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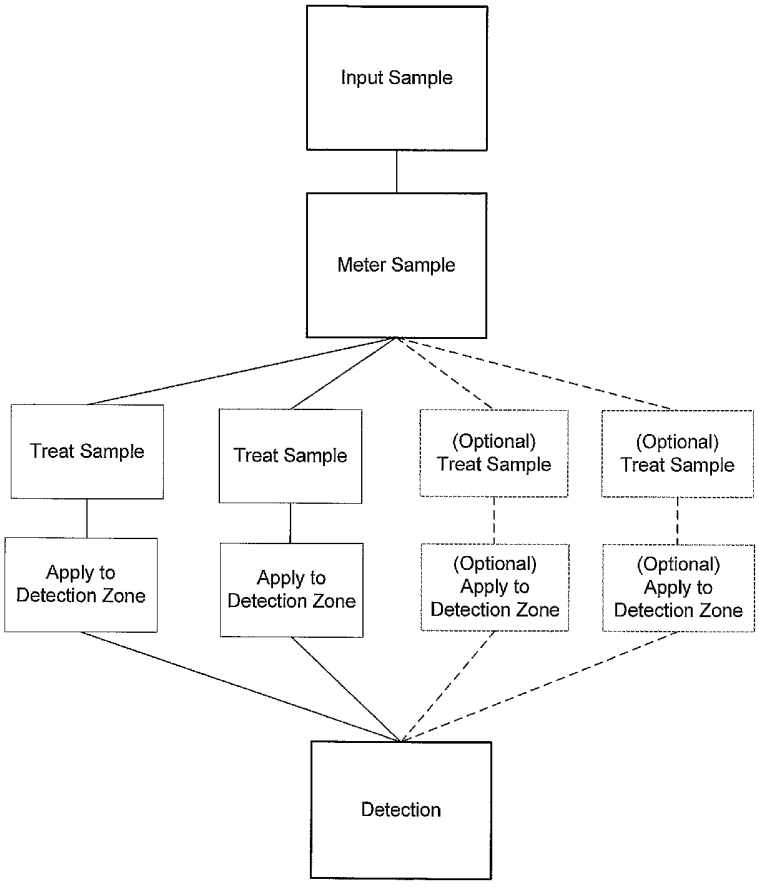

(57) Abstract: The present invention relates to sample processing using a microfluidic chip. A method for concurrent testing for at least two of RNA, DNA, antibody, and antigen in a sample is described, as are methods for testing for pre-selected pathogens and microfluidic methods.

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METHODS OF TESTING

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial Nos. 60/679,816, filed May 11, 2005, 60/679,798, filed May 11, 2005, and 60/679,797, filed May 11, 2005, and the disclosures of which are each incorporated herein by reference in their entireties.

BACKGROUND

While clinical laboratories excel at detecting proteins and nucleotides, including genetic information, disease-causing agents, and indicators of disease or disorders, there is always a delay between sample collection and communication of the results of testing. In certain circumstances, such as a highly infectious outbreak or incident of bioterrorism, such a delay could be catastrophic. In such cases, facilitating testing where the sample is collected is a highly important goal.

Even under less dramatic circumstances where such testing is already a reality, improved testing is very desirable. For example, there are known tests used to detect HIV via the presence of antibodies to HIV. However, there is a six to twelve week period between HIV infection and measurable antibody response, during which time an infected individual can transmit the virus. This presents an unacceptable lag. Testing by clinical laboratories does not remedy the lag, because of the above-mentioned delay between acquiring a sample and informing the individual of the test results. Also, some patients never return after providing a sample, whereas if a sample could be diagnosed on-site with an immediate result, the individual could be counseled and appropriate therapy initiated.

Thus, testing devices and methods capable of detecting both the pathogen (via antigen and/or nucleic acid) and antibody to the pathogen are needed and would have tremendous impact on the diagnosis and monitoring of HIV. Of course, such testing devices and methods would be

equally important for testing for other pathogens or diseases, or even pre-selected contaminants or pre-selected sequences, in fact, any nucleotide sequence, antigen, or antibody. Moreover, it is desirable that the testing devices and methods reduce costs. Finally, it is desirable that the testing be automated as far as possible to obtain the benefits of automation.

SUMMARY OF THE INVENTION

The present invention relates to sample processing using a microfluidic cassette. Microfluidic refers to the fact that a fluid is propelled through a system, allowing greater control. In some embodiments, the cassettes reduce processing time and materials. In some embodiments, the cassettes accommodate samples without pretreatment, or in a self-contained state to prevent cross-contamination. In some embodiments, the system allows for automatic processing. The present inventions also are suitable for use analyzing samples at the point of care, and in clinical laboratories, if the above-described delay is not a factor.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow chart of testing according to the present invention.

Fig. 2 is a schematic view of a developer receiving a cassette.

Fig. 3 is a schematic view of a chip contained by the cassette.

Fig. 4 is a chart showing the various paths for DNA detection, antibody detection, antigen detection, and RNA detection..

Fig. 5 is a schematic and images of an ice valve.

Fig. 6 is a schematic of filling a chamber in a cassette.

Figs. 7-9 are images of a portion of a cassette.

Fig. 10 is a chart showing detection by a cassette.

Fig. 11 is an image of a gel.

Fig. 12 is an image of a gel.

Fig. 13 is a schematic for metering in one embodiment.

It is understood that the figures are merely to illustrate certain features, and in no way limit the invention.

DETAILED DESCRIPTION

In one embodiment, the present invention provides a method for testing for a pre-selected pathogen in a sample, comprising placing the sample in a microfluidic cassette; propelling the sample along a flow path in the cassette to a detection zone having at least one zone adapted to interact with the pre-selected pathogen; and detecting the presence or absence of interaction. In one embodiment, there is a pre-selected pattern of zones on the detection zone, each for interacting with a different sequence. In one embodiment, the method further comprises applying a portion of the sample to a pre-selected pattern of zones on at least one further detection zone, each zone for interacting with a different sequence of RNA, DNA, antigen, or antibody.

Turning to Fig. 1, a method of testing is shown, comprising obtaining a sample, metering the sample, treating portions of the sample, applying the portions to a detection zone, and detecting interactions that would indicate the presence of a disease or a disease-indicator.

Referring to Fig. 2, a developer is shown having a chamber for receiving a cassette, such as a microfluidic chip containing cassette. In one embodiment, the chamber is temperature controlled. It is understood that the propulsion provided by the developer is hydraulic (either pressure or suction), pneumatic, electric, or magnetic. In Fig. 3, an exemplary microfluidic chip contained in the cassette is depicted. In some embodiments, the microfluidic flow path channels have a diameter of about 1mm or less.

In some embodiments, the developer supplies reagents that can be used in sample processing, sample treatment, or detection of interaction. In one embodiment, the developer dispenses a reagent for treating the sample. In some embodiments, the appropriate buffers and treatment fluids are pre-loaded on the cassette, and in some embodiments, some reagents are

preloaded and some dispensed. The developer also retains controls for controlling testing conditions and materials. Thus, in one embodiment, the developer provides electrical power. In another embodiment, the developer provides propulsion. In one embodiment, the developer includes a heater/cooler, such as a Peltier heater/cooler. In one embodiment, the cassette has a heater.

It is understood that the cassette and developer are in fluid communication. A sample inlet is disposed in the cassette for introduction of a sample into the cassette. The sample can be any material that might contain RNA sequences, DNA sequences, antibodies, or antigens. Examples of samples include foodstuffs, water, saliva, blood, urine, fecal samples, lymph fluid, breast fluid, CSF, tears, nasal swabs, and surface swabs. In one embodiment, the cassette finds use in testing for pathogens, so the pre-selected sequences, antibodies, or antigens are those associated with at least one known pathogen. In another embodiment, the pre-selected sequences, antibodies, or antigens are those associated with more than one pathogen. Likewise, in one embodiment, the pre-selected sequences, antibodies, or antigens are those associated with at least one known disorder. In one embodiment, the cassette further comprises at least one further detection zone for interacting with RNA, DNA, or antigen, to allow parallel testing.

The detection zone is contacted with capture sequences that are pre-selected for the pathogen. In some embodiments, multiple pathogens are tested for by providing complementary sequences pre-selected for the pathogens. Likewise, in one embodiment, the at least one further detection zone is a chromatographic detection zone. In one embodiment, the detection zone is nitrocellulose strip. The detection zone is contacted with capture sequences that are pre-selected for the pathogen or compound of interest. In some embodiments, multiple pathogens are tested for by providing complementary sequences pre-selected for the pathogens. It is understood that a sample lacking the pathogen(s) or compound(s) of interest will not interact with the detection zone. If present, the interaction between sample and sequence (s) is detectable.

It is understood that the developer could receive more than one cassette to process at a time. It is also understood that the developer could process cassettes of varying types, limited only by the reagents stored (unless the cassettes were pre-loaded), for example, an HIV test cassette, a cancer detection cassette (p-54 mutation or protein indicator), and a cassette for determining presence of a hair color gene could all be processed by the developer.

In one embodiment, the developer dispenses a reagent for diluting the sample. The dilution is optional, as it is understood that mixing the sample with buffer could serve a similar purpose. A flow path extends between the sample inlet and the detection zone. In one embodiment, the first mentioned detection zone is a chromatographic detection zone. In one embodiment, the first mentioned detection zone is in a lateral flow format. In one embodiment, the detection zone is nitrocellulose strip. Likewise, in one embodiment, the at least one further detection zone is a chromatographic detection zone. In one embodiment, the detection zone is in a lateral flow format, and in one embodiment, the detection zone is nitrocellulose strip. In one embodiment, the cassette further comprises a plurality of detection zones, wherein each detection zone independently interacts with RNA, DNA, antigen, or antibody. In one embodiment, the first mentioned detection zone has a pre-selected pattern of zones, each for interacting with a different sequence of RNA, DNA, antigen, or antibody. In one embodiment, the further detection zone has a pre-selected pattern of zones, each for interacting with a different sequence of RNA, DNA, antigen, or antibody. In some embodiments, the interaction is detectable, such as through reporter particles. All known reporter particles are contemplated, for example, the reporter particles may be phosphor particles (such as Up-Converting Phosphor Technology (UPT) particles), fluorescing particles, hybridization sensors, or electrochemical sensors.

In one embodiment, the cassette further comprises a waste reservoir to limit contamination by the sample, or cross-contamination between cassettes, as well as keeping the bioactive waste on the chip.

Various valve types are contemplated. It is understood that the valve could be any type of valve, including a phase change valve, piezo-electric valve, hydrogel valve, passive valve, check valve, or a membrane-based valve. In one embodiment, the valve is a phase change valve or a hydrogel valve. The temperature-responsive hydrogel, poly(N-isopropylacrylamide), when saturated with an aqueous solution, undergoes a significant, reversible volumetric change when its temperature is increased from room temperature to above the phase transition temperature of about 32°C. The hydrogel can be embedded in polycarbonate plates prior to the thermal bonding of the plates. The exposure of the hydrogel to the thermal bonding temperatures does not have any apparent adverse effect on the gel. Moreover, one important advantage of the hydrogel valve is that when dry, it allows free passage of gases. In pneumatic systems, the dry hydrogel valve will allow the displacement of air from cavities and conduits upstream of an advancing liquid slug. Once the aqueous liquid arrives at the hydrogel's location, it will saturate and swell the gel, blocking the flow passage. Thus, the valve is self-actuated. The valve can be opened by heating the hydrogel to above its transition temperature. The hydrogel proved to be biocompatible in our testing and did not to hinder PCR. Moreover, the hydrogel valves did not appear to absorb significant quantities of DNA and enzymes suspended in PCR buffer. Ice valves take advantage of the phase change of the working liquid itself- the freezing and melting of a portion of a liquid slug - to non-invasively close and open flow passages. An ice valve is electronically-addressable, does not require any moving parts, introduces only minimal dead volume, is leakage and contamination free, and is biocompatible. Moreover, in certain cases, the valve can operate in a self-actuated mode, alleviating the need for a sensor to determine the appropriate actuation time. For example, in a pneumatically driven system, the precooled conduit section would allow the free passage of air prior to the arrival of the liquid slug and would seal at the desired time when the slug arrives at the valve location. In one embodiment, the developer has means for controlling the valve. In one embodiment, the means is a heater/cooler, optionally controlled by logic.

Referring to Fig. 2, the developer may optionally have a detector for detecting interaction. Alternatively, the detector may be a stand alone detector, to allow the developer to remain dedicated to developing cassettes, allowing faster process times. Thus, in one embodiment, the system further comprises a detector for detecting the RNA, DNA, antibody, or antigen. The present invention, in one embodiment, provides a system, comprising a cassette having at least one port and a sample inlet in fluid communication with a detection zone for interacting with pre-selected RNA sequences, DNA sequences, antibodies, or antigens, or mixtures thereof, if present, in a sample; a developer for engaging the port of the cassette, wherein the developer propels the sample from said inlet to said detection zone; and a detector for detecting the RNA, DNA, antibody, or antigen. In one embodiment, the detector is a UPT detector.

Optionally, the cassette bears an identifier to indicate the type of pathogen(s) to be detected with the cassette. In one embodiment, the identifier is a barcode (either mechanical or optical), RFID tag, or mechanical change in the surface of the cassette. It can be appreciated that the identifier could be associated with certain information that is known at the time that the cassette is fabricated, for example, how many detection zones are on the cassette, what disease-causing agents or indicators of disease are being tested for, and whether each detection zone requires is detecting RNA, DNA, antibody, or antigen. The identifier could also be associated with certain information at the time of testing, for example, a unique patient identifier, sample type, and patient factors (age, health, suspected disorder).

Turning to Fig. 4, the developer can use the identifier to determine the appropriate analysis path. The analysis path for the detection of DNA will consist of the following main steps: pathogen lysis; DNA isolation and purification; PCR; isolation of the amplified DNA; mixing and incubation with target specific reporter particles; and capture of the labeled amplicon on a lateral flow strip. The analysis path for the detection of RNA comprises: cell lysis; RNA isolation and purification; Reverse Transcription PCR; isolation of the amplified DNA; mixing

and incubation with target specific reporter particles; and capture of the labeled amplicons on a lateral flow strip. The analysis path for the detection of human antibodies to select pathogens comprises: dilution of sample; mixing and incubation with target specific reporter particles; capture on a lateral flow strip. The analysis path for the detection of pathogen antigens comprises dilution; solubilization or release of antigen; mixing and incubation with target specific reporter particles; and capture of labeled antigen on a lateral flow strip. Of course, the analysis paths described above focused on the lateral flow format. The invention also includes consecutive flow assays for the detection of antibodies. In the case of the consecutive flow assay, the analysis path will comprise: dilution, capture/enrichment of specific antibodies on a lateral flow strip; wash step to remove unbound antibodies; and detection by flowing reporter particles over the lateral flow strip.

Thus, in one embodiment, the developer provides treating reagent directed to RNA isolation and amplification. In another embodiment, the developer provides treating reagent directed to DNA isolation and amplification. In another embodiment, the developer provides treating reagent directed to antibody detection. In another embodiment, the developer provides treating reagent directed to antigen detection. Likewise, unless the reagent has been pre-loaded, the developer dispenses a reagent for labeling the interacted RNA, DNA, antibody, or antigen with a reporter particle.

In one embodiment, the present invention provides a method for concurrent testing for at least two of RNA, DNA, antibody, and antigen in a sample, comprising applying a portion of the sample to a detection zone disposed on a microfluidic cassette for interacting with pre-selected RNA sequences, DNA sequences, antibodies, or antigens, or mixtures thereof; and applying at least one further portion of the sample to at least one further detection zone disposed on the microfluidic cassette for interacting with pre-selected RNA sequences, DNA sequences, or antigens.

In one embodiment, the method further comprises applying a portion of the sample to another detection zone, wherein the detection zone interacts with RNA, DNA, antigen, or antibody. In one embodiment, the method further comprises detecting the interaction. In one embodiment, the interaction is detected using UPT particles, fluorescing particles, hybridization sensors, or electrochemical sensors.

In one embodiment, the present invention provides a method of testing for pre-selected pathogens, comprising placing a sample in a cassette; and propelling the sample through the cassette under pressure, wherein a portion of the sample is directed to a detection zone for interacting with pre-selected RNA sequences, DNA sequences, antibodies, or antigens known to be associated with a pre-selected pathogen.

In one embodiment, the method further comprises controlling movement of the sample with a valve disposed in the cassette. In one embodiment, the method further comprises diluting the sample.

In one embodiment, the method further comprises metering the sample. In one embodiment, Fig. 13 shows schematic description of the displacement process. (A) A sample flows into the metering chambers and displaces air out of the downstream phase change valve; (B) The sample fills all the metering chambers and freezes at the phase change valve.

In one embodiment, the method further comprises treating the sample. In one embodiment, the method further comprises lysing cells in the sample. In one embodiment, the method further comprises isolating RNA or DNA in the sample. In one embodiment, the RNA or DNA are attached to a solid support.

In one embodiment, the method further comprises amplifying RNA or DNA in the sample. In one embodiment, the RNA or DNA is amplified using PCR.

In one embodiment, the method further comprises detecting the interaction by attachment of a label. In one embodiment, the label is UPT particles or fluorescing particles.

In another embodiment of the present invention, a method is provided for testing for HIV in a sample, comprising providing a microfluidic cassette having means for testing for RNA sequences associated with HIV and means for testing for antigens associated with HIV.

When the reaction chamber is a PCR chamber, the format can be stationary (sample held in a chamber that is alternately heated and cooled, continuous flow through (sample propelled through a serpentine channel passing through a plurality of heating zones), pneumatic oscillatory (sample propelled back and forth through a conduit passing through a plurality of heating zones), self actuated (sample propelled through a closed loop containing a plurality of heating zones), electrokinetic (sample propelled by an electric field), or magneto-hydrodynamically (MHD)-driven (flow induced by electric current in the presence of a magnetic field). The developer has logic to control the valve settings as listed, thereby allowing for proper treatment.

Referring to Fig. 5, in certain applications such as MHD driven circular chromatography, MHD-driven PCR, MHD stirrer, and self-actuated flow-cycling PCR, it is desirable to operate in a closed loop. Ice valving provides a unique solution to the filling and emptying of a closed loop without any influence on the flow pattern along the whole loop. The filling of the closed loop without creating gas bubbles can be easily carried out. In Fig. 5, a closed loop is depicted equipped with an ice valve to aid in the filling and withdrawal of a liquid sample. The loop is connected to an inlet conduit and an exit conduit at points A and B, respectively. The inlet and exit conduits divide the loop into a long arc segment and a short arc segment. The thermoelectric unit is installed to cool part of the shorter arc segment between the inlet and outlet conduits (A). (B) provides a photograph of the loop equipped with the thermoelectric unit and fabricated with polycarbonate. The (B)-(G) depict the sequence of steps needed to fill (first row) and empty (second row) the loop. Initially, the valve is open. A liquid slug enters through the inlet conduit and fills the short arc segment between the inlet and the exit conduits (B). This is the path of least resistance to the flow. Next, a portion of the liquid slug is frozen (the valve closes), and the slug must flow through the longer (right) arc (C) until the loop fills entirely with

liquid (D). At this point in time, the PC valve opens, and the two other valves (not shown here) upstream and downstream of the loop are closed. The sample can now circulate around the loop as many times as desired. To withdraw the sample from the loop, the upstream and downstream valves (not shown) are opened and the PC valve along the short segment of the loop is closed (E). A gas stream delivered through the inlet conduit (F) displaces the sample (G).

Thus, in another embodiment of the present invention, a method is provided for filling and emptying of a closed loop, comprising providing an ice valve in the loop between an inlet and outlet; closing the valve to fill the loop; opening the valve to circulate fluid; and closing the valve to empty the loop out the outlet.

In another embodiment of the present invention, a method is provided for mixing fluids in a chamber without bubble formation, comprising adding a fluid; freezing the fluid; adding at least one further fluid; and thawing the first fluid.

Referring to Fig. 6, in another embodiment of the present invention, a method is provided for automating flow control in a microfluidic cassette without the need for a sensor on the cassette, comprising propelling a fluid; freezing the fluid at the valve location via self-actuation (ice or hydrogel valve); external pressure sensor detecting the pressure increase; and stopping the fluid propelling.

In another embodiment of the present invention, a method is provided for performing PCR in a chamber without bubble formation, comprising providing a valve at each inlet and outlet of the chamber; and closing the valves. One mode of achieving cassette-based PCR is to hold the reagents in a chamber while cycling the chamber temperature (stationary PCR). One of the problems often experienced with stationary PCR microreactors is bubble formation. The bubbles are undesirable, as they may expel the reagents from the PCR chamber, thereby reducing the amplification efficiency. One way to minimize or eliminate the bubble formation is to pressurize the PCR chamber by sealing it. The PCR mixture is driven into the reaction chamber through the inlet phase change (PC) valve. During this process, the inlet valve is maintained at

room temperature, allowing unhindered passage of the liquid. The liquid fills the PCR chamber, displacing the air through the pre-cooled exit valve. Once the air has been displaced out of the chamber and the liquid arrives at the exit valve's location, it freezes and blocks the passage. Subsequently, the inlet PC valve is closed. Once both the upstream and downstream valves are closed, the temperature of the PCR reactor is cycled according to standard protocols. The subsequent increase in pressure suppresses bubble formation.

EXAMPLES

A cassette was designed, constructed, and successfully tested to carry out PCR and to detect the amplified DNA (Fig. 7). Fig. 8 shows a schematic of a partially integrated PCR and Detection cassette. This is the downstream component of the analysis path for pathogen RNA or DNA. The device demonstrates the partial integration of the PCR chamber, a mixing and incubation (37°C) chamber, and a detection compartment. Two phase-change valves were used to assist in the PCR chamber filling and sealing. The downstream valve was pre-cooled. When the PCR mixture arrived at the valve location, it froze and blocked the passage. Once the PCR chamber was filled, the upstream valve was closed. The PCR thermocycling was achieved with a thermoelectric module.

At the completion of the amplification process the PCR products were propelled to the mixing chamber where they mixed with buffer solution containing UPT particles for detection. Mixing was accomplished by cooling and heating the mixing chamber with two thermoelectric modules. After incubation at 37°C for 30 min, the mixture was pneumatically propelled into the loading pad of the detection strip. The solution was drawn into the strip by capillary forces and the presence of the UPT particles was detected by exciting the UPT particles and scanning the emitted signal. The control algorithms for the fluid flow, heating, and cooling were implemented in LabVIEW™.

Fig. 9 shows images taken during treatment.

Fig. 10 shows the comparable results of detection between benchtop and cassette based runs.

Fig. 11 shows PCR amplification of *B. cereus* DNA obtained by benchtop vs. cassette lysis and isolation. *B. cereus* (2 x 109 cell/ml) was lysed either by conventional benchtop methods (BT) or in the cassette (Figure 13A). Purified DNA was eluted in 7 fractions and they were used as templates for PCR. (A) shows the agarose gel results comparing cassette lysed/purified DNA to whole genomic DNA (control), BT (benchtop lysed /purified DNA and cassette lysed/purified fractions. B. Relative pixel density of the PCR product from each fraction. Quantization of the gel image captured with a Kodak Image station used ImageQuant V5.2 software.

Fig. 12 is an agarose gel image of cassette isolated B. cereus DNA PCR products from a partially integrated DNA isolation and PCR device. PCR was performed for 25 cycles producing the anticipated 305 bp B. cereus amplicon.

The disclosures of each patent, patent application, and publication cited or described in this document, if any, are hereby incorporated herein by reference in their entireties.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

CLAIMS:

1. A method for concurrent testing for at least two of RNA, DNA, antibody, and antigen in a sample, comprising:

applying a portion of the sample to a detection zone disposed on a microfluidic cassette for interacting with pre-selected RNA sequences, DNA sequences, antibodies, or antigens, or mixtures thereof; and

applying at least one further portion of the sample to at least one further detection zone disposed on the microfluidic cassette for interacting with pre-selected RNA sequences, DNA sequences, or antigens.

2. The method of claim 1, further comprising applying a portion of the sample to another detection zone, wherein the detection zone interacts with RNA, DNA, antigen, or antibody.

3. The method of claim 1, further comprising detecting the interaction.

4. The method of claim 3, wherein interaction is detected using UPT particles, fluorescing particles, hybridization sensors, or electrochemical sensors.

5. A method for testing for the presence of a pre-selected pathogen in a sample, comprising:

placing the sample in a microfluidic cassette;

metering the sample;

propelling the sample along a flow path in the cassette to a detection zone having at least one zone adapted to interact with the pre-selected pathogen; and

detecting the presence or absence of interaction.

6. The method of claim 5, wherein there is a pre-selected pattern of zones on the detection zone, each for interacting with a different sequence.

7. The method of claim 5, further comprising applying a portion of the sample to a pre-selected pattern of zones on at least one further detection zone, each zone for interacting with a different sequence of RNA, DNA, antigen, or antibody.

8. A method of testing for pre-selected pathogens, comprising:

placing a sample in a cassette; and

propelling the sample through the cassette under pressure, wherein a portion of the sample is directed to a detection zone for interacting with pre-selected RNA sequences, DNA sequences, antibodies, or antigens known to be associated with a pre-selected pathogen.

9. The method of claim 8, wherein the propulsion is hydraulic, electric, or magnetic.

10. The method of claim 8, further comprising controlling movement of the sample with a valve disposed in the cassette.

11. The method of claim 8, further comprising diluting the sample.

12. The method of claim 8, wherein at least one reagent is pre-loaded.

13. The method of claim 8, further comprising metering the sample.

14. The method of claim 8, further comprising treating the sample.

15. The method of claim 14, further comprising lysing cells in the sample.

16. The method of claim 14, further comprising isolating RNA or DNA in the sample.

17. The method of claim 16, wherein the RNA or DNA are bound to a solid phase.

18. The method of claim 16, further comprising amplifying RNA or DNA in the sample.

19. The method of claim 18, wherein the RNA or DNA is amplified using PCR.

20. The method of claim 19, wherein the PCR chamber is pressurized to suppress bubble formation.

21. The method of claim 8, further comprising detecting the interaction by attachment of a reporter particle.

22. A method for testing for HIV in a sample, comprising:

providing a microfluidic cassette having means for testing for RNA sequences associated with HIV and means for testing for antigens associated with HIV.

23. A method for filling and emptying of a closed loop, comprising:

providing an ice valve in the loop between an inlet and outlet;

closing the valve to fill the loop;

opening the valve to circulate fluid; and

closing the valve to empty the loop out the outlet.

24. A method for mixing fluids in a chamber without bubble formation, comprising:

adding a fluid;

freezing the fluid;

adding at least one further fluid; and

thawing the first fluid.

25. A method for performing PCR in a chamber without bubble formation, comprising:

providing a valve at each inlet and outlet of the chamber; and

closing the valves.

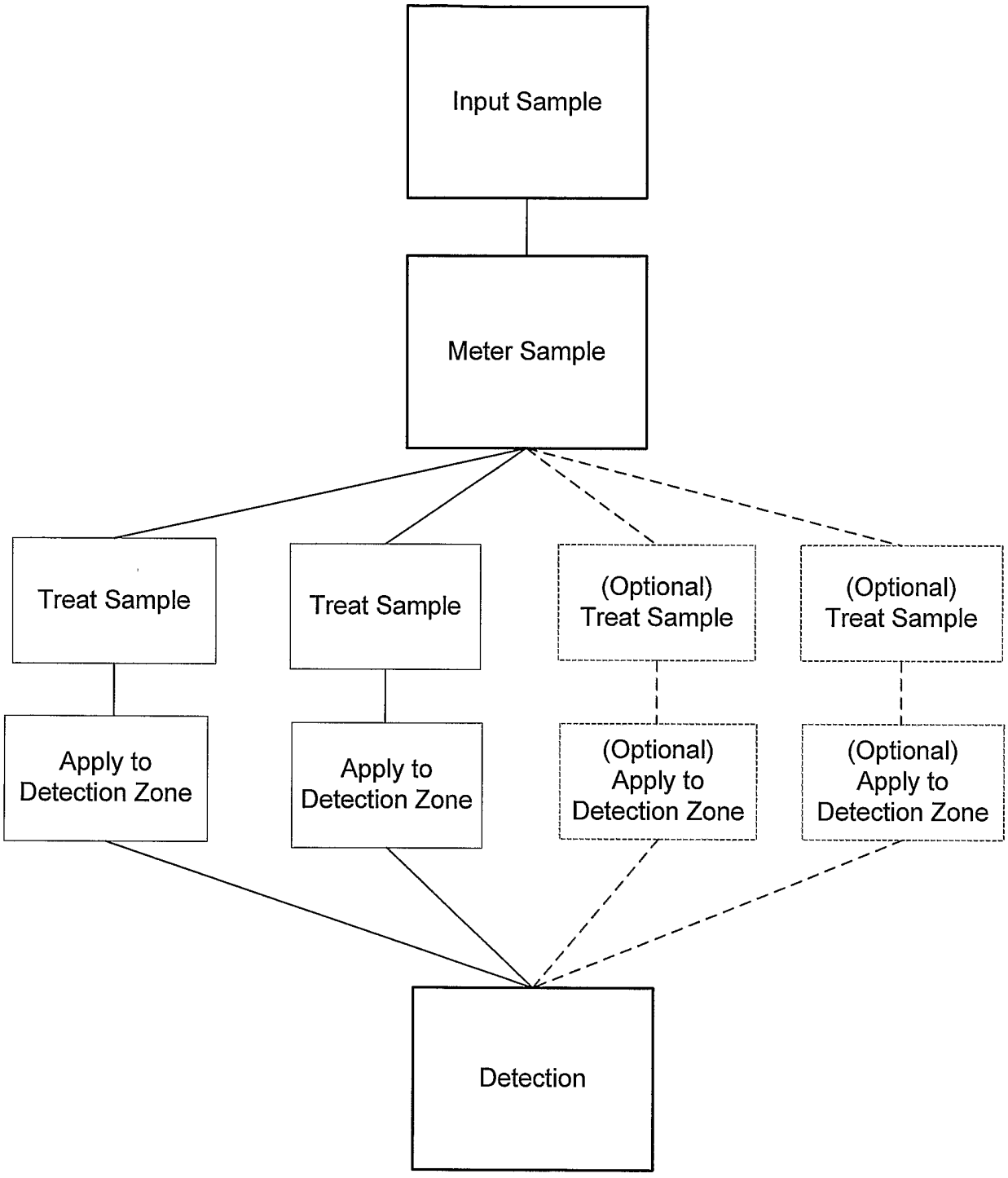

Input Sample
Meter Sample
Treat Sample
Treat Sample
(Optional) Treat Sample
(Optional) Treat Sample
Apply to Detection Zone
Apply to Detection Zone
(Optional) Apply to Detection Zone
(Optional) Apply to Detection Zone
Detection

FIG. 1

Fig. 2

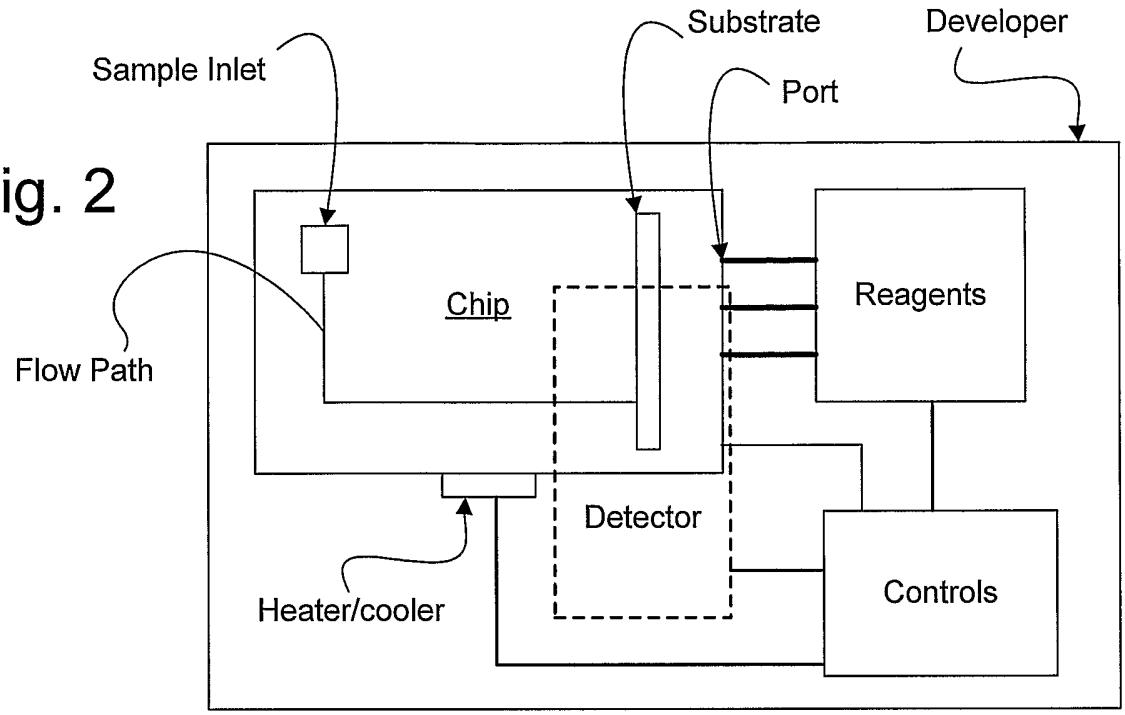
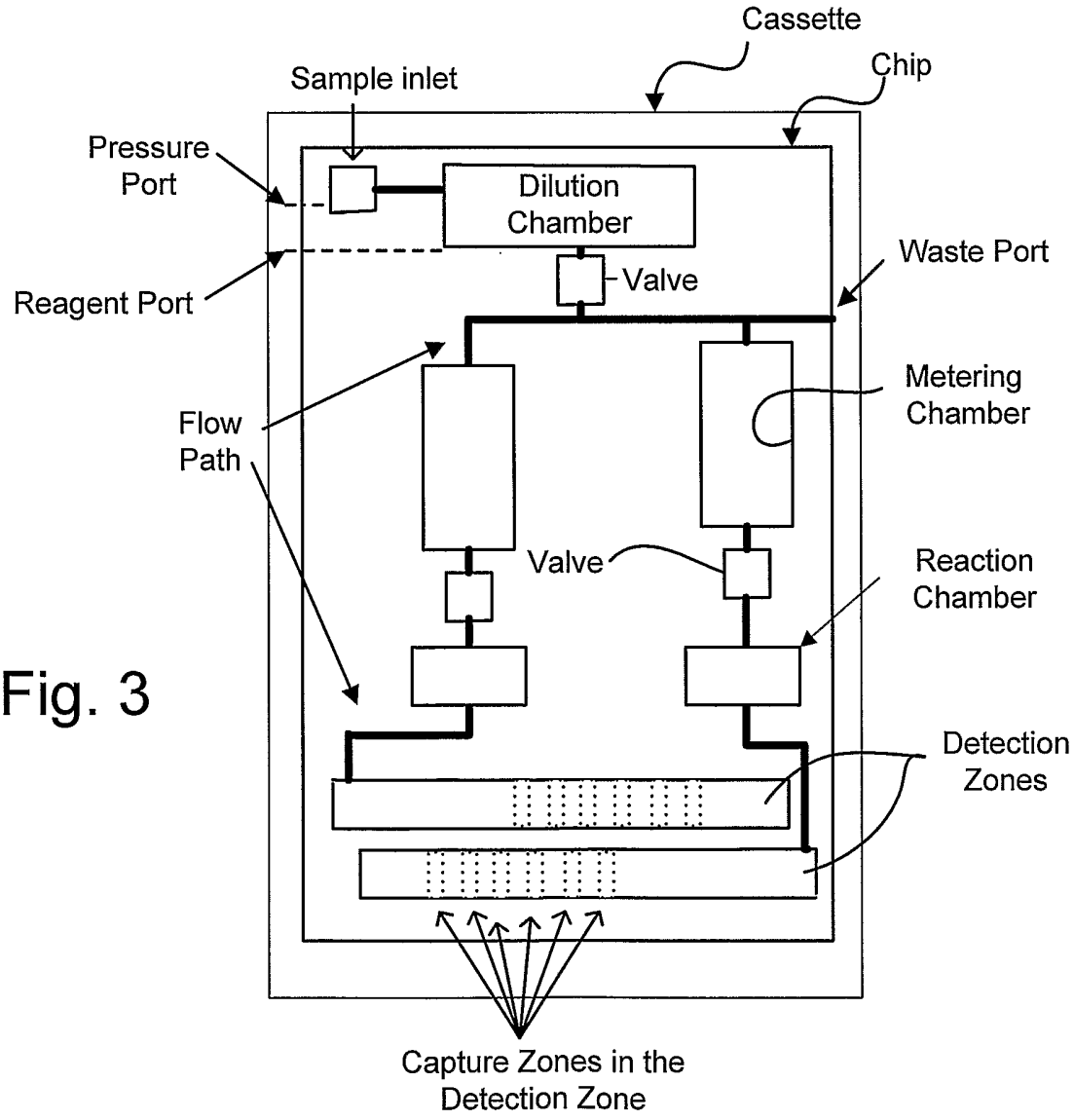
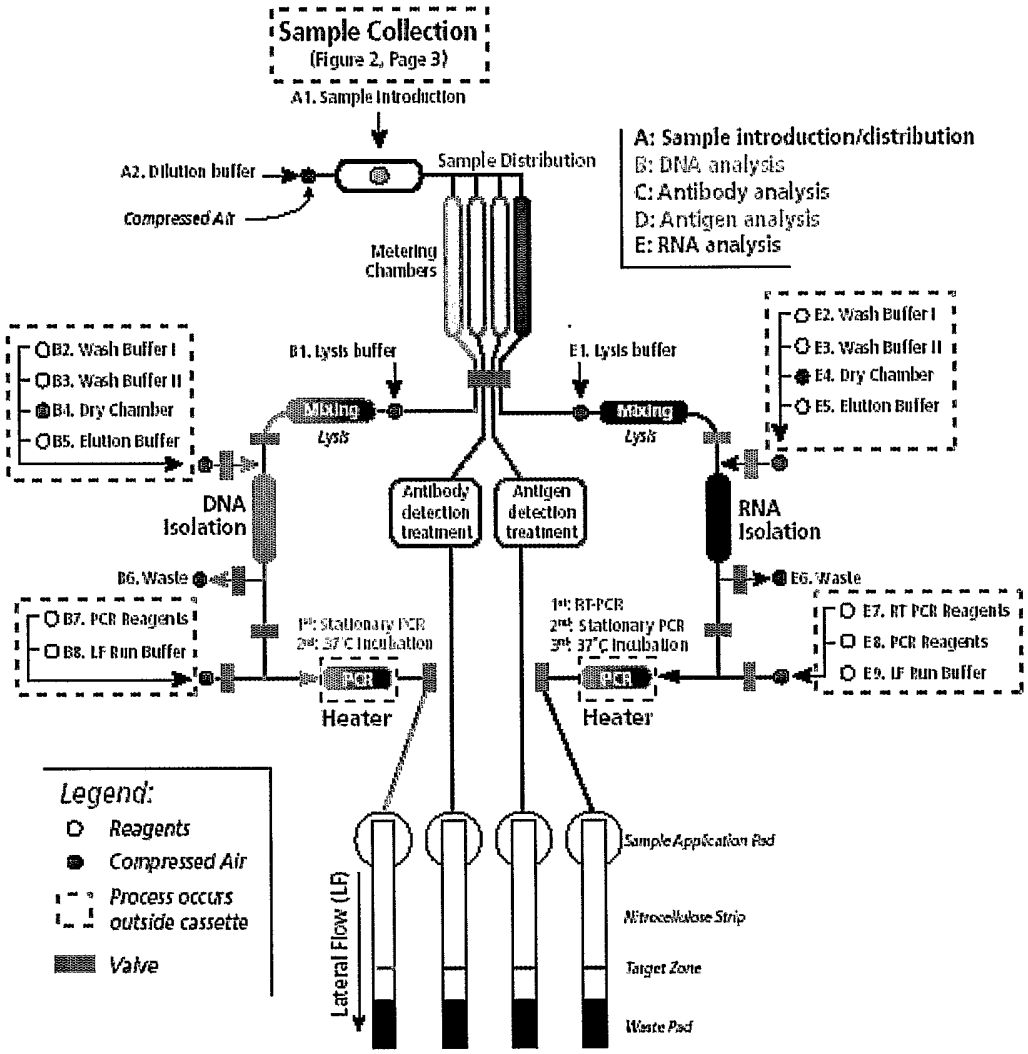

Sample Inlet
Substrate
Developer
Port
Chip
Reagents
Flow Path
Detector
Heater/cooler
Controls

Fig. 3


Cassette
Chip
Sample inlet
Pressure Port
Dilution Chamber
Waste Port
Valve
Reagent Port
Metering Chamber
Flow Path
Valve
Reaction Chamber
Detection Zones
Capture Zones in the Detection Zone



Target Detection via UPT

Fig. 4

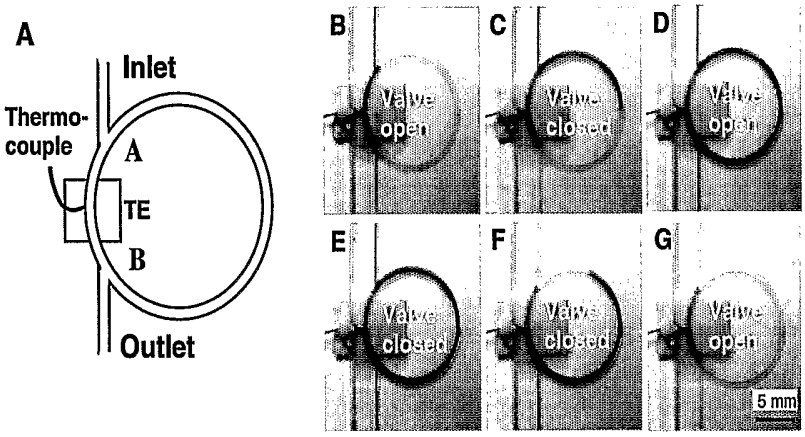

A
Inlet
Thermo-
couple
A
TE
B
Outlet
B
Valve
open
C
Valve
closed
D
Valve
open
E
Valve
closed
F
Valve
closed
G
Valve
open
5 mm

Fig. 5

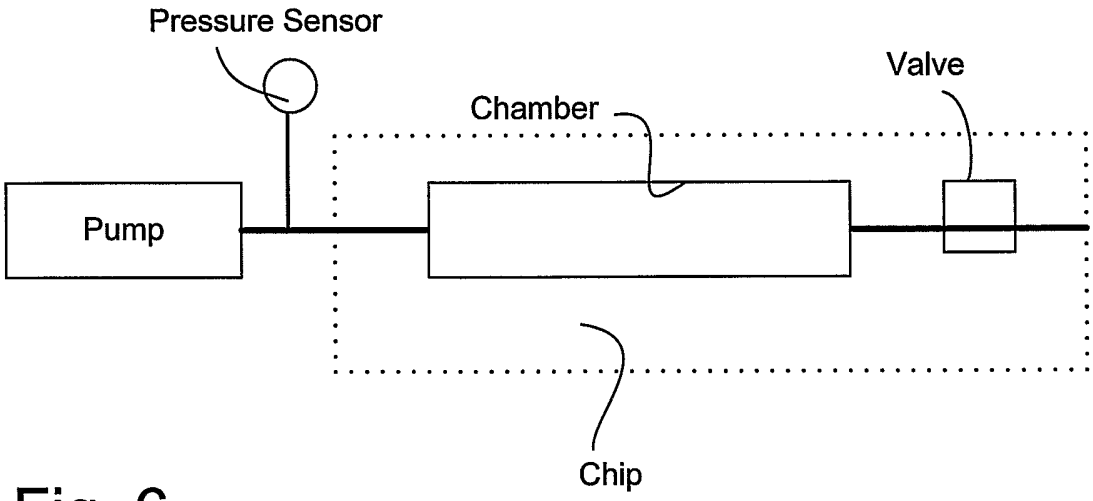
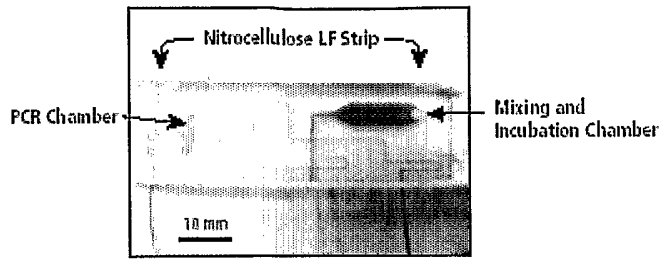

Pressure Sensor
Valve
Chamber
Pump
Chip

Fig. 6

Fig. 7



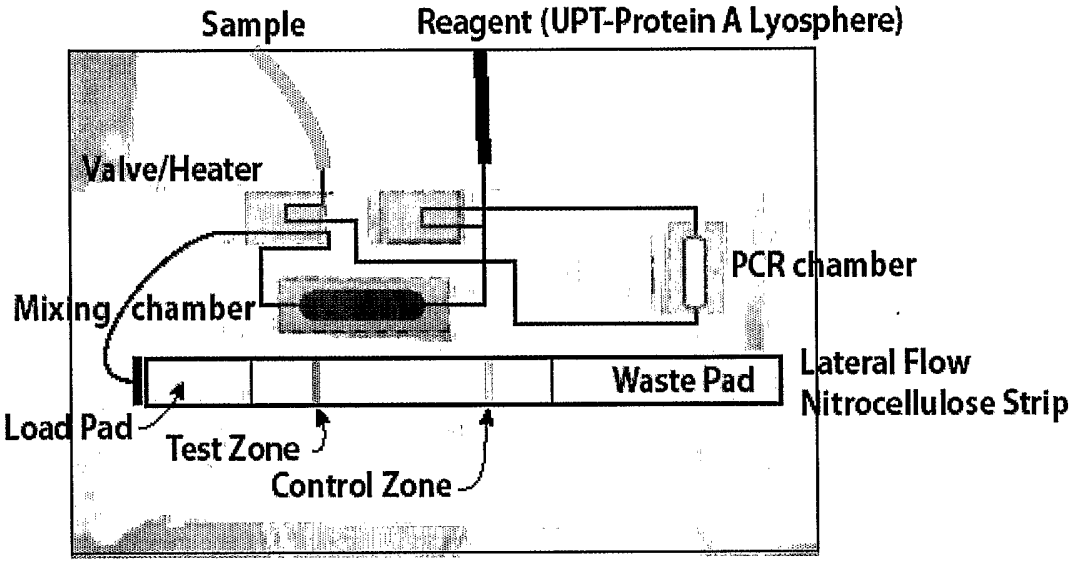


Fig. 8

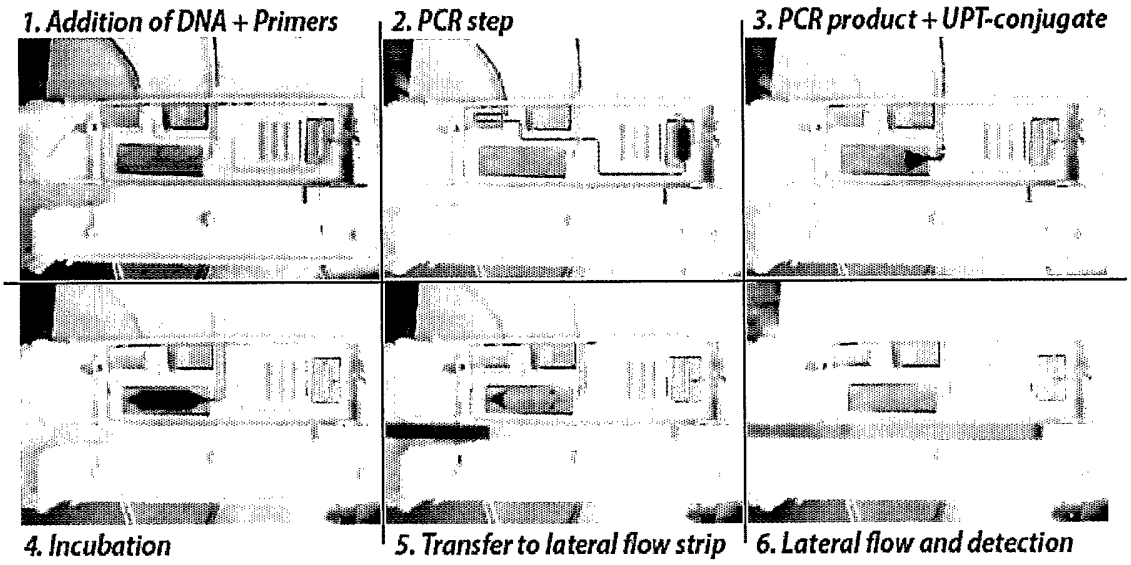


Fig. 9

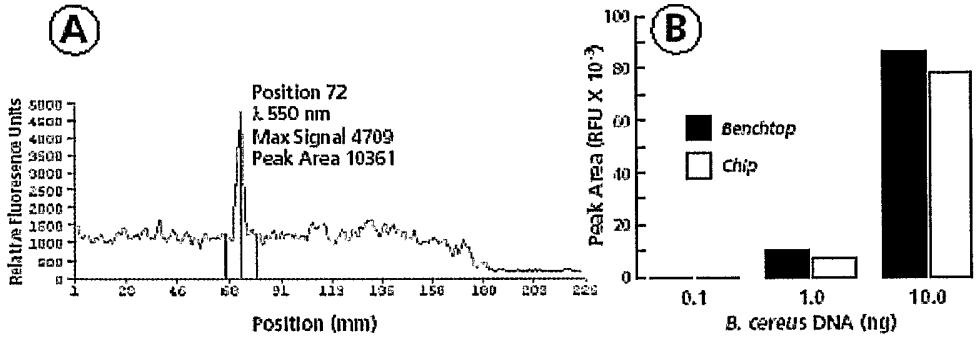


Fig. 10

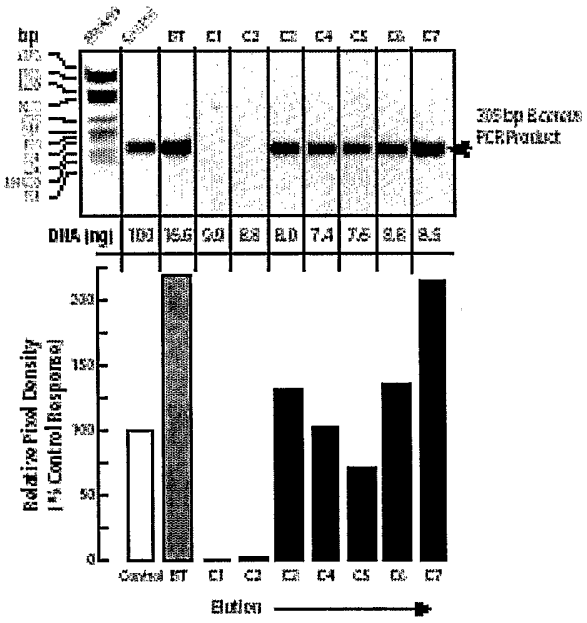


Fig. 11

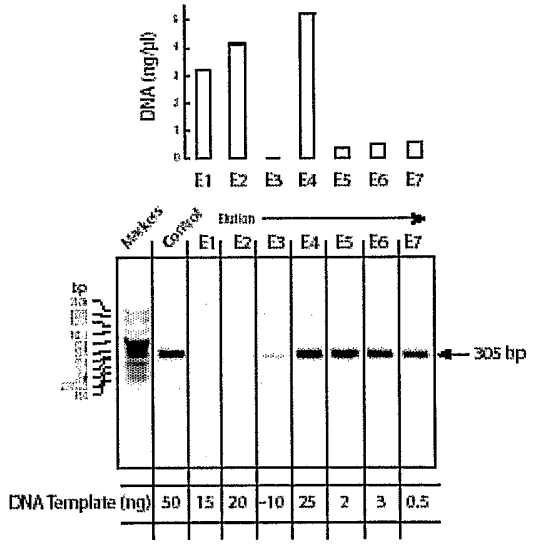


Fig. 12

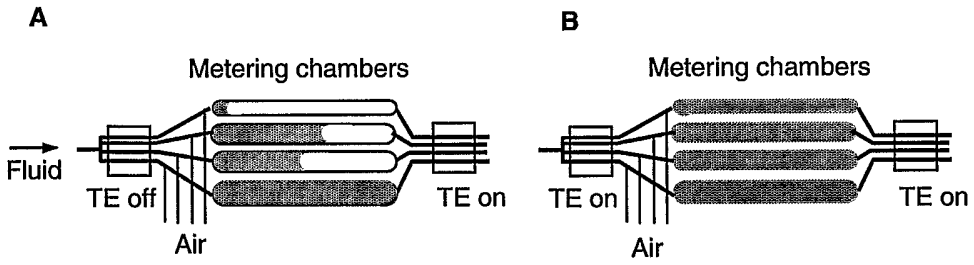

A
Metering chambers
Fluid
TE off
TE on
Air
B
Metering chambers
TE on
TE on
Air

Fig. 13