

Apparatus and method for detection of organismsTechnical Field

The present invention relates to an apparatus and method for the detection of organisms. In particular, the invention relates to an apparatus and method for the detection of organisms from a fluid such as, for example, a water sample.

Background

Microbial contamination of drinking water is a major problem in developing as well as developed countries. Outbreaks of and even fatalities from serious enteric disorders have been reported. The cause of each different outbreak may be due to different culprit microorganisms.

Water monitoring is very important, with a view to safeguarding public health by complying with stringent water standards. Water supply infrastructure has always been vulnerable to pathogenic contamination, either at the reservoir and/or in the distribution pipelines. As water can be very easily contaminated, it is crucial to check water quality at different stages of the distribution system.

There are two critical checkpoints for water monitoring – the water reservoir and the public distribution pipeline system. The water reservoir usually has a centralised system for treatment of water. Water treatment, including chlorination, ozone and UV treatment may be carried out at treatment plants to inactivate or eliminate pathogens and make water safer to drink.

In addition, the distribution channels may be prone to contamination by microorganisms and it is also important to monitor the distribution channels. As it is important to monitor water treatment and also what is entering homes of consumers, water authorities have implemented stringent water safety policies and water companies are under increasing pressure to comply with the strict regulations.

Conventional testing methods for the presence of microorganisms include cell culturing which may take a few days to obtain tests results, leading to delays or a failure to alert consumers or the water authorities until it is too late, by which time many cases of infection may have already been reported. A second method for testing for the presence of microorganisms is by staining a concentrated sample and observation by microscopy. However, this test requires a large number of cells to be present in the sample and there is the possibility that the pathogens may not be observed and remain undetected. In addition, such conventional test methods are manually conducted and

involve several sample transfers which may lead to loss in sensitivity at every stage and may thus generate unreliable results. There have been reported cases where hazardous bacteria like *Escherichia coli* O157:H7, *Salmonella*, *Shigella* come to light only after 6-8 days, following the reporting of a few cases of infection with disease.

- 5 *Cryptosporidium* is an example of a pathogen spread by contaminated water. *Cryptosporidium* is a protozoan human parasite that infects and invades the intestinal tract causing severe diarrhoea symptoms for a few weeks. *Cryptosporidium* oocysts (the infective form) are resistant to water treatment by chlorination but UV treatment may be effective. Contamination of the water supply may be by *Cryptosporidium*
- 10 oocysts which may have evaded conventional chlorine treatment or subsequent contamination of the water supply. Examples of current methods for detecting viable *Cryptosporidium* include the USEPA Method 1622 and also the USEPA Method 1623 for detection of *Cryptosporidium* together with *Giardia*. Basically, the USEPA 1622 method includes concentration by filtration, elution (by wrist action or agitation) and
- 15 viability determination by membrane permeability dye assays with 4', 6-diamindino-2-phenylindole (DAPI) and propidium iodide (PI) and microscopy. These assays require standalone equipment, numerous sample transfers and are time consuming and involve high costs to water companies, including field samples transportation costs, laboratory costs, certificates of inspection costs. Moreover, theses assays are also
- 20 highly dependent on the operator's technical efficiency in interpreting the results. Further, water treatment may also affect the monitoring of water for organisms post-treatment. For example, although UV treatment of water may cause *Cryptosporidium* oocysts to lose infectivity, this treatment alters the membranes of *Cryptosporidium* and renders them impermeable to the staining dyes and the presence of viable
- 25 *Cryptosporidium* oocysts may not be detected accurately. It is therefore desirable to develop an improved water monitoring system that is rapid, efficient and reliable.

Summary

The present invention relates to an apparatus and method for detecting organisms from

30 a fluid, for example a water sample.

According to a first aspect, there is provided an apparatus for detecting organisms from a fluid comprising at least the following components:

- (i) an inlet for introducing the fluid into the apparatus;

(ii) a filtration system for trapping particles including organisms from the fluid including a system configured to direct a flow of eluent to the filtration system at a pressure sufficient to release trapped organisms into the eluent; and

(iii) a detection cartridge for detecting different organisms comprising at least an array of a plurality of assay chambers connected via at least a first common channel, being a common inlet channel; and

(iv) at least one valve for controlling fluid flow through the apparatus; wherein at least components (i) to (iii) are in fluid communication.

According to another aspect, there is provided a method for detecting organisms from a fluid comprising:

(a) providing an apparatus comprising at least the following components:

(i) an inlet for introducing the fluid into the apparatus;

(ii) a filtration system for trapping particles including organisms from the fluid including a system configured to direct a flow of eluent to the filtration system at a pressure sufficient to release trapped organisms into the eluent; and

(iii) a detection cartridge for detecting different organisms comprising at least an array of a plurality of assay chambers connected via at least a first common channel, being a common inlet channel; and

(iv) at least one valve for controlling fluid flow through the apparatus; wherein at least components (i) to (iii) are in fluid communication;

(b) introducing a fluid via the inlet into the apparatus;

filtering the fluid through the filtration system to trap particles including organisms from the fluid and directing a flow of eluent through the filtration system at a pressure sufficient to release trapped organisms into the eluent;

(c) allowing the eluent to flow via the common inlet channel into at least one assay chamber of the cartridge; and

(d) performing at least one reaction to detect at least one species of organism.

The detection cartridge of the apparatus may further comprise at least a second common channel connected to the plurality of assay chambers, being a common outlet channel. Each assay chamber of the detection cartridge may further comprise at least one dedicated inlet channel only to that particular assay chamber.

The apparatus may be automated such that once the fluid is introduced into the apparatus, there is little or no requirement for further user intervention and the apparatus performs the detection reaction and subsequently provides the detection results. The user may then review and analyse the detection results to determine the presence and/or identity of organisms present in the fluid. Accordingly, the apparatus may include a processor for automation. The processor may control the entire process of performing the method of the invention, including fluid flow through the various components of the apparatus, the detection reaction and output of the detection results. Fluid flow through the various components of the apparatus may be controlled via valves, each of which may be actuated by at least one motor, which in turn may be controlled by the processor.

A more complete understanding of the present invention, as well as further features and advantages of the present invention, will become apparent from the detailed description and figures below.

Brief description of the drawings

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative drawings.

In the drawings:

Figure 1 shows a plan view of an exemplary embodiment of a base including various components of the apparatus, together with a cross-sectional side view of the apparatus along the line A-A.

Figure 2 shows a schematic representation of an example of the apparatus of the present invention.

Figure 3(A)-(F) illustrates the steps in the method of the present invention.

Figure 4 shows an example of a filter holder. Figure 4(A) depicts a perspective cut-away view of the filter holder and Figure 4(B) depicts a plan view, cross-sectional side view and an exploded view of the filter holder.

Figure 5 shows an example of a low pressure concentrator for concentrating a sample to a predetermined volume.

Figure 6 illustrates a first embodiment of the method of concentrating a sample to a predetermined volume with a low pressure concentrator.

Figure 7 illustrates a second embodiment of the method of concentrating a sample to a predetermined volume with a low pressure concentrator.

Figure 8 shows an example of the detection cartridge comprising two assay chambers.

Figure 8(A) shows a plan view of the detection cartridge and Figure 8(B) shows cross-sectional side view of the reaction cartridge along the line A-A of Figure 8(A).

Figure 9 illustrates the fluidic operation of loading primers and PCR mixture and draining of the detection cartridge. The arrows indicate the direction of fluid flow.

Figure 10 shows a photograph of the fluidic operation of loading primers and PCR mixture and draining of the detection cartridge. The arrows indicate the direction of fluid flow.

Figure 11 illustrates the fluidic operation of discharging a portion of the primers and washing of the detection cartridge prior to performing a subsequent round of PCR. The arrows indicate the direction of fluid flow.

Figure 12 shows a photograph of the fluidic operation of discharging a portion of primers and washing of the detection cartridge prior to performing a subsequent round of PCR. The arrows indicate the direction of fluid flow.

Figure 13 illustrates the initial steps of loading primers and PCR mixture for a subsequent round of PCR.

Figure 14 shows a photograph of the initial steps of loading primers and PCR mixture for a subsequent round of PCR.

Figure 15 illustrates an embodiment of the detection cartridge.

Definitions

Residual substances refer to any remaining substances other than the target substance (e.g. DNA or antigen) to be detected and include but are not limited to substances smaller than DNA, particles smaller than DNA, contaminants, impurities, PCR inhibitors, chemicals and metal ions.

Detailed description of the exemplary embodiments

The present invention relates to an apparatus and method for detecting organisms from a fluid sample. The reaction to detect the organism may be detecting for DNA or alternatively antigens specific to a particular species of organism. Detection of specific DNA sequences may be by any conventional method, for example hybridisation to specific probe sequences or using polymerase chain reaction (PCR). The detection of specific antigens may be by a specific antibody-antigen reaction. In one particular

embodiment, the detection reaction comprises the use of real-time PCR for detecting amplified DNA of specific organisms with specific primers.

In an exemplary embodiment, some components of the invention may be mounted onto a support. As shown in Figure 1, the support may be in the form of a base 1 mounted with various components of the apparatus. For example, the filter holder 2 and the detection cartridge 3 are mounted on the base 1 as shown in Figure 1. The base may also be mounted with a reservoir 4 for eluent, a reservoir 5 for collecting eluent comprising organisms exiting the filtration system, a reservoir 6 for storing PCR reagents and a mixer 7 for mixing of the PCR reagents. The additional components of the apparatus include at least one of the following components: a reaction chamber 8 for the reaction for differentiating live organisms, a cell lysis system 9, a filter 10 for substantially removing particles larger than DNA, a filter 11 (e.g. gel filtration column) for substantially removing residual substances (e.g. including but not limited to substances smaller than DNA, particles smaller than DNA, contaminants, impurities, PCR inhibitors, chemicals and metal ions) and a concentrator 12. Tubings 13 and/or channels 14 may be used for fluid communication between the components. The base may also include additional control components 15 comprising connectors 16, 17 and pinch valves. Each pinch valve typically comprises soft tubing 18 and bars 19 for pinching the tubing. The pinching bars 19 act like fingers to pinch the tubing 18 to shut the valve and reduce/stop fluid flow or, in the release position, to allow almost unobstructed fluid flow. The pinching bars 19 may each be independently actuated by at least one motor.

Figure 2 shows a schematic representation of an example of the apparatus of the present invention. Components of the apparatus mounted on the base 1 of Figure 1 are shown inside a dashed line box 20. These components include a reservoir 4 for storing the eluent, a filtration system including a filter holder 2 for trapping organisms, a reservoir 5 for collecting organisms after being released from the filtration system, a reaction chamber 8 for differentiating viable organisms, a cell lysis system 9, a filter 10 for substantially removing particles larger than DNA, a filter 11 for substantially removing residual substances (e.g. substances or particles smaller than DNA, contaminants, impurities, PCR inhibitors, chemicals or metal ions), a concentrator 12 for concentrating DNA test solution, a reservoir 6 for storing PCR master mix reagents, a mixer 7 for thorough mixing of the PCR master mix with the DNA test solution, and a

PCR detection cartridge 3 with an array of assay chambers. In particular, the filter 11 may be for removing chemicals, metal ions and other substances smaller than DNA that may inhibit the detection method (for example, PCR or other genetic detection). The filter may utilise any suitable filtration system, including but not limited to gel
5 filtration, a pore filtration membrane (e.g. which blocks DNA) or DNA adsorption and elution systems (e.g. silica medium or magnetic beads system).

In addition, a three-port pinch valve 21 and several two-way pinch valves 22-28 for fluidic control are also included as illustrated in Figure 2. Other components include a light emitting diode 29 for transmitting light to the reaction chamber 8, a heat and/or
10 ultrasonic wave generator 30 for cell lysis with lysis reactor 9, a heater 31 for the concentrator 12, a condenser 32 for collection of vapour from the concentrator 12, a thermal cycler 33 under the PCR detection cartridge 3 comprising an array of assay chambers 34, an illuminator 35 and a detector 36 for fluorescence signal detection of real-time PCR.

The fluid and chemicals inside the integrated apparatus may be driven pneumatically by a pneumatic system within the apparatus. The pneumatic system includes a pressure pump 37, an air reservoir 38, a filter 39, a first regulator 40 for a pressure level of ~ 2.5 bar, a second regulator 41 for a pressure level of ~ 0.01 bar, a vacuum pump 42, a drain filter 43 and several pneumatic solenoid valves 44-52. An additional
20 tank 53 for eluent may be connected to the eluent reservoir 4 through a valve 54. A tank 55 for PCR master mix may be connected to the PCR master mix reservoir 6 through valve 56. An inlet 57 for the fluid sample and an outlet 58 for the filtered fluid sample is also provided as shown. A pre-filter 59 for removing larger particles and dirt and a flow meter 60 for measuring the volume of the fluid sample is also illustrated.

25 An exemplification of the components of the apparatus together with their operation when the apparatus is in use and the method of the invention will now be described in detail below, with reference to the embodiment shown in Figure 3.

The reservoir 4 is loaded with eluent 61 (e.g. 10 ml of an aqueous solution with propidium monoazide) supplied from a tank 53. Another reservoir 6 is loaded with PCR
30 master mix 62 (e.g. 20 µl of buffer with dNTPs, fluorescence dye, PCR polymerase) from another tank 55. At the start of operation, all the two-way valves 22-28 are maintained in the closed position (Figure 3(A)). For example, the apparatus may be connected directly to a water supply, for example, from the tap. As illustrated in Figure

3(B), the first valve 22 that regulates incoming water sample is opened. The water sample flows into the sample inlet 57, passes through a pre-filter 59, a flow meter 60 at a pressure of ~ 2-5 bar (pressure dependent on water source). The water may be introduced into the apparatus directly from the tap or via a water pump. The pressure of the water flow depends on the pressure of tap water or may be adjusted using the water pump. The water then enters the filtration system.

Filtration system

The filtration system is designed for trapping and retrieving particles (e.g. organisms) and comprises a filter holder and a system configured to direct a flow of eluent through the filtration system under pressure to release trapped organisms into eluent exiting the filtration system.

An embodiment of the filtration system as described in SG 200902067-8 may be used for the present apparatus. The filtration system as described in SG 200902091-1 and PCT application entitled "A filter" which claims priority from SG200902061-1 may also be used for the present apparatus. In an exemplary embodiment, the filtration system comprises a filter holder 2 comprising a membrane filter comprising a plurality of pores of substantially the same size. In a particular embodiment, the membrane filter 64 is sandwiched between two support grids 63, 65 (Figure 4).

The openings 77 of the upper support grid 63 may be aligned with the openings 78 of the lower support grid 65. The lower support grid 65 is mounted on top of a base 74 with a gasket 73 between them to prevent leakage. The inner-upper surface of the base 74 may be shaped to facilitate fluid flow downwards and to reduce residue build-up. A lower opening 76 in the base 74 allows for inflow of the water sample and outflow of eluent.

A flow guide 71 is placed on top of the upper support grid 63, with a gasket 72 between them to prevent leakage. A lid 70 is fitted on top of the flow guide 71. In the lid 70 there is an upper opening 75 for outflow of the water sample and inflow of eluent (for back-flushing).

When these components are secured together, a lower chamber 80 is formed between the membrane filter 64 and the base 74, and an upper chamber 79 is formed between the lid 77 and the membrane 64, as shown in Figure 4(B).

The filter holder 2, including the membrane filter 64 and two support grids 63 and 65, is placed upright within the filtration system (Figure 3(A)). The upper opening 75 of the

filter holder 2 is connected to a common port 81 of a three-port valve 21. A second port 82 of the three-port valve 21 is connected to an outlet 58 and a third port 83 is connected to an eluent supply, for example, reservoir 4 containing eluent 61 (Figure 3(A)). At the start of operation of the apparatus, the three-port valve 21 is positioned
5 with the connection to the outlet open and the connection to the reservoir 4 closed (Figure 3(B)).

As illustrated in Figure 3(B), the water sample enters the filter holder 2 via the lower opening 76, flows through the lower chamber 80, the membrane filter 64 and its support grids 63, 65 and the upper chamber 79 and exits the filter holder 2 via the
10 upper opening 75. Typically, the water passes through the membrane filter 64 at a pressure of approximately 2-5 bar. The membrane filter 64 does not bend or break under the pressure due to the upper support grid 63 and/or lower support grid 65 and may thus withstand the large flux and pressure due to the water flow.

The water sample has been pre-filtered through pre-filter 59 to substantially remove
15 larger particles like dirt and other impurities which may clog the membrane filter 64. The remaining particles (including organisms) from the water sample, especially those larger than the pores of the membrane filter, may then be trapped by the membrane filter 64 as the fluid sample flows through the filter holder 2. The pores of the membrane filter 64 may be precisely fabricated to trap particles of specific sizes as
20 discussed in the above-referenced specifications. In general, organisms, which typically have a size bigger than $0.45\text{ }\mu\text{m}$ will be trapped by using a membrane filter 64 with a pore size of $0.45\text{ }\mu\text{m}$. The size of the pores may be varied according to requirement. For example, for trapping *Cryptosporidium* which are in the range of $3\text{--}6\text{ }\mu\text{m}$, a suitable pore size may be $\sim 2\text{ }\mu\text{m}$. For smaller organisms, the size of the pore
25 may be reduced as appropriate. The diameter d of the pores of the membrane filter may, for example, be in the range of: $0.1\text{ }\mu\text{m} \leq d \leq 10\text{ }\mu\text{m}$.

The filtered water flowing through the upper opening 75 of the filter holder 2 contains virtually no particles or organisms and eventually exits the apparatus via the outlet 58 (Figure 3(B)). The "purified" water may be collected on exit if required. In this step, 15
30 litres of a fluid sample (e.g. tap water) may be passed through the membrane filter relatively quickly, such as in 20 minutes, with almost all particles (organisms, pathogens etc) trapped by the membrane filter and/or retained in the lower chamber.

The filtration system also includes a system configured to direct a flow of eluent through the filtration system at a pressure sufficient to release trapped organisms into eluent exiting the filtration system. In the embodiment shown in Figure 3(A), there is a reservoir 4 for storing eluent 61. The reservoir 4 may for example, store 10 ml of eluent. In an exemplary embodiment, the eluent may comprise, for example, an aqueous solution containing propidium monoazide.

After a suitable amount of the water sample (for example, 15 litres) has entered the apparatus, the valve 22 controlling the incoming sample is closed. When the water sample has flowed through the membrane filter 64 and exited the apparatus, the three-port valve 21 is then adjusted to close the connection to the outlet 58 and open the connection to the reservoir 4 containing the eluent 61 (Figure 3(C)). The valve 23 controlling the connection to the second reservoir 5 is also opened. The air vent valve 45 and an air pressure control valve 44 are then opened and air at a pressure of ~ 2.5 bar enters the reservoir 4 and forces the eluent 61 through the filter holder 2. Inside the upper chamber of the filter holder, the eluent is guided through the openings of the upper support grid 64 and as the openings of the upper support grid 64 are aligned with the openings of the lower support grid 65 a jet flow of eluent is formed under pressure and the eluent passing through the membrane filter 64 flushes down particles (organisms, pathogens etc) deposited on the pores of the lower surface of the membrane filter 64. The particles flushed down from the membrane filter 64 together with the particles retained in the lower chamber form eluent comprising organisms 66. This process is known as "back-flushing". As the air pressure continues to be maintained, the eluent comprising organisms 66 flows to and collects in the second reservoir 5, as the valve 24 is in a closed position (Figure 3(C) and (D)). The airflow may be kept blowing through the membrane filter 64 for a further two minutes before the air pressure control valve 44 is closed to ensure almost complete collection of all the eluent comprising organisms 66 in the second reservoir 5. In addition, a reduced pressure may be generated by closing additional air vent 45 and opening additional vacuum control valve 46 to further assist the complete collection of the eluent comprising organisms in reservoir 5 (Figure 3(D)). The filtration system may also operate as a standalone component.

Apparatus for reaction for differentiating organisms

In the embodiment shown in Figure 3, the next component is an apparatus for a reaction for differentiating viable organisms.

An embodiment of an apparatus for a reaction for differentiating viable organisms has been described in PCT application entitled "A fluidic apparatus and/or method for differentiating viable cells" which claims priority from SG 200902057-9.

In the embodiment shown in Figure 3(E), the apparatus comprises a substantially optically transparent reaction chamber 8 configured in use to expose to light from a light source the reaction chamber 8 comprising the eluent comprising organisms and a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised organisms over viable and/or substantially intact organisms so as to bind the phenanthridium compound to nucleic acid molecules of penetrated organisms on exposure to light and at least one light source configured to transmit light to the reaction chamber 8. The reaction chamber component 8 may be detachable for cleaning or replacement.

The next step may thus include allowing the eluent comprising organisms exiting from the filtration system to flow into the substantially optically transparent reaction chamber 8, providing a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised over viable and/or substantially intact organisms to mix with organisms in the eluent; exposing the eluent comprising organisms and phenanthridium compound to light transmitted from a light source to the reaction chamber 8 to enable a reaction of phenanthridium compound covalently binding with nucleic acid molecules of penetrated dead or membrane-compromised organisms; and allowing reacted liquid sample to flow out of the reaction chamber 8. The phenanthridium compound may be any phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells. In an embodiment, the phenanthridium compound may be propidium monoazide which may be included in the eluent as described above. Alternatively, the phenanthridium compound need not be included in the eluent but may be introduced separately into the reaction chamber. In one such embodiment, the phenanthridium compound may be introduced separately into the reaction chamber 8 by preloading into the reaction chamber. The eluent comprising organisms mixes with the preloaded phenanthridium compound as it enters the reaction chamber. In another

embodiment, the eluent comprising organisms may enter the reaction chamber via a first inlet and the phenanthridium compound may enter the reaction chamber via a second inlet.

The following steps are then carried out to allow the eluent comprising organisms 66 to
5 flow from the second reservoir 5 into the reaction chamber 8 for the reaction for differentiating viable cells. The pinch valve 23 upstream of the second reservoir 5 is closed. The air vent 45 and the vacuum control valve 46 are also closed. A pinch valve 24 downstream of the reservoir 5 is opened. An air pressure control valve 47 may be operated in a pulsed manner such that air pressure of ~ 0.01 bar from a regulator 41
10 enters the reservoir intermittently. Under pressure, the eluent comprising organisms 66 from the reservoir 5 is pushed into the substantially optically transparent reaction chamber 8 and the light emitting diode (LED) 29 positioned below the reaction chamber 8 is also switched on as the eluent comprising organisms enters the reaction chamber. As the LED 29 transmits light to the reaction chamber, the phenanthridium compound,
15 for example, propidium monoazide binds covalently to the DNA of penetrated cells (a process known as "photo-crosslinking"). In a particular embodiment, the LED emits blue light for the photo-crosslinking reaction to occur.

The reaction chamber may comprise a straight channel or may include at least one bend which may slow the flow of liquid through the chamber. Slowing the flow of fluid
20 through the reaction chamber may thus enable the reaction to be substantially completed as the fluid flows through the reaction chamber while exposed to the light source. In the embodiment shown in Figure 3(E), the reaction chamber 8 includes a number of bends. In addition, by controlling the opening and closing of the air pressure control valve 47, the pulse ratio of the air pressure may be controlled and in turn, the
25 flow rate of the liquid through the reaction chamber 8 may be controlled. Accordingly, the flow of fluid through the reaction chamber 8 may be manipulated/controlled by either the channel design and/or valves. The LED 29 may be configured to transmit light to the reaction chamber 8 for a predetermined time. This predetermined time should be sufficient to allow for a substantially complete reaction as the fluid flows
30 through the reaction chamber 8, for example 10 mins. The reacted liquid sample may then exit the reaction chamber 8. Since most of the DNA of membrane-compromised and/or dead organisms would be covalently bound to the phenanthridium compound following the photo-crosslinking reaction, these covalently bound DNA will not be

available for the subsequent detection reaction(s). Only DNA from intact and/or viable cells would participate in the subsequent detection reactions. Accordingly, only DNA from intact and/or viable cells will subsequently be detected with the inclusion of the apparatus for a reaction to differentiate viable cells.

5 Cell lysis system and further filtration of liquid sample

After substantially the entire eluent comprising organisms from the reservoir 5 has passed through the reaction chamber 8 and undergone the photo-crosslinking reaction, the reacted liquid sample 67 may then directed to another optional component, the cell lysis system (Figure 3(F)). The pinch valve 24 and the air pressure control valve 47 are
10 then closed (Figure 3(F)). In one embodiment, the cells from the reacted sample may be lysed using heat. In the embodiment illustrated in Figure 3(F), the cell lysis system comprises a generator 30 which produces ultrasonic waves for lysing cells in contact with a lysis reactor 9. In addition (or alternatively), other methods, whether individually or in combination may also be performed for breaking up the membrane or surface, for
15 example: (i) adding any suitable lysis buffer into lysis reactor 9 or (ii) freeze-thawing (e.g. with liquid nitrogen). In this optional step, the previously viable cells may then be lysed to release DNA into the liquid sample.

Following cell lysis, two pinch valves 25 and 26 downstream of the lysis reactor 9 are opened, as illustrated in Figure 3(G). The three-port air valve 48 is then opened to
20 allow air pressure of ~ 2.5 bar to enter the lysis reactor 9, pushing the liquid sample out of the lysis reactor and through a filter 10 to remove particles and dirt. DNA is not retained by filter 10 and passes through the filter with the liquid sample. The liquid sample may then be allowed to pass through a gel filtration column 11 to remove impurities smaller than DNA molecules, e.g. inhibitors of PCR. The filtered liquid
25 sample 68 may then be collected in a concentrator chamber 12, displacing air from the concentrator chamber 12 which leaks out through air vent 50.

Low pressure concentrator

The concentrator, which is optional, may be a low pressure concentrator. After substantially all the liquid sample has collected in the concentrator chamber 12, the
30 pinch valves 25, 26 and the air vent 50 are closed (Figure 3(H)). A vacuum control valve 51 is then opened and a low pressure environment is generated inside the concentrator chamber to an absolute pressure level of ~ 0.1 bar. A heater 31 in contact with the concentrator chamber 12 is then turned on and heats the liquid sample 68 to

boiling at a temperature of $\sim 50^{\circ}\text{C}$ to reduce the volume to a concentrated sample 69 through evaporation without damaging the DNA. The liquid sample 68 may be reduced to a concentrated sample 69 with a predetermined volume. The vapour exiting the concentrator chamber may be condensed into liquid in condenser 32.

5 In the embodiment illustrated in Figure 3, the low pressure concentrator is a component integrated within the apparatus, for concentrating DNA in solution for further analysis. Apart from being integrated into an apparatus, the low pressure concentrator may also be a standalone apparatus and used in isolation.

More specifically, the low pressure concentrator is capable of concentrating a liquid
10 sample to a predetermined volume. A low pressure concentrator for concentrating a sample to a predetermined volume has been described in SG 200902058-7. The low pressure concentrator comprises (i) a concentrator chamber comprising at least a first region and at least a second region, the first region configured in use to be at a temperature higher than the second region, (ii) a heater for heating the first region and
15 (iii) a vacuum pump configured in use to provide a pressure environment lower than atmospheric pressure to the chamber. An embodiment of the low pressure concentrator in isolation is illustrated in Figures 5 and 6. The concentrator chamber 12 may also comprise at least one vapour outlet 93. The concentrator chamber 12 may further comprise an inlet 95 for a liquid sample 68 to be concentrated, and/or an outlet
20 96 for a concentrated sample 69. The inlet 95 and the outlet 96 may be connected to tubing 87, which may be of a material of low thermo-conductivity (e.g. PTFE or silicone tubing). Liquid sample and air may enter the concentrator chamber through tubing opening 92 while concentrated sample may exit or be collected via tubing outlet 91. The low pressure concentrator may further comprise a condenser 32 between the
25 concentrator chamber 12 and the vacuum pump 42 for condensing vapour exiting the concentrator chamber 12 into tubing 94 back into liquid. There is also provided three valves 88, 89 and 90 connected with tubing for controlling liquid and air flow.

The first region 84 (the "high-temperature" region) may be heat-conductive (for example, with walls made of metal) and may be heated by a temperature controlled
30 heater 31. The second region 85 (the "low-temperature" region) comprises a low thermo-conductivity region (for example, with walls made of polytetrafluoroethylene (PTFE), a compound also known by the trade name Teflon). The inside walls of the chamber 12 may be coated with a super hydrophobic or anti-adhesion layer 86 (e.g.

Teflon or cured poly-dimethylsiloxane (PDMS)). Cured PDMS may be prepared from Sylgard 184 Silicone Elastomer and Sylgard Curing Agent 184 (both from Dow Corning Corporation). Coating the walls may help to reduce sample adhesion or attachment and facilitate flowing down of any droplets. The first region ("high-temperature" region) 84 may be positioned above the second region 85 ("low-temperature" region)

During operation, the vacuum pump 42 generates a low pressure environment in the chamber and may thus enable the liquid sample 68 in the concentrator chamber to be boiled at a low temperature as the first region is heated. When evaporation has occurred such that the concentrated liquid sample 69 is reduced to a level 98 that is lower than the first region, the concentrated liquid sample 69 does not continue to boil at the temperature within the second region and the evaporation is drastically decreased. The sample may thus be reduced to a final volume in the micro-liter range.

A first embodiment of the low pressure concentrator and its operation is illustrated in Figure 6. Initially, all three valves 88, 89 and 90 are closed. Opening the valve 90 allows a liquid sample 68 (e.g. DNA in aqueous solution) to load into the interior of the concentrator chamber (Figure 6(A)). The valve 90 is then closed and the vacuum pump 42 and the heater 31 are turned on for degassing the chamber 12 and evaporating the liquid sample 68. As shown in Figure 6(A), during the evaporation process, the first region 82 (heat conductive region) of the chamber wall is hot, e.g. at $\sim 70^{\circ}\text{C}$, while the low thermo-conductivity region 85 and the tubing 87 is kept at a lower temperature. During the evaporation process, the liquid sample 68 does not enter the tubing 87 because of the air trapped in the region of the tubing 87 between the outlet and the closed valves 88, 89 and also because of capillary forces.

As the liquid sample evaporates, the volume decreases until the sample level 98 is lower than the first region 82 (heat conductive region). The concentrated sample 69 is now within the second region 85 ("low-temperature" region or low thermo-conductivity region of the chamber), as illustrated in Figure 6(B). Within this "low-temperature" region, the evaporation rate is drastically reduced and a final volume is reached. The final volume is dependent on the size of the low thermo-conductivity region 85 and the final volume may be adjusted by modifying the size of the low thermo-conductivity region 85 during the construction of the concentrator chamber. The endpoint of the concentration process (i.e. when the final volume is reached) may be detected optically or estimated based on time. On reaching the endpoint or the final volume of the

sample, the vacuum pump 42 and heater 31 are turned off. The valve 89 is kept closed, while the valves 88 and 90 are opened, and the concentrated liquid sample 69 is allowed to flow out of the chamber via outlet 94 and into tubing 87 and tubing outlet 91 either by introducing air pressure at tubing opening 92 or suction at tubing outlet 91.

5 Another embodiment of the low pressure concentrator and its operation is illustrated in Figure 7. The operation of this embodiment is similar to the embodiment of Figure 6 and as discussed above except that after the concentrator chamber 12 has been degassed for a short period, ~ 1 min, the valve 89 is opened and a portion of the liquid sample enters into tubing 87. As the liquid sample 68 inside the concentrator chamber and the tubing 87 are at the same pressure, the liquid sample inside the chamber and the tubing are at substantially the same level 97 due to the gravity and capillary forces, as shown in Figure 7(A). As the liquid sample evaporates, both these two levels drop but are maintained at substantially the same level. The sample level 98 within the chamber eventually drops below the heat-conductive region 84. The sample is now
10 within the second region 85 (the "low-temperature" region or low thermo-conductivity region) of the chamber wall, as shown in Figure 7(B) and the evaporation rate is drastically reduced and a final volume is reached. The final volume may be adjusted by modifying the size of the low thermo-conductivity region 85 or the size and/or curvature of the tubing 87.

20 As before, the endpoint of the concentration process, i.e. when the final volume is reached, may be detected optically or estimated based on time. On reaching the endpoint or the final volume, the vacuum and heater are turned off. The valve 89 is closed, while the valves 88 and 90 are opened. The liquid sample may be forced out by air pressure entering through tubing opening 92 or by suction through tubing outlet 91.
25 In the embodiments illustrated in Figure 5-7, the concentrated sample 69 exiting from the tubing 91 may be collected.

The low pressure concentrator may achieve a high concentration ratio, with a concentration from an initial volume of millilitres to a final volume of micro-litres, a concentration of a thousand fold or even more. The hydrophobic or anti-adhesion
30 coating may assist with high sample recovery with virtually no loss of sample. In addition, the low pressure concentrator may concentrate to the final volume at a high speed, for example from 15 ml to 5 μ l within 5 minutes. The final volume of the

concentrated sample 69 obtained may facilitate further downstream testing and detection processes.

The low pressure concentrator may be produced at a relatively low cost. In addition, control of the vacuum concentration by means of a few valves enables the low
5 pressure concentrator to be readily automated and integrated into other apparatus.

Detection

Referring to Figure 3(I), after concentration of the liquid sample 69 to a final volume in the low thermo-conductivity region, for example $\sim 20 \mu\text{l}$, the vacuum control valve 51 is closed. The air vent valve 50, the air pressure control valve 52 leading to the reservoir
10 6 and the pinch valve 27 are then opened, and air at a pressure of ~ 0.01 bar enters the reservoir 6 and causes PCR master mix 62 to enter the concentrator chamber to mix with the DNA sample.

In the next step as shown in Figure 3(J), the pinch valve 27 and air valves 50 and 52 are then closed. A pinch valve 28 downstream of the concentrator chamber 12 is then
15 opened, and intermittent air at a pressure of ~ 0.01 bar enters the concentrator chamber 12 by a pulsed operation of air pressure control valve 49 and causes the mixture of DNA sample and PCR master mix to enter into a mixer 7 for thorough mixing. The sample and PCR mixture then enters a detection cartridge 3 (Figure 3(K)). The detection cartridge comprises at least an array of a plurality of assay chambers 34
20 connected via at least a first common channel, being a common inlet channel. The assay chambers 34 of the detection cartridge may be used for separate PCR reactions with different primers or used for replicates for the same PCR reaction (for reliability and/or consistency). Multiplex PCR reactions may also be conducted in the assay chambers 34.

25 The detection cartridge may further comprises a second common channel connecting the assay chambers, being a common outlet channel and with a dedicated inlet to each assay chamber and has been described in SG 200902060-3. Exemplary embodiments of such a detection cartridge are also illustrated in Figures 8 and 15.

Referring to Figure 15, the detection cartridge may comprise a first test array of a
30 plurality of assay chambers 34 for analysis of test sample connected to a common inlet channel 99, a second negative control array of a plurality of assay chambers 122 for negative control (e.g. PCR with no DNA template) reactions connected to a common inlet channel 117 and at least one positive control assay chamber 123 for a positive

control reaction introduced through opening 116. Appropriate positive control reactions may be performed. For example, the positive control reaction for PCR may comprise at least one DNA template to which at least one pair of primers used to test the test samples is known to bind and subsequently amplify, basically to indicate whether the basic conditions of the PCR were able to produce a positive reaction. Alternatively, a number of positive control assay chambers may be used, each for a different pair of primers, as appropriate. Positive control reactions comprising more than one set of DNA template and primer pairs may also be performed in one positive control assay chamber, with the results of the positive control reaction analysed subsequently by another method, for example, electrophoresis.

An example of a passive exit valve 104 is also indicated in Figure 15. Each test assay channel may be connected to a common outlet channel 103 via outlet bridge 102. In the embodiment illustrated in Figure 15, both the test array and the negative control array have six assay chambers. It is understood that any number of test assay chambers and negative assay chambers may be included in the detection cartridge as appropriate.

The common inlet channel 99 of the test array is connected to inlet opening 100. Each assay chamber of the test array may be connected to the common inlet channel 99 via separate individual inlet bridges 101. The inlet opening 100 may be used for the loading of test sample and PCR master mix (without primers) which flow into the common inlet channel 99 and eventually flow into each individual assay chamber. Each test assay chamber 34 has its own dedicated inlet channel 106 with opening 107 where different primers may be introduced into each assay chamber 34 and in this way, each assay chamber 34 of the test array may be used for a different PCR reaction. Each outlet bridge 102 is connected to the common outlet channel 103 via a passive exit valve. Each passive exit valve 104 may prevent or reduce liquid from exiting into the outlet channel 103 during loading of each of the assay chambers.

The negative control array has a similar arrangement and negative control (no DNA) and PCR master mix (without primers) may be introduced via loading inlet opening 115, and then flowing through the common inlet channel 117 into each negative assay chamber 122 via inlet bridge 118. Each negative assay chamber 122 may be used as a negative control for different primers introduced into the chamber via its dedicated inlet channel 121 and opening 120. In practice, the primers for the negative control PCR

reactions correspond to the primers used for the test sample PCR reactions. After complete filling of the negative assay chambers 34 and 122, the contents of each assay chamber 34 or 122 may be isolated from each other by continuous removal of excess liquid in the test inlet channel 99 or negative control inlet channel 117 through the respective waste ports 105 or 119, respectively.

A detailed description of the detection cartridge for PCR will now be described with reference to the exemplary embodiment illustrated in Figures 8. Figure 8(A) is a schematic representation of the detection component including inlet channel(s) 99, outlet channel(s) 103, passive exit valve(s) 104, dedicated inlet channel(s) 106, inlet bridge(s) 101 and outlet bridge(s) 102 fabricated on poly(dimethylsiloxane) (PDMS) substrate. The inlet channel 99 connecting inlet opening 100 and assay chamber(s) 34 via inlet bridge(s) 101 is used for delivering PCR mixture (without primers) or washing buffer into the assay chambers 34. Excess PCR mixture or washing buffer may be removed from waste ports 105 at one end of the inlet channel. The passive valves 104 may prevent excess liquid from flowing into the outlet channel 103 during loading.

The designs of the inlet bridge(s) 101 and outlet bridge(s) 102 are intended to reduce evaporative loss during PCR thermal cycling. The dedicated inlet channels 106 may be used for loading primers into the assay chambers. The outlet channel 103 may be used for removing the liquid from the assay chambers 34, inlet bridges 101 and outlet bridges 102. In an exemplary embodiment, the assay chambers 34 may be 8 mm long and ~ 8 mm long and ~ 2 mm wide, with a volume of ~ 12.8 μ l.

Figure 8(B) is a schematic diagram of a cross-section of Figure 8(A) along the line A-A and illustrates a fabricated PDMS array 109 including inlet channel 99, assay chamber 34, outlet channel 103, and passive exit valves 104 with a PDMS bottom layer 108 and a PDMS top layer 110. In an embodiment, the thickness of the array 109 including the assay chamber 34, the inlet channel 99, outlet channel 103, inlet bridge 101, outlet bridge 102 and the passive exit valve 104 may be ~ 0.8 mm high.

Dedicated inlet channels to each assay chamber are optional. For example, in an embodiment where the assay chambers are used for replicates for the same PCR reaction mixture, the same PCR master mix comprising primers may be introduced into the assay chambers through the common inlet channel. Dedicated inlet channels to each assay chamber may accordingly be omitted in the detection cartridge. Dedicated inlet channels to each assay chamber are optional even if different PCR primers are

used in the different assay chambers as PCR primers may be introduced into each assay chamber during fabrication of the detection cartridge.

An example of a method of fabrication of the PDMS detection cartridge will now be further discussed. A PDMS sheet with a thickness of ~ 0.8 mm may first be prepared on an acrylic substrate by volume control casting. A pulsed CO₂ laser (VersaLaser VLS 2.30) may then be used to produce desired patterns including inlet channel 99, assay chambers 34, outlet channel 103, passive exit valves 104, inlet bridges 101 and outlet bridges 102 on the PDMS sheet. The laser may be at ~ 0.6 W with a resolution setting of 300 pulses per inch (PPI). The extra bits of the PDMS not forming part of the pattern may be peeled off from the acrylic substrate. A PDMS top layer 110 is then bonded to the laser-patterned PDMS sheet using a layer of PDMS prepolymer as adhesive. The two bonded PDMS layers are then removed from the acrylic substrate and bonded with a bottom layer of PDMS 108. PCR primers may be introduced into each assay chamber and substantially dried with a drying process before bonding the top PDMS layer.

Figure 9 illustrates as an example the fluidic operation of loading primers and PCR mixture and draining of the cartridge. PCR primers 111 may be dispensed into the assay chamber 34 via dedicated inlet channel 106 as illustrated in Figure 9(A). PCR mixture 112 comprising DNA template is loaded via inlet opening 100 into inlet channel 99 (Figure 9(B)). The PCR mixture 112 flows into the assay chamber 34 via the inlet bridge 101 by capillary action and mixes with the primers 111 displacing air out through outlet channel 103 (Figures 9(B) and (C)). After filling the assay chambers 34, the PCR mixture 112 flows into outlet bridge 102 but the flow stops at passive valve 104 due to a passive valve effect. As illustrated in Figure 9(D) and (E), excess PCR mixture 112 in the inlet channel 99 may be removed via waste port 105, and this may help in isolating the liquid inside one assay chamber from another assay chamber.

The filled assay chambers may then be subjected to PCR thermal cycling. The apparatus includes a thermal cycler 33 for the PCR reaction (Figure 3(K)). The thermal cycler 33 may be in contact with the detection cartridge 3. In one embodiment, the PCR may be real-time PCR and the apparatus includes an illuminator 35 and a detector 36 for fluorescence signal detection of the real-time PCR (Figure 3(K)).

After completion of thermal cycling, the PCR mixture 112 is then allowed to flow out of the assay chamber into the outlet channel as illustrated in Figure 9(F). The flow of the

PCR mixture 112 continues (Figure 9(G)) and the assay chamber(s) 34, outlet bridge(s) 102 and outlet channel(s) 103 are eventually drained or emptied as illustrated in Figure 9(H). The draining or emptying of the inlet bridge(s) 101, assay chamber(s) 34, outlet bridge(s) 102 and outlet channel(s) 103 may also be facilitated by capillary action and geometry of the detection cartridge. Valves controlling the dedicated inlet channel(s) 106 are closed during the draining process so as to prevent the primers 111 from being drained. As illustrated in Figure 9(H), some PCR mixture 112 may remain in the passive exit valve(s) 104. This residual PCR mixture 112 may be removed through evaporation by blowing hot air at the passive exit valves, for example using a hair dryer leaving the detection cartridge substantially dry as illustrated in Figure 9(I). Figure 10 shows a photograph of the operation of the detection cartridge.

After the detection cartridge is emptied, the cartridge may be washed so as to clean out contaminants or residual PCR mixture 112 from the previous reaction. Figure 11 illustrates the fluidic operation of loading and removing washing solution 113. In Figure 11(A), primers 114 which may be contaminated with DNA template from the previous amplification reaction are dispensed into the assay chamber(s) 34 by opening control valves controlling the dedicated inlet channel(s) 106. This step may prevent or reduce contamination in the subsequent amplification reaction. Washing solution 113 is loaded into the inlet opening 100 and flows into the inlet channel 99 (Figure 11(B)). The washing solution 113 then flows into the assay chamber(s) 34 and mixes with the contaminated primers 114 (Figure 11(C)). After filling the assay chamber(s) 34, the washing solution enters the outlet bridge(s) 102 and stops flowing at passive exit valve(s) 104 due to the passive valve effect. Excess washing solution 113 in inlet channel 99 may be removed via the waste port 105 at one end of the inlet channel 99 (Figure 11(D)). In the next step, washing solution 113 is allowed to flow into the outlet channel 103 (Figure 11(E)). Upon filling the outlet channel 103, the contents of the detection cartridge is then allowed to drain out, including the mixture of washing solution and primers from the assay chamber(s) 34. (Figure 11(F)). As already described above, the draining or emptying of the inlet bridge(s) 101, assay chamber(s) 34, outlet bridge(s) 102 and outlet channels 103 may also be facilitated by capillary action and geometry of the detection cartridge. During this draining step, the control valves of the dedicated inlet channel(s) 106 are closed to prevent the primers from being drained any further. After the draining of the washing solution from the detection

cartridge, some washing solution 113 may remain in passive exit valve(s) 104 (Figure 11(G)) and this may be removed through evaporation by blowing hot air at the passive exit valves, for example using a hair dryer leaving the detection cartridge substantially dry as illustrated in Figure 11(H). Figure 12 shows a photograph of the discharging of contaminated primers and washing of assay chambers.

The detection cartridge may thus be cleaned and made available for another reaction. A subsequent PCR amplification may thus be performed using the cleaned detection cartridge. The detection cartridge may also be detachable from the apparatus for additional cleaning if necessary. Alternatively, a new detection cartridge may also be used to replace the used detection cartridge.

After washing, the detection cartridge 3 may be used for another round of PCR amplification. Similar to Figures 9(A) and (B), Figures 13(A) and (B) illustrate the loading of primers 111 and PCR mixture 112 into the detection cartridge. Primers 111 may again be dispensed into the assay chamber(s) 34 through dedicated inlet channel(s) 106. PCR mixture 112 is then loaded into the inlet channel 99 via inlet opening 100 for another round of PCR amplification. Figure 14 shows a photograph of the fluidic operation of loading PCR primers, DNA and PCR master mix for the next round of PCR. Those of skill in the art will recognise that the detection cartridge may be used once or repeatedly. As an example, the outlet bridge and/or the second common channel, the outlet channel, may be omitted if the detection cartridge is for single use.

Those of skill in the art will also recognise that other components of the integrated apparatus may also be cleaned by allowing washing solution to flow through the entire apparatus. The integrated apparatus may accordingly be used repeatedly.

Whilst the foregoing description has described exemplary embodiments, it will be understood by those skilled in the technology concerned that many variations in details of design, construction and/or operation may be made without departing from the present invention.

Claims

1. An apparatus for detecting organisms from a fluid comprising at least the following components:
 - (i) an inlet for introducing the fluid to the apparatus;
 - 5 (ii) a filtration system for trapping particles including organisms from the fluid including a system configured to direct a flow of eluent to the filtration system at a pressure sufficient to release trapped organisms into the eluent; and
 - (iii) a detection cartridge for detecting different organisms comprising at least an array of a plurality of assay chambers connected via at least a first common
 - 10 channel, being a common inlet channel; and
 - (iv) at least one valve for controlling fluid flow through the apparatus; wherein at least components (i) to (iii) are in fluid communication.
2. The apparatus according to claim 1, wherein the detection cartridge comprises at least a second common channel connected to the plurality of assay
- 15 chambers, being a common outlet channel.
3. The apparatus according to claim 1 or 2, wherein each assay chamber further comprises at least one dedicated inlet channel only to that particular assay chamber.
4. The apparatus according to any one of the preceding claims, wherein the
- 20 filtration system comprises a filter holder comprising a membrane filter comprising a plurality of pores of substantially the same size.
5. The apparatus according to any one of the preceding claims, further comprising a three-port valve for controlling in a first position flow of fluid passing out from the filtration system through a first common port of the three-port valve to a
- 25 second port of the three-port valve connected to an outlet and in a second position flow of eluent passing through a third port of the three-port valve to the first common port through to the filtration system to release trapped particles.
6. The apparatus according to any one of the preceding claims wherein the detection cartridge is for performing polymerase chain reaction (PCR).
- 30 7. The apparatus according to claim 6, further comprising a thermal cycler for performing PCR.
8. The apparatus according to claim 6 or 7, wherein the common inlet channel is for introducing PCR master mix and DNA template into the assay chambers.

9. The apparatus according to any one of claims 3 to 8, wherein each dedicated inlet channel is for introducing PCR primers into each corresponding assay chamber of the detection cartridge.
10. The apparatus according to any one of claims 6-9, wherein the PCR is real-time PCR and the apparatus further comprises an illuminator and a detector for fluorescence signal detection of real-time PCR.
11. The apparatus according to any one of the preceding claims, further comprising at least one additional component selected from the group consisting of: a component for a reaction to differentiate viable organisms, a cell lysis system, a filter for substantially removing particles larger than DNA, a filter for substantially removing residual substances and a concentrator; in fluid communication with components (i) to (iii).
12. The apparatus according to claim 11, wherein the apparatus comprises all of the additional components in the group.
13. The apparatus according to any one of claims 10 to 12, wherein the component for a reaction to differentiate viable organisms comprises a fluidic cartridge comprising:
- (a) a substantially optically transparent reaction chamber configured to expose to a light source a sample comprising cells and a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to the nucleic acid molecule on exposure to a light source,
- (b) at least one inlet in fluid communication with the reaction chamber; and
- (c) at least one outlet in fluid communication with the reaction chamber; and at least one light source, the reaction chamber being configured such that in use it will receive light transmitted from the at least one light source.
14. The apparatus according to any one of claims 10 to 13, wherein the cell lysis system comprises a heat lysis system or an ultrasonic lysis system.
15. The apparatus according to any one of claims 10 to 14, wherein the concentrator comprises a low pressure concentrator.
16. A low pressure concentrator for concentrating a sample to a predetermined volume, comprising: (i) a concentrator chamber comprising at least a first region

and least a second region and at least one outlet for vapour, the first region configured in use to be at a temperature higher than the second region, (ii) a heater for heating the first region and (iii) a vacuum pump configured in use to provide a pressure environment lower than atmospheric pressure to the concentrator chamber.

17. The low pressure concentrator according to claim 16; wherein the first region is positioned above the second region.

18. The low pressure concentrator according to claim 16 or 17, wherein the concentrator chamber comprises an inlet for introducing a sample to be concentrated and/or an outlet for exit of a concentrated sample.

19. The apparatus according to any one of claims 1 to 18; wherein the apparatus is an automated apparatus.

20. A method for detecting organisms from a fluid comprising the steps of:

(a) providing an apparatus comprising at least the following components:

(i) an inlet for introducing the fluid into the apparatus;

(ii) a filtration system for trapping particles including organisms from the fluid including a system configured to direct a flow of eluent to the filtration system at a pressure sufficient to release trapped organisms into the eluent; and

(iii) a detection cartridge for detecting different organisms comprising at least an array of a plurality of assay chambers connected via at least a first common channel, being a common inlet channel; and

(iv) at least one valve for controlling fluid flow through the apparatus; wherein at least components (i) to (iii) are in fluid communication;

(b) introducing a fluid via the inlet into the apparatus;

(c) filtering the fluid through the filtration system to trap particles including organisms from the fluid and directing a flow of eluent through the filtration system at a pressure sufficient to release trapped organisms into the eluent;

(d) allowing the eluent to flow via the common inlet channel into the assay chambers of the cartridge; and

(e) performing at least one reaction to detect at least one species of organism.

21. The method according to claim 20; wherein steps (b) and (c) comprise providing a three-port valve comprising a first common port connected to the filtration

system, a second port connected to an outlet for the fluid and a third port connected to an eluent supply, positioning the three-port valve in a first position with the second port opened and the third port closed to allow the fluid to flow into the apparatus through to the filtration system and out of the apparatus via the outlet, followed by positioning the three-port valve in a second position with the second port close to stop the flow of fluid and the third port open to allow eluent to flow through the filtration system at a pressure sufficient to release trapped organisms.

22. The method according to claim 20 or 21; wherein step (d) comprises performing at least one polymerase chain reaction (PCR).

23. The method according to claim 22; comprising introducing PCR primers into each assay chamber via a dedicated inlet channel to that particular assay chamber and PCR master mix without primers into the assay chambers via the common inlet channel.

24. The method according to claim 22 or 23; wherein the PCR comprises real-time PCR.

25. The method according to any one of claims 20 to 24; further comprising performing between step (c) and (d) at least one of the following steps:

(c)(i) performing a reaction to differentiate viable organisms;

(c)(ii) performing a cell lysis reaction;

(c)(iii) performing a further filtration to substantially remove particles larger than DNA;

(c)(iv) performing a further filtration to substantially remove residual substances; and

(c)(v) concentrating the eluent prior to detecting the organisms.

26. The method according to claim 25; comprising performing all of the steps (c)(i) to (c)(v).

27. The method according to claim 25 or 26; wherein step c(ii) comprises allowing the eluent comprising organisms to enter via an inlet into a substantially optically transparent reaction chamber in the presence of a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cell over intact viable and/or substantially intact cells; exposing the reaction chamber to a light transmitted from a light source to covalently bind

the phenanthridium compound to the DNA of the dead or membrane-compromised cells and allowing the eluent to exit from the reaction chamber via an outlet.

28. A method of concentrating a liquid to a predetermined volume comprising:

- 5 (a) providing a low pressure concentrator comprising (i) a concentrator chamber comprising at least a first region positioned above at least a second region and at least one outlet for vapour, the first region configured in use to be at a temperature higher than the second region, (ii) a heater for heating the first region and (iii) a vacuum pump configured in use to provide a pressure lower
- 10 than atmospheric pressure to the concentrator chamber;
- (b) heating the first region of the concentrator chamber at a pressure lower than atmospheric pressure to concentrate the sample to a final surface level below the heated first region; wherein the evaporation of the sample is substantially
- 15 reduced within the unheated second region.