radially outward on the cartridge.

[0036] In one embodiment the centrifugal force ensures that no fluid reaches the second zone or third zone.

[0037] In one embodiment the cartridge is stationary or rotating slowly, gravity will influence the fluid and move the fluid towards the second zone or third zone.

[0038] In one embodiment the cartridge is rotated or agitated on an inclined plane with respect to a horizontal plane to create a downward slope for the fluid sample to flow under the influence of gravity.

[0039] In one embodiment, the cartridge is further configurable to be agitated to overcome any effects of surface tension that may prevent the fluid from flowing under the influence of gravity.

[0040] In one embodiment the cartridge rotates on an inclined plane at an angle of θi from the horizontal plane and wherein the angle is between 10° to 60°.

[0041] In one embodiment a buffer reservoir is positioned close to the centre of rotation of the cartridge and a module configured for applying a sample directly to the cartridge.

[0042] In one embodiment the dominant force on the fluid sample meniscus is the centrifugal force such that the centrifugal force is parallel to the upper and lower surface of the first detection zone to provide a meniscus evenly on both surfaces.

[0043] In one embodiment the second zone comprises a dried reagent.

[0044] In one embodiment the third zone comprises a dried reagent.

[0045] In one embodiment the dried reagent remains intact until the second or third zones are rehydrated with the fluid sample and a buffer solution.

[0046] In one embodiment the dried reagent can be spotted in singular or multiple spots in said second and/or third zones.

[0047] In one embodiment the second or third zone comprises multiple dried reagents.

[0048] In one embodiment the cuvette comprises a single volume cuvette configured to allow for optical measurement of the buffer solution, the fluid sample and the rehydrated reagents used in each phase of an assay.

[0049] In one embodiment the system is configured for performing an immunoturbidimetric or an enzyme-based clinical chemistry assay.

[0050] In one embodiment there is provided a sample metering chamber configured to receive the fluid sample and meter a pre-defined volume of the sample and a buffer metering chamber configured to meter a pre-defined volume of buffer solution.

[0051] In one embodiment there is provided a sample mixing chamber coupled to the sample metering chamber and coupled to the buffer metering chamber, wherein the sample mixing chamber is configured to mix the sample volume transferred from the sample metering chamber with the volume of buffer solution transferred from the buffer metering chamber to form a dilution of the sample.

[0052] In one embodiment there is provided a diluted sample metering chamber coupled between the sample mixing chamber and the reaction chamber, wherein the diluted sample metering chamber is configured to meter a pre-defined volume of the dilution of the sample for transfer to the reaction chamber.

[0053] In one embodiment there is provided a reaction chamber coupled to the diluted sample metering chamber.

[0054] In one embodiment there is provided two or more reaction chambers each reaction chamber comprising at least the first zone, and wherein at least one reaction chamber has the at least three zones.

[0055] In one embodiment there is provided a sample dilution chamber for mixing the fluid sample and a buffer solution, and a distribution channel coupled between the sample dilution chamber and the two or more reaction chambers, wherein the distribution channel is configured to deliver a diluted sample from the sample dilution chamber downstream to each of the two or more reaction chambers in sequence.

[0056] In one embodiment there is provided a delivery channel associated with each reaction chamber, wherein the diluted sample is delivered from the distribution channel to each reaction chamber by means of its delivery channel.

[0057] In one embodiment there is provided an overflow chamber coupled to the distribution channel for receiving the diluted sample which remains after delivery to the two or more reaction chambers.

[0058] In one embodiment there is provided a buffering chamber coupled to the distribution channel, wherein the buffering chamber is configured to prevent cross contamination between two or more of the reaction chambers.

[0059] In one embodiment there is provided an intermediate sample metering chamber coupled between one of the reaction chambers and its delivery channel, wherein the intermediate sample metering chamber is configured to prevent cross-contamination between the two or more reaction chambers.

[0060] In one embodiment there is provided an intermediate chamber coupled between each delivery channel and its reaction chamber.

[0061] In one embodiment each intermediate chamber comprises a metering chamber and an overflow chamber configured such that the metering chamber is filled with diluted sample from the distribution channel until the centrifugal pressure applied to the delivery channel is equal to the pressure in the overflow chamber.

[0062] In one embodiment there is provided a buffering chamber coupled to the distribution channel, wherein the buffering chamber comprises a first section and a second section linked by a capillary channel.

[0063] In a further embodiment there is provided a microfluidic system comprising:

[0064] a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge, wherein the cartridge is configured to rotate on an inclined plane with respect to a horizontal plane;

[0065] the cartridge comprises a chevron shaped or substantially V shaped reaction chamber having at least three zones, wherein a first zone is positioned near the apex of the V shaped reaction chamber to define a detection zone, a second zone positioned near a first end of the V shaped reaction chamber and a third zone positioned near a second end of the V shaped reaction chamber, wherein each of the second zone and the third zone comprise a reagent zone; and

the motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the at least three zones

[0066] In another embodiment there is provided, a microfluidic system comprising:

[0067] a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge;

[0068] the cartridge comprises a chevron shaped or substantially V shaped reaction chamber having at least three zones, wherein a first zone is positioned near the apex of the V shaped reaction chamber to define a detection zone, a second zone positioned near a first end of the V shaped reaction chamber and a third zone positioned near a second end of the V shaped reaction chamber; and

[0069] the motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the at least three zones.

[0070] In yet another embodiment there is provided, a microfluidic system comprising:

[0071] a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge, wherein the cartridge is configured to rotate on an inclined plane with respect to a horizontal plane;

[0072] the cartridge comprises a reaction chamber having at least three zones, a first zone positioned near one end of the reaction chamber to define a detection zone, a second zone positioned proximal to the first zone and a third zone positioned near the other end of the reaction chamber, wherein each of the second zone and the third zone comprise a reagent zone; and the motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the at least three zones.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] The invention will be more clearly understood from the following description of an embodiment thereof, given by way of example only, with reference to the accompanying drawings, in which:

[0074] FIG. **1** is a flow chart illustrating a number of sequential steps required to transfer a 2-step dried reagent assay onto a self-contained/single-use/disposable point-of-care (POC) cartridge;

[0075] FIG. **2** shows a cartridge design embodiment to perform the assay sequence according to a first embodiment of the invention;

[0076] FIG. **3** illustrates a normal view of the cartridge surface showing reagent rehydration;

[0077] FIG. **4** illustrates a chevron shaped or substantially V shaped reaction chamber having at least three zones, according to one embodiment;

[0078] FIG. **5** shows a side view of the cartridge mounted on a motor platform during operation;

[0079] FIG. **6**, FIG. **7** and FIG. **8** illustrate the benefit of filling the cuvette by centrifugal force;

[0080] FIG. **9** shows a cartridge design embodiment to perform the assay sequence according to an embodiment of the invention which uses a second dried reagent spot in the third reagent zone;

[0081] FIG. **10** shows a cartridge design embodiment to perform the assay sequence illustrated in the flow chart of FIG. **1**;

[0082] FIG. **11***a* illustrates an alternative cartridge design to FIG. **10** incorporating an additional rehydration chamber;

[0083] FIG. **11***b* shows a cartridge design embodiment based on FIG. **11***a* where an additional rehydration chamber is used;

[0084] FIG. **12** illustrates another cartridge design and a variation of the embodiment shown in FIG. **11***a;*

[0085] FIG. **13** illustrates another cartridge design and a variation of the embodiments shown FIGS. **11***a* and **12**;

[0086] FIG. **14***a* illustrates another embodiment showing a plurality of reaction is chambers on a single cartridge design;

[0087] FIG. **14***b* shows a cartridge design embodiment based on FIG. **14***a*; and

[0088] FIG. **14***c* shows a cartridge design embodiment based on FIG. **14***a*.

DETAILED DESCRIPTION OF THE DRAWINGS

[0089] FIG. **1** illustrates a number of sequential steps required to transfer a 2-step dried reagent assay onto a self-contained/single-use/disposable point-of-care (POC) cartridge. This sequence can be applied to immunoturbidimetric and enzyme-based clinical chemistry assays that require two-step addition & rehydration of reagents R**1** and R**2** to complete a test measurement. A similar test sequence can be used for a 1 step assay where reagents R**1** or R**2** are used only.

[0090] The POC cartridge can include a buffer reservoir and will have a means to apply a sample (for example whole blood, plasma, serum) to the cartridge. The cartridge may contain dried, immobilised reagents (R**1** and R**2**) stored in specific locations on the cartridge that can be rehydrated independently. Depending on where the sample is added in the sequence (option (a) or (b) in FIG. **1**), R**1** can be rehydrated by either diluted sample (buffer+sample) or buffer only. R**2** is then rehydrated by this same fluid volume.

[0091] FIG. **2** shows a cartridge design embodiment to perform the assay sequence illustrated in the flow chart of FIG. **1**, according to a first embodiment of the invention. The cartridge design employs a combination of centrifugal and gravitational microfluidics to move fluids to multiple locations on the cartridge. The cartridge **5** includes a buffer reservoir **10** that will sit at or close to the centre of rotation **25**. There is also provided a means for applying a sample directly to the cartridge (not shown in FIG. **2**). The cartridge, layout described in more detail below, resolves the following problems:

- **[0092]** Single volume reaction, i.e. removes the need for any or all of the steps including: dilution, aliquoting or metering of reagents which simplifies operation and potentially improves test precision
- **[0093]** Sequential optical measurements in a single cuvette for each assay phase to improve precision
- **[0094]** Location of R**1** and R**2** reagents in distinct zones for sequential rehydration
- **[0095]** Homogenous mixing of sample and buffer and the ability to carry out an optical measurement on buffer and/or sample

[0096] Referring to FIG. **2** the cartridge **5** comprises a chevron shaped or substantially V shaped reaction chamber **15** having at least three zones. A first zone is positioned near the apex of the V shaped reaction chamber to define a detection zone. A second zone is positioned near a first end of the V shaped reaction chamber and a third zone is positioned near a second end of the V shaped reaction chamber. The motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the three zones.

[0097] In operation, centrifugal force is used to control the delivery of a stored buffer from its reservoir **10** and/or subsequent buffer chambers prior to being delivered to the reaction chamber **15**. The reaction chamber **15** is sized such that it is much greater than the buffer reaction volume that will be used. The reaction chamber **15** incorporates three distinct zones: A) cuvette detection zone, B) R**1** reagent zone and C) R**2** reagent zone. The cuvette **45** is located at the radial extent of the reaction chamber **15** (typically close to the cartridge outer diameter **20**). The chamber extends radially inward on two sides to create two zones that can be independently filled with fluid for the R**1** and R**2** reactions. It is beneficial that each zone is sized such that when occupied by buffer they can hold the entire volume within the zone, i.e. the volume of zone A, B or C is equal or greater than the buffer volume and the entire reaction chamber **15** is at a minimum of 3× greater than the buffer volume.

[0098] Typically it is very difficult to move fluids radially inward using centrifugal microfluidics as the primary means of fluid movement. This can limit/restrict the options available to allow a sequential assay to be performed. To overcome this problem, a combination of centrifugal force and gravity are used to move fluids radially outward and inward respectively. When the cartridge **5** rotates at velocities where the relative centrifugal force (RCF) is much greater than gravity, centrifugal forces will dominate and fluid can be moved radially outward on the cartridge. When the cartridge **5** is stationary or rotating slowly, gravity will still influence the fluid and can be used to move the fluid. To take advantage of this, the cartridge **5** is rotated on an inclined plane (from the horizontal) such that the cartridge **5** can be positioned statically to create a downward slope for fluid to flow. This method can be employed to move fluids radially inward on the cartridge when it is aligned in particular orientations. The flow of fluid under gravity can also be aided by gentle agitation/shaking to overcome any effects of surface tension that may prevent fluids from flowing.

[0099] In FIG. **2**, the buffer stored centrally in the buffer chamber is delivered to the reaction chamber **15** (via a capillary valve **30**) by centrifugal force. This buffer volume fills the cuvette **45** (Zone A) and a blank measurement of buffer can be performed. Next, the applied sample in the sample chamber **35** is also delivered by centrifugal force (via a capillary valve **40**) into the reaction chamber **15** (Zone A) where it is mixed with the buffer. It is appreciated that the sample chamber may include additional sample processing steps such as but not limited to plasma separation or whole blood lysis. A sample measurement can be taken at this point in the test sequence if required (may be used as an internal control). During both buffer and sample delivery steps, the centrifugal force ensures that no fluid reaches Zones B or C and the dried reagents remain intact until R**1** and R**2** are to be rehydrated.

[0100] The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required). The sample and buffer suspension wets reagent R**1** and begins rehydrating it. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a cali-

bration measurement can be performed on this suspension. FIG. **3** illustrates a normal view of the cartridge surface showing reagent rehydration.

[0101] Similar to the rehydration of reagent R**1**, the cartridge **5** is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagent(s) are wetted by the buffer, sample and R**1** suspension. Again, rehydration continues for a defined period of time until both dried reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge **5**. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) where the final reaction can be monitored. It is worth noting that reagents R**1** and/or R**2** can be spotted in singular or multiple spots.

[0102] Illustrated in FIG. **4** are the radii r**1** and r**2**, the angles θ and θ**2** and the length L. The reagent spot locations are not shown for simplicity. r**1** is the radius at which the distal wall of the reaction chamber in Zone B and Zone C is located while r**2** is the radius at which the cuvette is centered in Zone A. The length L is the length of distal wall of the reaction chamber. θ is the angle at which the wall is defined from the centerline (created through the center of rotation **25** and the center of the cuvette) and θ**2** is the angle formed between a notional centerline (through the center of rotation) and the distal wall of the reaction chamber at the extent of the chamber. In this embodiment, the reaction chamber is designed symmetrically about the centerline which can be advantageous but is not a requirement and can be designed asymmetrically. It is preferred that the length of the chamber wall (L) does not extend beyond a point such that the angle θ**2** is <90°. When the angle θ**2** remains ≥90°, this ensures that the radius r**1**<r**2**. Under centrifugal force, this allows fluid to return to the cuvette region at r**2** since fluid will tend towards the outer radius.

[0103] FIG. **5** shows a side view of the cartridge **5** mounted during operation. The cartridge rotates on an inclined plane at an angle of θi (from horizontal). It is ideal that the inclined angle is between 10° to 60°, preferably 30° (provides sufficient gravity and is beneficial for ease of use). Also highlighted are the directions of the centrifugal force and gravity force. The centrifugal force will always be perpendicular to the axis of rotation, i.e. acts in the radial direction (outward) upon rotation.

[0104] For example FIG. **3** shows the cartridge rotated to align at an angle of 120° from a zero position. In one embodiment the zero position can be the lowest point of the cartridge plane with respect to the center of rotation to enable operation. In this location, Zone B can be filled with fluid from Zone A since the cartridge is secured on an inclined plane. After reagent rehydration is performed in Zone B, the fluid can be returned to Zone A (cuvette) for detection by centrifugal or gravity driven methods. However, it is highly preferred that centrifugal force is used to achieve consistent filling of the cuvette.

[0105] FIG. **6**, FIG. **7** and FIG. **8** illustrate the benefit of filling the cuvette by centrifugal force as opposed to gravity. The optical detection path is normal to the cartridge surface and so is aligned perpendicular to the angle at which the cartridge **5** is inclined. It is important that the cuvette is filled entirely and consistently by a column of fluid to ensure that there are no optical irregularities arising from partially or badly filled cuvettes. If the cuvette is filled by gravity, the dominant force on the liquid meniscus is gravity and so the meniscus shape will be uneven and is likely to wet the upper and lower cuvette surfaces to varying levels (FIG. **6**). However, when filled by centrifugal force (FIG. **7**), the dominant force on the liquid meniscus is the centrifugal force. Since the centrifugal force is parallel to the upper and lower surface of the cuvette, the meniscus is formed evenly on both surfaces. This ensures that the detection zone will always be sufficiently filled with fluid during optical measurements. FIG. **8** shows the formed meniscus when viewing the cartridge normal to the axis of rotation. The optical path (which may be larger or smaller than shown) can be filled entirely by centrifugal force. Additionally, filling by centrifugal force also ensures that the cuvette is entirely free from air by preventing any trapped air bubbles forming within the optical window.

[0106] FIG. **9** shows a cartridge design embodiment to perform the assay sequence according to an embodiment of the invention which uses a second dried reagent spot in the third reagent zone. In FIG. **9**, the buffer stored centrally in the buffer chamber **10** is delivered to the reaction chamber **15** (via a capillary valve **30**) by centrifugal force. This buffer volume fills the cuvette **45** (Zone A) and a blank measurement of buffer can be performed. Next, the applied sample in the sample chamber **35** is also delivered by centrifugal force (via a capillary valve **40**) into the reaction chamber **15** (Zone A) where it is mixed with the buffer. A sample measurement can be taken at this point in the test sequence if required (may be used as an internal control). During both buffer and sample delivery steps, the centrifugal force ensures that no fluid reaches Zones B or C and the dried reagents remain intact until R**1** and R**2** are to be rehydrated.

[0107] The cartridge is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required). The sample and buffer suspension wets reagent R**1** and begins rehydrating it. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0108] Similar to the rehydration of reagent R**1**, the cartridge is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagents (split in to reagents R**2**-A and R**2**-B) are wetted by the buffer, sample and R**1** suspension. Again, rehydration continues for a defined period of time until both dried reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) where the final reaction can be monitored. Reagents R**1** and/or R**2** can be spotted in singular or multiple spots.

[0109] In the context of the present invention the term 'zone' can be interpreted as an area within a chamber than can be wholly filled with fluid without wetting or filling neighbouring zones within the same chamber. In practice, this means that the volume of fluid used is typically much less than the total volume of the chamber and is only sufficient to occupy a single zone at any given time. The fluid is then manipulated between each zone by centrifugal or gravitational force. Each zone can be further distinguished or protected from neighbouring zones by physical barriers incorporated in the shape and design of the reaction chamber.

[0110] FIG. **10** shows a cartridge design embodiment to perform the assay sequence illustrated in the flow chart of FIG. **1**, according to one embodiment of the invention. The cartridge design employs a combination of centrifugal and gravitational microfluidics to move fluids to multiple locations on the cartridge. The cartridge **5** includes a buffer reservoir **10** that will sit at or close to the centre of rotation **25**. There is also provided a means for applying a sample directly to the cartridge.

[0111] The cartridge comprises a reaction chamber **15** having at least three zones. The reaction chamber **15** as shown is substantially oblong in the radial direction but it is understood that the shape can be modified for optimal performance such as elliptical, circular, zig-zag or other desired shape to accommodate the three zones. The reaction chamber **15** may also have additional mechanical features (not shown) to better distinguish the individual zones in operation. For example, the centre of the chamber may have a restriction in width and/or depth in relation to either end of the reaction chamber.

[0112] A first zone A is positioned at the radial extent (i.e. furthest from the centre of rotation **25**) of the reaction chamber **15** and defines the detection zone containing the cuvette **45** for optical interrogation of fluid. A second zone B is positioned radially inward of Zone A and contains the first reagent spot location R**1**. A third zone C can be positioned at the most radially inward end of the reaction chamber **15** and contains a second reagent spot location R**2**. It will be appreciated that the third zone can also be positioned in the same radial position as the second zone if required. The motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the three zones.

[0113] In operation, centrifugal force is used to control the delivery of a stored buffer from its reservoir **10** and/or subsequent buffer chambers prior to being delivered to the reaction chamber **15**. The reaction chamber **15** is sized such that it is much greater than the buffer reaction volume that will be used. The reaction chamber **15** incorporates three distinct zones: A) cuvette detection zone, B) R**1** reagent zone and C) R**2** reagent zone. The cuvette **45** is located at the radial extent of the reaction chamber **15** (typically close to the cartridge outer diameter **20**). The chamber is dimensioned to allow for the creation of two zones that can be independently filled with fluid for the R**1** and R**2** reactions. It is beneficial that each zone is sized such that when occupied by buffer they can hold the entire volume within the zone, i.e. the volume of zone A, B or C is equal or greater than the buffer volume and the entire reaction chamber **15** is preferably at a minimum of 3× greater than the buffer volume.

[0114] Typically it is very difficult to move fluids radially inward using centrifugal microfluidics as the primary means of fluid movement. This can limit/restrict the options available to allow a sequential assay to be performed. To overcome this problem, a combination of centrifugal force and gravity are used to move fluids radially outward and inward respectively. When the cartridge **5** rotates at velocities where the relative centrifugal force (RCF) is much greater than gravity, centrifugal forces will dominate and fluid can be moved radially outward on the cartridge. When the cartridge **5** is stationary or rotating slowly, gravity will still influence the fluid and can be used to move the fluid. To take advantage of this, the cartridge **5** is rotated on an inclined plane (from the horizontal) such that the cartridge **5** can be positioned statically to create a downward slope for fluid to flow. This method can be employed to move fluids radially inward on the cartridge when it is aligned in particular orientations. The flow of fluid under gravity can also be aided by gentle agitation/shaking to overcome any effects of surface tension that may prevent fluids from flowing.

[0115] In FIG. **10**, the buffer stored centrally in the buffer chamber **10** is delivered to the reaction chamber **15** by centrifugal force. This buffer volume fills the cuvette **45** (Zone A) and a blank measurement of buffer can be performed. Next, the applied sample in the sample chamber **35** is also delivered by centrifugal force into the reaction chamber **15** (Zone A) where it is mixed with the buffer. It is appreciated that the sample chamber may include additional sample processing steps such as but not limited to plasma separation or whole blood lysis. A sample measurement can be taken at this point in the test sequence if required (may be used as an internal control). During both buffer and sample delivery steps, the centrifugal force ensures that no fluid reaches Zones B or C and the dried reagents remain intact until R**1** and R**2** are to be rehydrated.

[0116] The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required). The sample and buffer suspension wets reagent R**1** and begins rehydrating the reagent. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0117] Similar to the rehydration of reagent R**1**, the cartridge **5** is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagent(s) are wetted by the buffer, sample and R**1** suspension. Again, rehydration continues for a defined period of time until both dried reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge **5**. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) where the final reaction can be monitored. It is worth noting that reagents R**1** and/or R**2** can be spotted in singular or multiple spots.

[0118] FIG. **11***a* illustrates an alternative cartridge design which uses an additional rehydration chamber **46** to rehydrate an R**1** reagent (R**1**-X). In operation, the buffer chamber **10** and the rehydration chamber **46** can be filled from a stored buffer reservoir (not shown) that can be located at a smaller radial location than chambers **10** and **46**, i.e. closer to the centre of rotation **25**. Once these chambers are filled with buffer, the remainder of the assay sequence can proceed in two ways.

[0119] Firstly, the buffer volume can be delivered from the buffer chamber **10** to the reaction chamber **15** where it fills Zone A under centrifugal force. At this point, an optical measure or blank can be taken of the buffer volume in the cuvette **45**. Sample is then delivered from the sample chamber **35** to the reaction chamber **15** under centrifugal force where it mixes with the buffer volume already contained in Zone A. A sample measurement can be taken at this point.

[0120] The rehydrated reagent R**1**-X can then be delivered from the rehydration chamber **46** to the reaction chamber **15** to mix with the diluted sample and buffer volume already

present in Zone A. The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity if a secondary R**1** reagent is present. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0121] The cartridge **5** is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagent(s) are wetted by the buffer, sample and R**1** suspension. Again, rehydration continues for a defined period of time until both dried reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge **5**. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) where the final reaction can be monitored.

[0122] Secondly/alternatively, the above sequence can be altered such that the rehydrated R**1**-X volume can be delivered from the rehydration chamber **46** to the reaction chamber **15** before the buffer or sample. This allows for a reagent blank to be measured optically in the cuvette **45** prior to further dilution with buffer or the addition of sample. The advantages of this method are:

[0123] Reagent R**1**-X can be rehydrated in parallel with other assay processes such as blank measurement, sample/buffer delivery reducing the total assay time.

[0124] Alternatively, the rehydrated R**1**-X may be delivered prior to sample allowing for a reagent blank measurement. This can be advantageous as a control for reagents sensitive to storage conditions.

[0125] FIG. **11***b* illustrates one embodiment of the cartridge design of the embodiment of FIG. **11***a* which uses an additional rehydration chamber to rehydrate a R**1** reagent (R**1**-X). This embodiment is used to perform a glycated haemoglobin (HbA1c) assay but can equally be adapted for other such immunoturbidimetric assays or an enzyme-based clinical chemistry assay. The sample is loaded into the sample chamber **35** and the buffer is loaded into buffer chamber **10**. It will be appreciated that the sample could be delivered using a sample applicator and the buffer chamber could be a stored buffer reservoir.

[0126] Using centrifugal force, the sample in chamber **35** is delivered to a sample metering chamber **54**, where a pre-defined sample volume required for the test is metered. In parallel, centrifugal force is also used to deliver an aliquot of buffer from chamber **10** to a first buffer metering chamber **52**. Subsequently, a second aliquot of buffer is delivered to a second buffer metering chamber **53** and the excess of buffer from chamber **10** is delivered to an overflow metering chamber **58**. Chamber **58** can be used as a procedural control to determine if buffer has been delivered to chambers **52**, **53** and **58**.

[0127] The buffer siphons in the first buffer metering chamber **52** and the second buffer metering chamber **53** are then primed using an acceleration profile provided by the motor attached to the cartridge at **25**. These accelerated primed siphons do not require hydrophilic coatings to function. When the buffer siphons are primed, centrifugal force is used to move the buffer in the first buffer metering chamber **52** downstream to a sample mixing chamber **55** and in parallel move the buffer in the second buffer metering chamber **53** to the rehydration chamber **46**. A suction effect is then used to transfer the sample aliquot from the sample metering chamber **54** into the sample mixing chamber **55** after the buffer aliquot from the first buffer metering chamber **52** has been delivered.

[0128] Sample and buffer are then mixed in the sample mixing chamber **55** to lyse the sample (for HbA1c) and homogenise the dilution. Other assays require the use of plasma instead of whole blood (as required for HbA1c) and the lysis step would not be required in this case. In parallel to sample mixing, the R**1**-X reagent in the rehydration chamber **46** is rehydrated by the buffer aliquot from the second buffer metering chamber **53**.

[0129] The next operation in the cartridge is to prime the siphon exiting the sample mixing chamber **55** (on its left hand side) using an acceleration profile from the motor. This transfers the dilution downstream to the diluted sample metering chamber **56** where an aliquot of this dilution is metered. The excess of this dilution is also transferred to a reaction chamber **57** where it can be used as a procedural control to ensure sufficient sample has been delivered and/or to monitor a reaction after reagent R**3** (if required) has been rehydrated.

[0130] A final acceleration profile from the motor is used to prime the siphon exiting the diluted sample metering chamber **56** and in parallel the siphon exiting the rehydration chamber **46**. Using centrifugal force, the metered volume of the diluted sample from the diluted sample metering chamber **56** and the rehydrated reagent dilution from the rehydration chamber **46** are delivered simultaneously to the reaction chamber **15**. This final dilution is then homogenised using a mixing profile from the motor in Zone A and an optical measurement of sample and R**1**-X is taken from the optical cuvette **45**. A secondary reagent R**1** in Zone B can also be rehydrated and mixed with this dilution. Similarly reagent R**1**-X could be placed at R**1** and rehydrated in the reaction chamber **15** instead. For a HbA1c test this corresponds to the lysed sample being mixed with latex beads (R**1**-X and/or R**1**).

[0131] The cartridge **5** is then orientated to allow the fluid to flow from the cuvette **45** (and sitting in Zone A and B) to Zone C where the R**2** reagent(s) are wetted by the buffer, sample and R**1**-X (and/or R**1**) suspension. Again, rehydration continues for a defined period of time until the reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge **5**. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) using centrifugal force where the final reaction can be monitored. For the HbA1c test, this corresponds to the antibody complex reagents being rehydrated by the dilution of the lysed sample and latex beads. This agglutination phase is then optically monitored at cuvette **45**.

[0132] FIG. **12** illustrates another cartridge design and a variation of the previously described embodiment of FIG. **11***a*. Similar to the rehydration chamber described in FIG. **11***a*, a rehydration chamber **47** as shown contains a dried reagent R**2**-Y. In operation, the buffer chamber **10** and the rehydration chamber **46** can be filled from a stored buffer reservoir (not shown) that can be located at a smaller radial location than chambers **10** and **46**, i.e. closer to the centre of rotation **25**.

[0133] The buffer volume can be delivered from the buffer chamber **10** to the reaction chamber **15** where it fills Zone A under centrifugal force. At this point, an optical measure or blank can be taken of the buffer volume in the cuvette **45**. The sample is then delivered from the sample chamber **35** to

the reaction chamber **15** under centrifugal force where it mixes with the buffer volume already contained in Zone A. A sample measurement can be taken at this point.

[0134] The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity where reagent R**1** is present and can be rehydrated. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0135] The rehydrated R**2**-Y volume can then be delivered from the rehydration chamber **47** to the reaction chamber **15** where it can be mixed with the buffer/sample/R**1** suspension already present in the reaction chamber. Mixing of these volumes can be further enhanced by centrifugal or gravitational means before the mixed suspension is returned to Zone A where the endpoint reaction can be monitored in the cuvette **45**. The advantage of this embodiment is that the reagent R**2**-Y can be rehydrated in parallel with other assay processes such as blank measurement, sample/buffer delivery and R**1** rehydration, thus reducing the total assay time.

[0136] FIG. **13** illustrates another cartridge design and a variation of the previously described embodiment of FIGS. **11***a* and **12**. Similar to the rehydration chamber described in FIG. **11***a*, the rehydration chamber **46** as shown contains a dried reagent R**1**-X and the rehydration chamber **47** as shown contains a dried reagent R**2**-Y. In operation, the buffer chamber **10** and the rehydration chambers **46** and **47** can be filled from a stored buffer reservoir (not shown) that can be located at a smaller radial location than chambers **10** and **46**, i.e. closer to the centre of rotation **25**.

[0137] The buffer volume can be delivered from the buffer chamber **10** to the reaction chamber **15** where it fills Zone A under centrifugal force. At this point, an optical measure or blank can be taken of the buffer volume in the cuvette **45**. The sample is then delivered from the sample chamber **35** to the reaction chamber **15** under centrifugal force where it mixes with the buffer volume already contained in Zone A. A sample measurement can be taken at this point. In parallel to the above steps, the reagents R**1**-X and R**1**-Y have been fully rehydrated in their respective chambers **46** and **47**.

[0138] The rehydrated reagent R**1**-X can then be delivered from the rehydration chamber **46** to the reaction chamber **15** to mix with the diluted sample and buffer volume already present in Zone A. The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity if a secondary R**1** reagent is present. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0139] The rehydrated R**2**-Y volume can then be delivered form the rehydration chamber **47** to the reaction chamber **15** where it can be mixed with the buffer/sample/R**1** suspension already present in the reaction chamber. If present, a secondary R**2** reagent can be rehydrated in Zone C at this point. Mixing of these volumes can be further enhanced by centrifugal or gravitational means before the mixed suspension is returned to Zone A where the endpoint reaction can be monitored in the cuvette **45**. The advantages of this embodiment are:

[0140] Reagent R**1**-X can be rehydrated in parallel with other assay processes such as blank measurement and sample/buffer delivery, thus reducing the total assay time.

[0141] Reagent R**2**-Y can be rehydrated in parallel with other assay processes such as blank measurement, sample/buffer delivery and R**1** rehydration, thus reducing the total assay time.

[0142] FIG. **14***a* illustrates a further variation of the present invention. Shown are a sample dilution chamber **51** and a plurality of reaction chambers **15** (two are shown). Although not shown in the figure, it should be understood that a sample chamber **35** and a buffer chamber **10** can be present radially inward of the dilution chamber **51**. Once the sample has been diluted, it can be delivered through a distribution channel **48** to each reaction chamber **15**, **15**A, **15**B etc. It should be understood that two or more separate reaction chambers can be present per cartridge. The diluted sample is delivered to each sequential reaction chamber via individual delivery channels **49**, **50**.

[0143] As the diluted sample is delivered to each reaction chamber under centrifugal force, Zone A is filled where a sample measurement can be performed in the cuvette **45**. The cartridge **5** is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required). The sample and buffer suspension wets reagent R**1** and begins rehydrating it. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the sample, buffer and R**1** suspension back to the cuvette **45** (Zone A) where a calibration measurement can be performed on this suspension.

[0144] Similar to the rehydration of reagent R**1**, the cartridge **5** is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagent(s) are wetted by the buffer, sample and R**1** suspension. Again, rehydration continues for a defined period of time until both dried reagents are fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge **5**. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) where the final reaction can be monitored. It is worth noting that reagents R**1** and/or R**2** can be spotted in singular or multiple spots. The advantage of this embodiment is that a multiplexed assay can be performed on a single cartridge in isolated reaction chambers preventing the risk of cross contamination.

[0145] FIG. **14***b* illustrates one embodiment of the cartridge design of the embodiment of FIG. **14***a*. This embodiment is used to perform a triplex of immunoturbidimetric or enzyme-based clinical chemistry assays. The sample is loaded into the sample chamber **35** and the buffer is loaded into the buffer chamber **10**. It will be appreciated that the sample could be delivered using a sample applicator and the buffer chamber could be a stored buffer reservoir.

[0146] Using centrifugal force, the sample in chamber **35** is delivered to a plasma separation and metering chamber **59**, where a pre-defined blood sample volume is first metered. In parallel this centrifugal force is used to deliver an aliquot of buffer from chamber **10** into the first buffer metering chamber **52** and the excess of buffer from chamber **10** is delivered to the overflow metering chamber **58**. Chamber **58** can be used as a procedural control to determine if buffer has been delivered to chambers **52** and **58**. The

centrifugal force is then increased to separate the cellular components from the plasma in the plasma separation and metering chamber **59**.

[0147] The plasma siphon exiting the plasma separation and metering chamber **59** and the buffer siphon exiting the first buffer metering chamber **52** are then primed using an acceleration profile provided by the motor attached to the cartridge at **25**. When the siphons are primed, centrifugal force is used to move the metered plasma from the plasma separation and metering **59** and the metered buffer from the first buffer metering **52** downstream to the sample dilution chamber **51** where the plasma and diluent is mixed.

[0148] Once the sample has been diluted and mixed, it is delivered downstream through a distribution channel **48** to each reaction chamber **15**, **15**A, and **15**B and to a buffering chamber **62**, which prevents cross-contamination between **15**A and **15**B, and an overflow chamber **63**. It should be understood that two or more separate reaction chambers can be present per cartridge. The diluted sample is delivered to each sequential reaction chamber via individual delivery channels **49**, **50** and **60**. Between the delivery channel **49** and the reaction chamber **15**, there is an intermediate sample metering chamber **61** which is used to prevent cross-contamination between reaction chamber **15**, **15**A and **15**B. The siphon connecting the intermediate sample metering chamber **61** and reaction chamber **15** is primed using an acceleration profile provided by the motor and the metered sample is then delivered to the reaction chamber **15** using centrifugal force.

[0149] This diluted sample volume fills cuvette **45** (Zone A) in reaction chambers **15**, **15**A and **15**B respectively and an individual blank measurement can be performed in each. During the diluted sample delivery steps, the centrifugal force ensures that no fluid reaches Zone B or Zone C (in reaction chamber **15** only) and the dried reagents remain intact until R**1** and R**2** (in reaction chamber **15** only) are to be rehydrated.

[0150] The cartridge is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required) in reaction chambers **15**, **15**A and **15**B. The diluted sample wets reagent R**1** in all three reaction chambers **15**, **15**A and **15**B and begins rehydrating them in parallel. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move the diluted sample and R**1** suspension back to the cuvette **45** (Zone A) where measurements can be performed on these suspensions.

[0151] For two-phase reactions that contain a second reagent R**2**, as shown in reaction chamber **15** only, the cartridge is then orientated to allow the fluid to flow from the cuvette **45** to Zone C where the R**2** reagent is wetted by the diluted sample and R**1** suspension. Again, rehydration continues for a defined period of time the R**2** reagent is fully rehydrated. The rehydration can again be aided by mixing agitation on the cartridge. Finally, the entire fluid volume is returned to the cuvette **45** (Zone A) in reaction chamber **15** where the final two-phase reaction can be monitored. Reagents R**1** and/or R**2** can be spotted in singular or multiple spots.

[0152] FIG. **14***c* illustrates another embodiment of the cartridge design of the embodiment of FIG. **14***a*. This embodiment, similar to FIG. **14***b*, is used to perform a triplex of immunoturbidimetric or enzyme-based clinical chemistry assays. The sample is loaded into sample chamber **35** and buffer is loaded into buffer chamber **10**. It will be appreciated that the sample could be delivered using a sample applicator and the buffer chamber could be a stored buffer reservoir.

[0153] Using centrifugal force, the sample in chamber **35** is delivered to the plasma separation and metering chamber **59**, where a pre-defined blood sample volume is first metered. In parallel this centrifugal force is used to deliver an aliquot of buffer from chamber **10** into the first buffer metering chamber **52** and the excess of buffer from chamber **10** is delivered to the overflow metering chamber **58**. Chamber **58** can be used as a procedural control to determine if buffer has been delivered to chambers **52** and **58**. The centrifugal force is then increased to separate the cellular components from the plasma in the plasma separation and metering chamber **59**.

[0154] The buffer siphon exiting the first buffer metering chamber **52** is then primed using an acceleration profile provided by the motor attached to the cartridge at **25**. This accelerated primed siphon does not require a hydrophilic coatings to function. When the buffer siphon is primed, centrifugal force is used to move the metered buffer from the first buffer metering **52** downstream to the sample dilution chamber **51**. A suction effect is then used to transfer the plasma volume downstream from the plasma separation and metering chamber **59** into the sample dilution chamber **51** where the plasma and diluent is mixed.

[0155] Once the sample has been diluted and mixed, it is delivered downstream through a distribution channel **48** sequentially to a buffering chamber **66**, reaction chamber **15**, **15**A, and **15**B and to the sample dilution overflow chamber **63**. It should be understood that two or more separate reaction chambers can be present per cartridge. Buffering chamber **66** is placed at the beginning of the distribution channel **48** to ensure non-homogeneous diluted sample flows in here instead of into the reaction chambers **15**, **15**A and **15**B. The buffering chamber comprises a first section **66***a* and a second section **66***b* linked by a capillary channel **67**. The diluted sample passes through a delivery channel **49** using centrifugal force and into the first intermediate chamber **61** which contains a metering chamber and an overflow chamber. The metering chamber is filled with diluted sample first before the overflow fills and blocks its vent. The pressure in the overflow chamber will then increase and the diluted sample flow through delivery channel **49** will stop when the centrifugal pressure being applied to the delivery channel **49** is equal to the pressure in the overflow chamber. When the diluted sample stops flowing through the delivery channel **49**, a second intermediate chamber **64** is filled in the same way through delivery channel **50**. A third intermediate channel **65** is filled in the same manner through the delivery channel **60** prior to the remaining diluted sample being transferred to the overflow chamber **63** via the distribution channel **48**.

[0156] When all of the diluted sample is delivered from the sample dilution chamber **51**, the centrifugal force produced by the motor is increased to break the capillary channel **67** in the buffering chamber **66** so that the diluted sample passes radially outward from the first section **66***a* to the second section **66***b*. In parallel the centrifugal pressure in delivery channels **49**, **50** and **60** will increase and the diluted sample remaining in these channels will be flushed out and

the pressure in the overflow chambers of the first, second and third intermediate chambers, **61**, **64** and **65** respectively will return to normal atmospheric pressure. This allows the downstream fluidics to operate as expected and ensure the transfer of fluids from **61** to **15**, **64** to **15**A and **65** to **15**B when required.

[0157] For two-phase assays, a first reagent R**1** can be placed in the first, second and third intermediate chambers **61**, **64** and **65** and these dried reagents are rehydrated by the metered volumes of diluted sample. An acceleration profile from the motor is then used to transfer this dilution from the first, second and third intermediate chambers **61**, **64** and **65** via their exit siphons to the reaction chambers **15**, **15**A and **15**B downstream. This dilution volume fills cuvette **45** (Zone A) in reaction chambers **15**, **15**A and **15**B respectively and an individual blank measurement can be performed in each. During the dilution delivery steps, the centrifugal force ensures that no fluid reaches Zone B in the reaction chambers **15**, **15**A and **15**B and the dried reagents in Zone B (first or second reagents R**1**, R**2**) remain intact until they are to be rehydrated.

[0158] The cartridge is then aligned to allow the fluid within Zone A to flow to Zone B under gravity (aided by gentle agitation if required) in reaction chambers **15**, **15**A and **15**B. The dilution wets these reagents in all **3** reaction chambers **15**, **15**A and **15**B and begins rehydrating them in parallel. The rehydration continues for a defined period of time until full rehydration has been achieved. This rehydration can be aided by mixing/agitation. When fully rehydrated, centrifugal force is used to move this dilution back to the cuvette **45** (Zone A) where measurements can be performed on these suspensions.

[0159] will be appreciated from the above description that microfluidic system of the present invention is suitable for performing any type of immunoturbidimetric and enzyme-based clinical chemistry assay. Furthermore, the microfluidic system of the present invention is very flexible, as it can be used to perform an assay that requires the addition and rehydration of a single reagent, as well as to perform an assay that requires the addition and rehydration of multiple reagents. This is due to the fact that the second and/or third reagent zones of the cartridge can each be provided with multiple reagent spots.

[0160] In the specification the terms "comprise, comprises, comprised and comprising" or any variation thereof and the terms include, includes, included and including" or any variation thereof are considered to be totally interchangeable and they should all be afforded the widest possible interpretation and vice versa.

[0161] The invention is not limited to the embodiments hereinbefore described but may be varied in both construction and detail.

1. A microfluidic system comprising:

a cartridge coupled to a motor and adapted to move a fluid sample to a plurality of locations on the cartridge, wherein the cartridge is configured to rotate on an inclined plane with respect to a horizontal plane;

the cartridge comprises a reaction chamber having at least three zones, a first zone positioned near one end of the reaction chamber to define a detection zone, a second zone positioned proximal to the first zone and a third zone positioned near the other end of the reaction chamber, wherein each of the second zone and the third zone comprise a reagent zone; and

the motor and a control module is configured to provide a combination of centrifugal force and gravitational force to move said fluid sample between the at least three zones, wherein the first zone comprises a single cuvette positioned adjacent to the outer diameter of the cartridge and configured to allow for optical measurement of each phase of a reaction.

2. The microfluidic system as claimed in claim **1** wherein the first zone is positioned at a radial extent and at a furthest point from a centre of rotation of the reaction chamber,

wherein the second zone is positioned radially inward with respect to the first zone and comprises first reagent spot location R**1**, and

wherein the third zone is positioned between the most radially inward end of the reaction chamber and the radial inward position of the second zone and the third zone comprises a second reagent spot location R**2**.

3. (canceled)

4. (canceled)

5. The microfluidic system as claimed in claim **1** comprising a first separate rehydration chamber to rehydrate an R**1** reagent (R**1**-X) or a different reagent.

6. The microfluidic system as claimed in claim **5**, further comprising a buffer metering chamber coupled to the first separate rehydration chamber configured to meter a pre-defined volume of buffer solution for transfer to the first separate rehydration chamber to rehydrate the R**1** reagent (R**1**-X) or a different reagent in the rehydration chamber.

7. The microfluidic system as claimed in claim **1** comprising a second separate rehydration chamber to rehydrate an R**2** reagent (R**2**-Y) or a different reagent.

8. (canceled)

9. (canceled)

10. (canceled)

11. The microfluidic system as claimed in claim **1** wherein the cartridge is configured to rotate on the inclined plane at a velocity such that a combination of centrifugal force and gravity influence the movement of the fluid sample radially outward and inward in operation.

12. (canceled)

13. The microfluidic system as claimed in claim **1** wherein the cartridge is configured such that no fluid reaches the second zone or third zone when the fluid sample is under the influence of the centrifugal force, and

wherein when the cartridge is configured to be stationary or rotate slowly, gravity will influence the fluid and move the fluid towards the second zone or third zone.

14. (canceled)

15. (canceled)

16. The microfluidic system as claimed in claim **1** wherein the second zone and/or the third zone comprises a dried reagent.

17.-**22**. (canceled)

23. The microfluidic system as claimed in claim **1**, further comprising a sample metering chamber configured to receive the fluid sample and meter a pre-defined volume of the sample and a buffer metering chamber configured to meter a pre-defined volume of buffer solution.

24. The microfluidic system as claimed in claim **23**, further comprising a sample mixing chamber coupled to the sample metering chamber and coupled to the buffer metering chamber, wherein the sample mixing chamber is configured to mix the sample volume transferred from the sample

metering chamber with the volume of buffer solution transferred from the buffer metering chamber to form a dilution of the sample.

25. The microfluidic system as claimed in claim **24**, further comprising a diluted sample metering chamber coupled between the sample mixing chamber and the reaction chamber, wherein the diluted sample metering chamber is configured to meter a pre-defined volume of the dilution of the sample for transfer to the reaction chamber.

26. The microfluidic system as claimed in claim **25**, further comprising a reaction chamber coupled to the diluted sample metering chamber.

27. The microfluidic system as claimed in claim **1**, comprising two or more reaction chambers each reaction chamber comprising at least the first zone, and wherein at least one reaction chamber has the at least three zones.

28. The microfluidic system as claimed in claim **27**, further comprising a sample dilution chamber for mixing the fluid sample and a buffer solution, and a distribution channel coupled between the sample dilution chamber and the two or more reaction chambers, wherein the distribution channel is configured to deliver a diluted sample from the sample dilution chamber downstream to each of the two or more reaction chambers in sequence.

29. The microfluidic system as claimed in claim **28**, further comprising a delivery channel associated with each reaction chamber, wherein the diluted sample is delivered from the distribution channel to each reaction chamber by means of its delivery channel.

30. The microfluidic system as claimed in claim **29**, further comprising an overflow chamber coupled to the distribution channel for receiving the diluted sample which remains after delivery to the two or more reaction chambers.

31. The microfluidic system as claimed in claim **30**, further comprising a buffering chamber coupled to the distribution channel, wherein the buffering chamber is configured to prevent cross contamination between two or more of the reaction chambers.

32. The microfluidic system as claimed in claim **31**, further comprising an intermediate sample metering chamber coupled between one of the reaction chambers and its delivery channel, wherein the intermediate sample metering chamber is configured to prevent cross-contamination between the two or more reaction chambers.

33. The microfluidic system as claimed in claim **30**, further comprising an intermediate chamber coupled between each delivery channel and its reaction chamber.

34. The microfluidic system as claimed in claim **33**, wherein each intermediate chamber comprises a metering chamber and an overflow chamber configured such that the metering chamber is filled with diluted sample from the distribution channel until the centrifugal pressure applied to the delivery channel is equal to the pressure in the overflow chamber.

35. (canceled)

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