(54) Title: CARTRIDGE AND SYSTEM FOR LIQUID HANDLING AUTOMATION

Fig. 1

(57) Abstract: Disclosed herein is a cartridge (100) for liquid handling automation of chemical or biological reactions comprising: a reagent chamber, a sample chamber, reaction chamber and a distribution valve (140) allowing the reagent chamber and the sample chamber liquidly to communicate with the reaction chamber, wherein the reaction chamber comprises a vent for communication with a pump (300) capable of withdrawing air from the reaction chamber for liquid from the reagent and/or the sample chamber to enter the reaction chamber. The cartridge of the invention (100) requires few manual liquid manipulation steps, is suitable for field use and by untrained or only lightly trained individuals. Also disclosed herein is a system comprising the cartridge and an operation unit designed to accommodate the cartridge, wherein the operation unit comprises a pump adapted to access the cartridge through the vent of the reaction chamber.
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CARTRIDGE AND SYSTEM FOR LIQUID HANDLING AUTOMATION

Introduction

The present invention relate to a cartridge for liquid handling automation of chemical or biological reactions and a system in which the cartridge is a part. The simple and elegant system includes a cartridge with several chambers and a non-disposable operation unit that controls the reagent flow inside the cartridge.

Background art

Biochemical assays are methods to analyse chemical substances through the application of biological molecules, e.g. proteins or DNA. An immunoassay is a biochemical assay involving antibodies which are proteins with the property of being able to bind a substance, an antigen, with great specificity and high affinity. The antigen may be a protein, or a peptide, a carbohydrate, a small organic molecule etc. This property is utilised in an immunoassay, where antibodies are used to bind corresponding antigens that are present in a sample. For an introduction please refer to The Immunoassay Handbook edited by Wild (2005). Thus, immunoassays are used to quantify analytes such as biomarkers, toxins, drugs, pesticides, viruses etc.

Applications of immunoassay span a large area. Examples could be found in medical diagnostics, environmental and forensic analysis, food quality control etc. Most state-of-the-art quantitative immunoassays are carried out using automated instrument in centralized laboratories or in specially qualified laboratories. There is an increasing demand to bring specific, sensitive and accurate immunoassays to the end user, such as the doctor’s office, water works, and smaller laboratories.

Several requirements are necessary for quantitative in-situ immunoassays. The instrument and consumables must be user-friendly, e.g. the number of manual liquid manipulations should be restricted to a minimum. Preferably, a single liquid manipulation is required, i.e. the
application of sample into the instrument. The number of consumables must be kept to a minimum, preferably one. Since many samples could contain hazardous substances, waste should be stored properly. The cost of instrument, cost per assay and instrument maintenance should be reasonable, ideally, as low as possible.

A few systems are available on the market. The Triage system from Biosite, San Diego, USA is an example of a point of care (POC) instrument. The disposable system described in US patent application 2005/0136552A1 is based on lateral flow device including non-membrane based microstructures. The sample is applied onto the device and it is dragged by capillary action into a reagent zone where it is mixed with a freeze dried reagent predeposited in the reaction zone. The predeposited reagents are labelled with a tag and binds to analytes in the sample forming a complex. The reaction time is controlled using time gates which are sophisticated microstructures. Reagent flow control is further controlled using zones with different surface properties. The mixture is then further dragged to a capture zone with immobilized capture molecules where the complex is bound. The tag is now bound to the capture zone. It is read and a quantitative measure is obtained. Possible washing steps are done using the excess sample. For the Triage Meter-Plus system a non-disposable system is not required, since the liquid is driven by capillary force due to the porous structure of the membrane. Being one of the current market leaders, nevertheless, the Biosite system has inherent limitations:

a) The physical design of the disposable unit has to be adapted to fit requirements of different assays with e.g. reaction time.

b) High manufacturing costs of the disposable device since sophisticated manufacturing steps are required for the fabrication and different surface treatments are required for flow control.

c) Washing steps are carried out using the excess sample instead of a well defined buffer, which could influence the final results.

d) Since capillary flow is highly dependent on the device surface, viscosity, surface interactions and the physical dimensions of the device, extremely high requirements must be meet for the develop-
ment of quantitative assays.

Another product is the i-STAT. The electrochemical instrument is handheld and battery operated. The disposable system is a cartridge with predeposited reagents and integrated electrodes. However, due to the complicated design of the cartridge, the cost per analysis is very high. Also, only a single analyte can be tested in each analysis.

US patent application 2004/0063217A1 describes a cartridge with possibilities of reagent storage and the liquid is actuated using a combination of check valves that only permit liquids to move in one direction and pneumatic pumps. The reaction chamber contains microarrays enabling multianalyte analysis using a single disposable cartridge. However, this system has its limitations since it need 5 external actuators to address the 5 pneumatic pumps. Another drawback is that under normal circumstances pneumatic pumps does not deliver accurate amount of reagents. The lack of reagent precision propagates into the final results of analysis. Hence, the system only provides semi quantitative analysis.

PCT patent application 2005/072858A1 describes a disposable device where the reagents are stored in a tube as a sequence of liquid plugs separated by air. By connecting the tube to a reaction channel accurate amounts of reagents could be applied over the reaction channel in a sequential fashion using a single pump and one valve. The external actuators are reduced to one or two, nevertheless, two major issues are not resolved using this approach. The first issue is the problem of evaporation from the tube and the diffusion between the liquid plugs. Secondly, the PCT application lacks sample control. The sample must be mixed with a buffer reagent since antibodies require defined salinity and pH for optimal binding. The buffered sample applied to the reaction area must be well controlled either in terms of volume or volume equivalents, since variations in the sample volume will propagate into the results of analysis.

Several publication addresses immunoassays on chip i.e. Sato et al. 2000, Dodge et al. 2001, Linder et al. 2002, however, common for all similar systems, the disposable system does not include reagent stor-
age. Also, often the non-disposable liquid actuation system is extremely complicated and bulky.

**Disclosure of the invention**

The present invention relates to a cartridge for liquid handling automation of chemical or biological reactions comprising: a reagent chamber, a sample chamber, reaction chamber and a distribution valve allowing the reagent chamber and the sample chamber liquidly to communicate with the reaction chamber,

wherein the reaction chamber comprises a vent for communication with a pump capable of withdrawing air from the reaction chamber for liquid from the reagent and/or the sample chamber to enter the reaction chamber.

The cartridge of the invention allows for a simple and efficient handling of liquids. The cartridge may be prefilled with reagents before distribution to a user. The user needs only to add the sample to the cartridge. Thus, the invention is suited for point-of-care or field analysis carried out under non-laboratory conditions by untrained or lightly trained personal.

In a preferred aspect of the invention the valve is capable of assuming two or more positions allowing liquid communication between the reaction chamber and various compartments of the cartridge. The positions of the valve allow for distribution of liquids from compartments to the reaction chamber independent of each other, making it possible for a measured mount of the e.g. the reagent to enter the reaction chamber. The valve may be rotatable and containing a central channel for distribution of liquid.

In an aspect of the invention the cartridge is further comprising a mixing chamber, wherein the mixing chamber via a position of the valve can be in fluid communication with the reaction chamber allowing a pumping between the mixing and reaction chambers, thereby obtaining a mixing of the liquid.

The presence of a mixing chamber makes it possible to obtain an
easy and convenient mixing of the reagent and sample liquids to provide for a more accurate measurement.

In an embodiment of the invention the cartridge is further comprising a waste reservoir, wherein the waste reservoir via a position of the valve can be in fluid communication with the reaction chamber allowing an emptying of the reaction liquid into the waste.

Having the waste reservoir on the cartridge is especially an advantage when handling noxious samples that needs special disposal. Furthermore, it makes the overall design of the system simpler.

Two or more reagent chambers may be present in the cartridge in case complex reactions requiring several reactants are anticipated. One of the reagent chambers may also be used for washing liquids, buffers or similar liquids allowing for pre or post treatment of the liquid reactions performed in the reaction chamber.

In an aspect of the invention the reaction chamber is positioned in the cartridge in proximity to a face of the cartridge opposite to the reagent and sample chambers. The position of the reaction chamber at the opposition face as the notably the effluent chambers makes it possible to use a three dimensional design of the liquid flow path.

When the cartridge is supplied with one or more chambers filled with liquids it may be advantageous to provide the liquid filled chamber with a breakable membrane. When the membrane is broken, air is allowed to enter and the internal pressure is equalized to the ambient pressure. As the internal pressure of the chamber equal to ambient pressure liquid flow is unrestrained when air is withdrawn from the reaction chamber, provided the distribution valve is positioned to allow for a proper liquid communication.

The present invention also relates to a system comprising a cartridge as disclosed herein and an operation unit designed to accommodate the cartridge, wherein the operation unit comprises a pump adapted to access the cartridge through the vent of the reaction chamber. The pump is preferably selected such that it can displace a well-defined volume of air. A piston pump has this ability and is, therefore, a preferred choice. The pump is in a preferred embodiment able to with-
draw air as well as blowing air into the chamber. The blowing of air is advantageous when mixing of liquids is performed and when spent reaction fluid is emptied into the waste reservoir.

The operation unit may additionally comprise an actuator having an adaptor for engaging a corresponding adaptor of the distribution valve, said actuator being capable of adjusting the valve to at least two positions for distribution of liquids between the reaction chamber and the various compartments. Furthermore the operation unit may comprise a pointed tool capable of breaking the membrane to form a vent allowing ambient air to flow in and out of the chamber.

The present invention displays one or more of the following advantages:
- The cartridge is user-friendly and may be used under non-laboratory conditions.
- All reagents, buffers and waste are stored inside the cartridge.
- The user needed to make one liquid manipulation, which is the application of sample into the sample chamber.
- The sample treatment is integrated into the sample chamber.
- Liquids could be mixed using the pump and the mixing chamber,
- The accurate pump injects precise amounts of liquids into the reaction chamber.
- The waste chamber collects reagents such that all hazardous chemicals and sample is contained within the cartridge.
- By integrating microarrays into the reaction chamber, the numbers of analyte analyzed using the same cartridge could be extremely high.
- Chemical amplification steps could be implemented into the cartridge.
- Evaporation problems are minimized due to larger amounts of reagents could be stored and the surface to volume ratio is reduced.
- The sample could be mixed with a reagent or a suitable buffer.
- The sample volume could be accurately measured using the pump.

As pointed out by US patent application 2005/0136552 by Biosite, it is generally to recommend for immunoassays that the sample
and reagent volumes being dispensed are well-defined. Large random or systematic errors in the volume measurements propagate to the final results.

Since the exact volume is measure using the pump, the reagent volumes that is filled into the device during cartridge manufacturing does not have to be accurate. This allows additional reagents to be added to the cartridge to compensate for possible reagent evaporation.

The cartridge is composed of only four parts that could be injection moulded and welded using generic equipment, hence the manufacturing costs will be kept very low.

Since the cartridge only requires two external actuators the cost of the non-disposable system will also be very low.

The total liquid automation system is very simple and elegant; hence the inherent reliability will also be excellent.

The liquids in the disposable device does not come into contact with the non-disposable system, this reduces any cross contamination.

**Detailed description of the invention**

An immunoassay instrument could be partitioned into three subsystems,

I. a biosensor where the antibody – antigen reaction takes place,

II. a transducer that converts the biochemical reaction to physical signal that we could measure and present, and

III. an automatic liquid handling system that transports different reagents to the biosensor.

The present invention relates to the third subsystem. The liquid automation contains two main components, a disposable system, which carries the reagents, the sample and the reaction waste, and a non disposable system which actuates the liquids inside the disposable component. The liquid handling system should achieve several tasks

a) reagent storage
b) sampling

c) sample treatment

d) mixing liquids

e) transfer sample and reagents to reaction area

f) remove and store used reagents

The current invention describes a disposable cartridge suitable for liquid handling automation of chemical and biochemical reactions that require a sequence of steps. The current invention also presents non-disposable components required to successfully operate the cartridge.

Please refer to Figure 1 for an illustration of the invention.

The disposable cartridge (100) is composed of four pieces:
1. the middle part 101
2. the cover part 120
3. the bottom part 130
4. the distribution valve 140

The cartridge includes several chambers for sample application, reagent storage or mixing, and waste, which are connected to a reaction chamber using a distribution valve (140). A sample processing unit could be included in the sampling chamber. The distribution valve is actuated using an external valve actuator (200). The reaction chamber, where the reaction takes place, is connected to a pump (300) which displaces precise volumes of air which in turn moves accurate volumes of liquid. A detection system (400) monitors the reaction inside the cartridge.

The system is designed such with the aid of gravity that air bubbles do not interfere with the reaction, and all reagents and sample are kept inside the disposable device.

Figure 2 shows 4 different views of a simplified example of the middle part. The front side (Figure 2A) of the middle part includes several chambers which could be used for, but not restricted to, sample application (102), reagent storage (103), or mixing (104) and waste (105). The chambers in the front side are connected to a reaction chamber (107) on the back side (Figure 2D) using a distribution valve (140). The
sample is applied into the cartridge through a sample access (109). The mount of the distribution valve (106) has a conical shape to insure a tight sealing, inert valve grease could be required for optimal sealing. Each chamber is connected to the mount by capillaries (108). The reaction chamber is connected to the pump via a venting channel (110). Figure 2B is a side view and 2C is the top view.

The middle part could have other forms and more or less numbers of chambers. An example is illustrated in Figure 3. The sample chamber is a capillary (102), which is advantageous in applications where the sample volume is limited. E.g. for in-vitro diagnostic applications the sample is blood from finger sticks. The total sample volume is often less than 10 μL. There are two reagents (103) and two mixing chambers (104), more chambers could be included. It is very convenient to empty triangular chambers with one of the apex pointing down. Such chambers are very useful if all the reagents in the chamber must be consumed or used for reagent mixing. The reaction chamber (107) is divided into 2 compartments interconnected using one or more channels. The advantage of having several reaction chambers is useful if e.g. several sensors are involved. Another advantage is that the uppermost reaction chamber could serve as an air buffer such that there is plenty of room for air between the reagents in the cartridge and the pump (300). This air buffer would obstruct the reagent from getting into the pump and cause cross contamination.

A sample processing unit could be included in the middle part as a part of the sample chamber (102) or as an attachment to the cartridge connecting to the sample access (109).

Dissimilar samples could be significantly different in terms of viscosity, particle and cellular content etc. Thus, different samples must often be treated differently before carrying out immunoassays. Different application using the same sample could also require unlike preparation procedures.

In certain applications using whole blood as the sample, cellular and particle contents must be removed before possible analysis. For such applications, filters of various kinds are often used, e.g. as dis-
closed in US patent 4477575 and US patent application 20030035758A and the included references. In certain applications much more rigorous sample preparations must be carried out. E.g. to determine Microcystin which is a toxin from cyanobacteria, the sample must be boiled and then filtered before analysis. In such application it should be evaluated if it is more effective to conduct parts or entire sample processing independent of the cartridge.

The cover part in Figure 4 fits the middle part in Figure 2. It includes a hole (121) for the valve and small apertures (122) used to pre-fill reagents. After the reagent reservoirs are filled the apertures are sealed, preferably by aluminium foil. With the valve in a closed position, the foil will keep reagents inside the reservoirs until the application of the cartridge. After sampling, the foils covering the apertures are punctured at the same time or in sequence such that the pressure in the reagent chambers is identical to the surrounding atmosphere. Hence, the apertures both function as reagent filling ports and air vents during analysis.

Immunosensors could be mounted onto the bottom part such that the sensors are located in the reaction chamber. Such sensors could be electrochemical, piezoresistive or piezoelectric, electromagnetic, paramagnetic etc. Preferably, the sensor is a microarray.

Figure 5 shows an example of such a valve. The distribution valve is preferably conical such that it forms a water tight sealing with the middle part (101). The valve has an internal a capillary (141) such that it is able to connect the reaction chamber on the back side of the middle part to the front side of the middle part where different chambers are situated. The valve will have to be fixed to the middle part. In Figure 5 this is done by using a conical valve and a ring structure (142). A “handle” is needed for the connection between the valve actuator (200) and the valve itself. A cross structure (143) is implemented shown in Figure 5.

To be able to control the valve (140) on the cartridge, a valve actuator (200) is implemented. Such actuator could be magnetic, electrostatic, mechanical or of other type. Preferably, the valve actuator is a
stepper motor such that the degree of rotation is well controlled. Controls are implemented such that the position of the stepper motor is accurately known.

The pump used for actuation could be of positive displacement or rotodynamic type. It could be a diaphragm pump, peristaltic pump, syringe pump, centrifugal pump, etc. Preferably, it is a piston pump which displaces a well defined volume of air that displaces a well defined volume of liquid. A pipette of manual or electronic type is an example of such a pump. For the electronic pipette, the moment of the piston that displaces air is executed by a stepper motor. Unlike e.g. a syringe pump, where the pump is often in direct contact with the reagents, a pipette pump does not have liquid contact with the reagents. This avoids cross contamination.

The detection system translates the signal from the sensor located on the bottom part (130) into data that the end-user could interpret. Hence, the detection system is matched with the sensor and the immunoassay of interest.

Preferably, this invention is used to carry out a microarray assay such that several analytes could be analyzed using the same disposable cartridge. In such an application, preferably, the detection system is based on fluorescence detection including a illumination source and a imaging device such as a charge coupled device (CCD) or a CMOS based device. In the above example of application the microarray with immobilized reagents is referred to as the sensor and the detection system is the fluorescence detector.

Operation of the cartridge is stipulated below. The cartridge is assembled and the reagents are prefilled at the manufacturing facilities. The reagent reservoirs are filled by injecting reagents through the air vents (122). The air vents are sealed with metallic membranes, such that evaporation is avoided. The cartridge is then packed appropriately, and it is ready to use.

The user opens the packed cartridge and applies a treated or untreated sample into the sample reservoir through the sampling access (109). The cartridge should remain upright; such gravity will keep the
sample in its reservoir.

Please refer to Figure 1 for a side view illustration of a cartridge inserted into the device. After the sampling step the cartridge (100) is inserted into the device, such that the distribution valve (140) is coupled with the valve actuator (200), the metallic membranes on the air vents will be punctured such that the pressure inside the reagent chambers are identical to its environment during operation. An air tight contact is established between the pump (300) and the cartridge. The cartridge remains upright during the operation such that gravity will keep the sample and reagents in their reservoirs. Since the pipette pump is located above the all the chambers, there is an air plug between reagents and the pump. Together with gravity, the cartridge will keep any liquid from entering the pump itself. This will avoid cross contamination.

The sample is then aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the sample reservoir and the reaction reservoir. The reaction chamber is partially filled.

A first reagent is then aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the reagent reservoir and the reaction reservoir. The liquid volume in the reaction chamber is increased. More reagents could be added in the same fashion.

There are different methods of mixing the reagents with the sample. An example thereof being the integration of a magnetic stirring rod into the reaction chamber of the cartridge. The mixing magnetic rod inside the cartridge is turned using an external rotating magnet, which my a part of the non disposable liquid automation system. The reagent is then mixed with the sample by rotating the magnetic stirrer in the cartridge. The reagent mixing unit could also be integrated into the cartridge using passive mixing structures.

Preferably, the mixing action is carried out by pumping two or more different liquids from the reaction chamber into a mixing chamber (103), repetitions of pumping the mixture back and force between the chambers results in efficient mixing.
After possible incubation, the reaction mixture is pushed out of the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the waste reservoir and the reaction reservoir.

The reaction chamber is now ready for a new sequenced of reactions. The detection could be carried out between or after different steps of cartridge operation.

**Examples**

**Example 1**
Fluorescence microarray immunoassay using the cartridge presented in Figure 3.

Step 1. *Filling of cartridge with reagents*

The cartridge is assembled, reagents are prefilled. The two reagent reservoirs (104) are filled with washing buffer and the fluorescence labelled antibody reagent. One of the mixing reservoirs (103) is used for mixing and the other will remain unused in this example. The reagent reservoirs are filled by injecting reagents through the air vents (122). The air vents are sealed with metallic membranes, such that evaporation is avoided.

Step 2. *Sampling*

The user opens the packed cartridge and applies a sample into the sample reservoir (102) through the sampling access (109). The cartridge should remain upright; such gravity will keep the sample in its reservoir.

Step 3. *Insert into device*

After the sampling step the cartridge is inserted into the device, where the distribution valve is coupled with the valve actuator, the metallic membranes on the air vents will be punctured such that the reagents could be released, and an air tight contact is established between the pipette pump and the cartridge (Figure 8). Note: the cartridge should remain upright; such gravity will keep the sample and reagents in its res-
ervoir. Since the pump is above the all the chambers and liquids, gravity will keep any liquid away from the pump itself.

Step 4. **Injection of sample into reaction chamber (107) with the microarray**
The sample is then aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the sample reservoir and the reaction chamber. The reaction chamber is half filled.

Step 5. **Injection of antibody into reaction chamber**
The antibody reagent is the aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the reagent reservoir and the reaction reservoir. The reaction chamber is now full.

Step 6. **Mixing reagent and sample**
Mixing action is carried out by pumping the reagent and sample mixture into the mixing chambers (103). Repetitions of pumping the mixture back and force between the chambers results in efficient mixing.

Step 7. **Removal of the reaction mixture**
After incubation, the reaction mixture is pushed out of the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the waste reservoir (105) and the reaction reservoir.

Step 8. **Microarray wash**
To remove unbound antibodies the microarray is washed by aspiration of washing buffer into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the washing buffer reservoir and the reaction reservoir. The buffer is then pushed out of the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such
that there is a connection between the waste reservoir and the reaction reservoir. This washing step is repeated.

Step 9. Microarray reading

Microarrays are read using a fluorescence detector.

Example 2

Fluorescence microarray immunoassay using labelled secondary antibodies and the cartridge presented in figure 3

There are to main advantages of using a labelled secondary antibody.
1. It is not necessary to develop labelled primary antibody reagents.
2. Since many secondary antibodies could bind to one primary antibody the signal could be increased and the detection limit could be improved up to 10 times.

However, using a secondary antibody introduces an additional reaction step, and therefore will increase the assay error and complexity. Also such assays are normally only compatible with competitive assays.

Step 1. Filling of cartridge with reagents

The cartridge is assembled, reagents are prefilled. The two reagent reservoirs (104) are filled with washing buffer and the primary antibody reagent. One of the mixing reservoirs is used for mixing and the other will be used to store the fluorescence labelled secondary antibody reagent in this example. The reagent reservoirs are filled by injecting reagents through the air vents. The air vents are sealed with metallic membranes, such that evaporation is avoided.

Step 2 – 8 is similar to Example 1.

Step 9. Adding the labelled secondary antibody reagent.

The secondary antibody reagent is then aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the secondary
antibody reservoir and the reaction reservoir.

Step 10. *Incubation*

Step 11. Microarray wash – similar to step 8

Step 12. *Microarray reading*
Microarrays are read using a fluorescence detector.

**Example 3**
Colorimetric or chemiluminescence microarray immunoassay using the cartridge presented in figure 3.

Colorimetric and chemiluminescence detection have certain advantages compared to fluorescence detection. The detector requirements is very low for colorimetric assays, hence costs for the detector could be greatly reduced. Chemiluminescence assays provide unmatched sensitivity. For both types of assays the antibody is labelled with an enzyme, typically alkaline phosphatase or horseradish peroxidase. By adding a substrate the enzymes generate a colour reaction or emit light. A typical substrate for colorimetric reactions is the non-soluble TMB (3,3′,5,5′-tetramethylbenzidine), which stains the microarray with a blue colour. Examples of chemiluminescence substrates are AMPPD (Adameantyl 1,2-dioxygen arylphtate) and Lumigen TMA-6 from Lumigen Inc.

Step 1. *Filling of cartridge with reagents*
The cartridge is assembled, reagents are prefilled. The two reagent reservoirs (104) are filled with washing buffer and the enzyme labelled antibody reagent. One of the mixing reservoirs is used in this example as a reagent reservoir for the enzyme substrate. The reagent reservoirs are filled by injecting reagents through the air vents. The air vents are sealed with metallic membranes, such that evaporation is avoided.

Step 2 – 8 is similar to Example 1.
The substrate is then aspirated into the reaction chamber by displacing a well-defined volume using the pipette pump and turning the valve such that there is a connection between the substrate reagent reservoir and the reaction reservoir.

Step 10. *Microarray reading*
After the addition of the substrates the microarray could be read in different modes. For chemiluminescence the detector could be set to integrate the emitted light over a fixed period of time. For colorimetric reactions the detector could be set to follow the staining of the microarray in real time or to take one measurement after a certain reaction time. An optional washing step could be included.
PATENT CLAIMS

1. Cartridge for liquid handling automation of chemical or biological reactions comprising: a reagent chamber, a sample chamber, reaction chamber and a distribution valve allowing the reagent chamber and the sample chamber liquidly to communicate with the reaction chamber, wherein the reaction chamber comprises a vent for communication with a pump capable of withdrawing air from the reaction chamber for liquid from the reagent and/or the sample chamber to enter the reaction chamber.

2. The cartridge according to claim 1, wherein the valve is capable of assuming two or more positions allowing liquid communication between the reaction chamber and various compartments of the cartridge.

3. The cartridge according to claim 1 or 2 further comprising a mixing chamber, wherein the mixing chamber via a position of the valve can be in fluid communication with the reaction chamber allowing a pumping between the mixing and reaction chambers, thereby obtaining a mixing of the liquid.

4. The cartridge according to any of the preceding claims, further comprising a waste reservoir, wherein the waste reservoir via a position of the valve can be in fluid communication with the reaction chamber allowing an emptying of the reaction liquid into the waste.

5. The cartridge according to any of the preceding claims, wherein two or more reagent chambers are present in the cartridge.

6. The cartridge according to any of the preceding claims, wherein the reaction chamber is positioned in the cartridge in proximity to a face of the cartridge opposite to the reagent and sample chambers.

7. The cartridge according to any of the claims 1 to 6, wherein the reagent chamber comprises a breakable membrane.

8. A system comprising the cartridge according to any of the claims 1 to 7 and an operation unit designed to accommodate the cartridge, wherein the operation unit comprises a pump adapted to access the cartridge through the vent of the reaction chamber.

9. A system according to claim 8, wherein the operation unit further comprises an actuator having an adaptor for engaging a corre-
sponding adaptor of the distribution valve, said actuator being capable of adjusting the valve to at least two positions for distribution of liquids between the reaction chamber and the various compartments.

10. A system according to any of the claims 8 to 9, wherein the operation unit comprises tool capable of breaking the membrane.

11. A system according to any of the claims 8 to 10, further comprising a detector means for monitoring the reaction inside the cartridge.
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

**INV.** BOIL3/00

According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

BOIL

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
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<td>X</td>
<td>EP 1 535 667 A1 (SYSMEC CORP [JP]) 1 June 2005 (2005-06-01) paragraph [0016] - paragraph [0061]; figure 2</td>
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<td>X</td>
<td>WO 03/104771 A1 (CHEMPAQ AS [DK]; LARSEN ULRIK DARLING [DK]; HANSEN STINE HALLBERG [DK]) 18 December 2003 (2003-12-18) page 15, line 7 - line 33, paragraph 19; figures 6-8</td>
<td>1-6, 8-11</td>
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<td>X</td>
<td>US 5 731 212 A (GAVIN MICHAEL [US] ET AL) 24 March 1998 (1998-03-24) column 10, line 36 - column 12, line 14; figures 6-7d</td>
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Date of the actual completion of the international search: 28 October 2009

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