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(54) **Title:** INTEGRATED DEVICE FOR NUCLEIC ACID DETECTION AND IDENTIFICATION

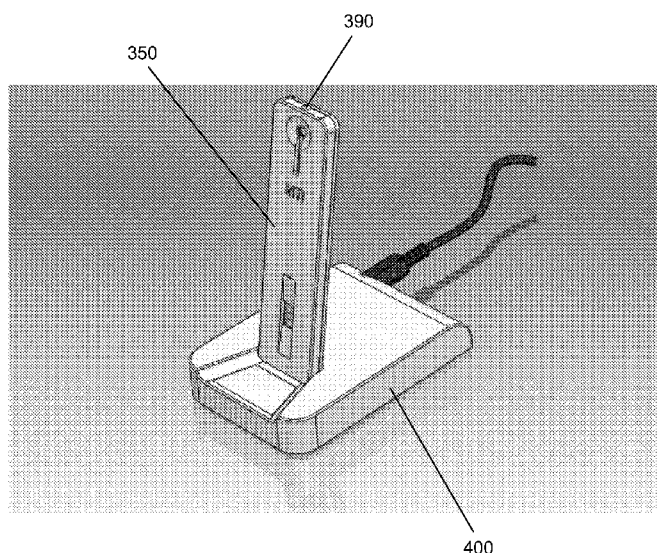


FIG. 15

(57) **Abstract:** A disposable assay platform for detecting a target nucleic acid comprising multiple chambers and a method for operating the assay platform. Solutions containing the target nucleic acid move from one chamber to the next chamber by opening a vent pocket. The resulting pressure change enables the solution to flow to the next chamber. The platform comprises an electronic layer and one or more fluid layers bonded together. All heating operations can be performed by using resistive heating elements in the platform. All cooling operations are preferably passive. The platform is preferably operated when in a vertical orientation and can be docked to an external docking station that controls the operation of the platform.



— *with information concerning incorporation by reference  
of missing parts and/or elements (Rule 20.6)*

**INTEGRATED DEVICE FOR NUCLEIC ACID DETECTION AND IDENTIFICATION**

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to and the benefit of filing of U.S. Provisional Patent Application Serial No. 61/477,357, entitled "Integrated Device for Nucleic Acid Detection and Identification", filed on April 20, 2011, and U.S. Provisional Patent Application Serial No. 61/477,437, entitled " Oscillating Amplification Reaction for Nucleic Acids", filed on April 20, 2011, which are incorporated herein by reference.

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REFERENCE TO A SEQUENCE LISTING, A TABLE, OR COMPUTER PROGRAM

Applicant hereby submits a sequence listing as a text file titled 042012\_ST25.txt created on April 20, 2012 having 2K kbytes that is ASCII compliant and is hereby incorporated by reference.

15

BACKGROUND OF THE INVENTIONField of the Invention (Technical Field):

Embodiments of the present invention relate to an integrated device and related methods for detecting and identifying nucleic acids. The device may be fully disposable or may comprise a disposable portion and a reusable portion.

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Background Art:

Note that the following discussion refers to a number of publications and references. Discussion of such publications herein is given for more complete background of the scientific principles and is not to be construed as an admission that such publications are prior art for patentability determination purposes.

25

As the public health impact and awareness of infectious and emerging diseases, biothreat agents, genetic diseases and environmental reservoirs of pathogens has increased, the need for more informative, sensitive and specific point-of-use rapid assays has increased the demand for

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polymerase chain reaction (PCR)-based tools. Nucleic acid-based molecular testing by such methods as PCR-based amplification is extremely sensitive, specific and informative.

Unfortunately, currently available nucleic acid tests are unsuitable or of limited utility for field use because they require elaborate and costly instrumentation, specialized laboratory materials and/or multiple manipulations dependent on user intervention. Consequently, most samples for molecular testing are shipped to centralized laboratories, resulting in lengthy turn-around-times to obtain the required information.

To address the need for rapid point-of-use molecular testing, prior efforts have focused on product designs employing a disposable cartridge and a relatively expensive associated instrument. The use of external instrumentation to accomplish fluid movement, amplification temperature control and detection simplifies many of the engineering challenges inherent to integrating the multiple processes required for molecular testing. Unfortunately, dependence upon elaborate instrumentation imposes tremendous economic barriers for small clinics, local and state government and law enforcement agencies. Further, dependence upon a small number of instruments to run tests could cause unnecessary delays during periods of increased need, as occurs during a suspected biowarfare agent release or an emerging epidemic. Indeed, the instrument and disposable reagent cartridge model presents a potentially significant bottleneck when an outbreak demands surge capacity and increased throughput. Additionally, instrumentation dependence complicates *ad hoc* distribution of test devices to deployment sites where logistic constraints preclude transportation of bulky associated equipment or infrastructure requirements are absent (*e.g.* reliable power sources).

Gravity has been described as a means of fluid movement in existing microfluidic devices. However, the typical device does not allow for programmable or electronic control of such fluid movement, or the mixing of more than two fluids. Also, some devices utilize a pressure drop generated by a falling inert or pre-packaged fluid to induce a slight vacuum and draw reactants into processing chambers when oriented vertically, which increases storage and transport complexities to ensure stability of the pre-packaged fluids. Existing devices which teach moving a fluid in a plurality of discrete steps require frangible seals or valves between chambers, which complicates operation and manufacture. These devices do not teach the use of separate, remotely located vents for each chamber.

Typical microfluidic devices typically make use of smaller reaction volumes than are employed in standard laboratory procedures. PCR or other nucleic acid amplification reactions such as loop mediated amplification (LAMP), nucleic acid based sequence amplification (NASBA) and other isothermal and thermal cycling methods are typically conducted in testing and research laboratories using reaction volumes of 5 to 100 microliters. These reaction volumes accommodate test specimen volumes sufficient to ensure the detection of scarce assay targets in dilute specimens. Microfluidic systems that reduce reaction volumes relative to those employed in traditional laboratory molecular testing necessarily also reduce the volume of specimen that can be added to the reaction. The result of the smaller reaction volume is a reduction in capacity to accommodate sufficient specimen volume to ensure the presence of detectable amounts of target in dilute specimens or where assay targets are scarce.

#### SUMMARY OF THE INVENTION

An embodiment of the present invention is a disposable platform for detecting a target nucleic acid, the disposable platform comprising a sample chamber for receiving a sample comprising the target nucleic acid; an amplification chamber connected via a first channel to the sample chamber and connected via a second channel to a first vent pocket; a labeling chamber connected via a third channel to the amplification chamber and connected via a fourth channel to a second vent pocket; a detection subsystem connected to the labeling chamber via a fifth channel and connected via a sixth channel to a third vent pocket; a plurality of resistive heating elements; and one or more temperature measuring devices; wherein the vent pockets are each sealed from the atmosphere by a heat labile membrane located in a vicinity of one of the resistive heating elements. The disposable platform optionally further comprises a sample preparation stage comprising an output in direct fluid connection with an input of the sample chamber. Dimensions of a substantially flat surface of the amplification chamber are preferably approximately the same as dimensions of a substantially flat surface of a resistive heating element in thermal contact with the amplification chamber. The amplification chamber is preferably not cooled by an active cooling device. The amplification chamber optionally contains an amplification solution, the sample chamber optionally comprises a liquid amplification reagent mix or a lyophilized amplification reagent mix, and/or the labeling chamber optionally comprises detection particles. The labeling

chamber is preferably heatable using one of the resistive heating elements. The detection subsystem comprises a lateral flow strip that preferably does not comprise detection particles. The chambers, the channels, and the vent pockets are preferably located on a fluid assembly layer and the electronic elements are preferably located on a separate layer comprising a printed circuit board, the separate layer bonded to the fluid assembly layer. The detection subsystem is preferably located on the fluid assembly layer or optionally on a second fluid assembly layer. The volume of at least one of the chambers is preferably between approximately 1 microliter and approximately 50 microliters. The disposable platform preferably further comprises a connector for docking the disposable platform with a base unit that is not an external instrument and that maintains the disposable platform in a vertical or tilted orientation.

An embodiment of the present invention is a method for detecting a target nucleic acid, the method consisting of disposing a sample comprising the target nucleic acid in a sample chamber of a disposable platform; orienting the disposable platform vertically or at a tilt; reacting the sample with a liquid or previously lyophilized amplification reagent mix; opening a first vent pocket connected to an amplification chamber to atmosphere, thereby enabling the reacted sample to flow into the amplification chamber; amplifying the target nucleic acid in the amplification chamber; opening a second vent pocket connected to a labeling chamber to atmosphere, thereby enabling the amplified target nucleic acid to flow into the labeling chamber; labeling the amplified target nucleic acid using detection particles in the labeling chamber; opening a third vent pocket connected to a detection subsystem to atmosphere, thereby enabling the labeled target nucleic acid to flow into the detection subsystem; and detecting the amplified target nucleic acid. The amplifying step preferably comprises amplifying the target nucleic acid using a resistive heating element located within the disposable platform in a vicinity of the amplification chamber. The method preferably further comprises passively cooling the amplification chamber. The method preferably further comprises heating the labeling chamber during the labeling step using a resistive heating element located within the disposable platform in a vicinity of the labeling chamber. The detection subsystem preferably does not comprise detection particles. The method preferably further comprises controlling operation of the disposable platform by using a docking station which is not an external instrument.

Objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification, illustrate embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating certain embodiments of the invention and are not to be construed as limiting the invention. In the drawings:

FIG. 1 is a drawing illustrating the fluidic and electronic layers for an embodiment of the present invention. Prepared sample fluid enters the sample chamber where it is mixed with preferably lyophilized reagents. In the vertical orientation, pressure of the fluid column equilibrates with the sealed volume of air below it. Capillarity prevents the escape of air and further advancement of fluid. When the appropriate vent seal underlying the corresponding vent pocket is ruptured, fluid moves through the outlet channel to the next chamber for further processing. Temperature and fluid control is preferably achieved using standard printed circuit assembly (PCA) components and assembly techniques.

FIG. 2A is a schematic representation of a vent mechanism employed in an embodiment of the present invention to accomplish controlled fluid movement within the fluidic layer. FIG. 2B is a drawing illustrating the vent location and construction in an embodiment of the present invention. A membrane holds the pressure of the fluid column above ambient. When sufficient heat is applied, the membrane ruptures and allows pressure to equilibrate. Fluid moves along the hydrostatic pressure gradient. Pressures can be less than a few mBar.

FIG. 3 shows further resistive heater details of an embodiment of the present invention. Two 2512 sized thick-film surface mount device (SMD) resistors (heating element) flank a 0402 sized thermistor (temperature sensor) on the printed circuit board (PCB). A thin layer of thermal

compound at the interface of the resistor(s) and the amplification chamber maintains thermal conductivity to the heaters and sensor. Dimensions of the chamber are preferably chosen to maximize the area/volume ratio.

FIG. 4 depicts embodiments of the present invention which support either thermal cycling or isothermal-based nucleic acid amplification methodologies. FIG. 4A shows a PCA with four resistor/thermistor pairs. Four surface mount resistors serve as four independently controllable heaters (arrows). FIG. (B shows a fluidic assembly attached to the PCA of FIG. 4A consistent with the resistive heater detail of FIG. 3. The fluidic layer interfaces with the surface mount resistors of the PCA to provide reaction chambers for nucleic acid amplification. FIG. 4C shows gel electrophoresis of amplification reactions producing a ~150 bp (base pair) product from a PCR machine (LAB) or by an embodiment of the present invention ( $\mu$ Heater) by thermal cycling. The left most lane is size standard. FIG. 4D is a graph of temperature versus time in seconds for fluid within the amplification chamber of the present embodiments. The darker line indicates temperature of solution in the reaction chamber obtained by thermocouple. The lighter line is the temperature measured by the thermistor used by the microcontroller for temperature control. 40 cycles of a two-temperature PCR reaction can be accomplished in less than 20 minutes using a 20  $\mu$ L reaction volume. FIG. 4E shows gel electrophoresis of isothermal Nucleic Acid Sequence Based Amplification (NASBA) reactions producing an ~150 bp product from a PCR machine (Positive Control), or by use of an embodiment of the present invention. Four separate reactions indicate both the setting of the temperature sensor, and a particular surface treatment applied to the interior of the fluidic chamber.

FIG. 5 illustrates an embodiment of the present invention comprising the technique of transporting fluid without the use of a vent. By heating the chamber below the fluid column, gas will expand and purge itself through the inlet channel. Upon cooling, the gas in the chamber volume will contract and draw in a volume of fluid proportional to that of the purged gas. The fluid drops to the chamber floor. Successive iterations of this cycle can move the full reaction volume. The operation of is technique depends on channel size, length, heat time and temperature, chamber volumes, and surface energies of components.

FIG. 6 shows the detail and function of a labeling chamber of an embodiment of the present invention. Fluid containing amplicon enters the labeling chamber through the inlet channel



and contacts detection particles. Sufficient mixing is accomplished by heating or boiling of fluid. Rising bubbles nucleated at the bottom and sides of the chamber, preferably by a textured feature such as a laser etched line or series of lines, preferably effectively stir the mixture. In this embodiment, SMD components are the same as those used in the amplification heater.

5           FIG. 7 shows the components of the fluidic layer of an embodiment of the present invention. A wall component of chosen thickness is bonded on two sides by face components. In one embodiment, the wall component is 0.5 mm laser cut acrylic and the faces are 0.004" polyester (PET) film. The parts are preferably bonded together with a silicone transfer adhesive. Interior surfaces are treated to control wetting. Reagents and lateral flow assembly are added  
10 during fabrication. An adhesive membrane is preferably sealed over the vent pockets.

          FIG. 8 shows the PCA side facing the fluidic assembly of an embodiment of the present invention in which the heating elements are thick-film resistors. The temperature sensor is a small SMD thermistor positioned in close proximity to the heaters. The PCA may optionally incorporate indicator LEDs for monitoring assay progression, heating, and vent opening.

15           FIG. 9 is a drawing illustrating the fluid cassette bonded to the PCA with an adhesive shim in accordance with an embodiment of the present invention. The shim thickness can be important to proper distancing and function of the vents and heaters.

          FIG. 10A shows the disposable PCA of a semi-disposable invention configuration embodiment of the present invention. The PCA contains only electronic components that interface  
20 with the disposable fluidic assembly. This includes the heating elements, temperature sensors, and optionally LED indicators. A connector is present to complete circuitry and optionally to add support in the vertical orientation.

          FIG. 10B shows the disposable PCA/fluidic assembly of FIG. 10A in place in a docking station. The docking station contains the control electronics and power supply and is optionally  
25 easily portable and handheld. The disposable portion containing the PCA and fluidic assemblies are inserted in the connector, preferably in a vertical orientation. A user interface including indicating LEDs, LCD, and user controls may optionally be present in some embodiments. The docking station may include buttons to initiate electronic processes required for the assay.

          FIG. 11A is drawing of the front side of the PCA of a disposable invention configuration  
30 embodiment of the present invention. This side faces the fluidic assembly. Heating and sensor

elements as well as user interface components such as LED indicators are present in this embodiment.

FIG. 11B is a drawing illustrating the layout of the back side of the PCA of the disposable invention configuration of FIG. 11A. This side of the PCB holds the control circuitry such as the  
5 microcontroller, MOSFET switches, and ancillary circuitry. In this embodiment, terminals are present for a 9V battery, as well as optional user interface devices such as tactile switches useful for assay initiation.

FIG. 11C is a drawing of the PCA of FIG. 11B with 9V battery installed. Plastic housing is not shown. Battery placement is preferably as shown to lower the center of mass and to help  
10 prevent tipping or overturning of device during operation.

FIG. 12 is an illustration of a semi-disposable embodiment of the present invention wherein a sample preparation subsystem is interfaced with the amplification and detection fluidics and electronics.

FIG. 13 shows the components of an embodiment of a multilayer fluidic layer incorporated  
15 into a disposable assay.

FIG. 14 shows an exploded view of a disposable assay cartridge incorporating the fluidic layer of FIG. 13.

FIG. 15 is an illustration of the assembled disposable PCA/fluidic assembly of FIG. 14 in place in a docking station.

FIG. 16 are photographs of the fluidic layers of an embodiment of the present invention  
20 which supports thermal cycling based nucleic acid amplification and detection. A reaction solution containing all reagents necessary to support nucleic acid amplification was added to the sample chamber. In FIG. 16A, required enzymes were added to the reaction solution in liquid form. In FIG. 16B, required enzymes were supplied by incorporation of a lyophilized pellet into the sample  
25 chamber. The amplification and detection of nucleic acid was performed as described in Example 1. The top line of the detection strip assembly represents the positive control, an oligonucleotide complementary to the detection probe. The line immediately below the positive control represents the capture line, an immobilized oligonucleotide complementary to the same amplification product strand as the detection probe.

FIGS. 17A and 17B are photographs of an integrated sample-to-result nucleic acid testing device fabricated by interfacing a sample preparation sub-system with the invention. Embodiments of the present invention support nucleic acid isolation, amplification, and detection in a single integrated device. Nucleic acid isolation, amplification, and detection was performed as described in Example 2. The top line of the lateral flow assembly represents the positive control, an oligonucleotide complementary to the detection probe. The line immediately below the positive control represents the capture line, an immobilized oligonucleotide complementary to the same amplification product strand as the detection probe. The device is shown following completion of processing macerated leaf tissue from a citrus tree suffering from citrus greening disease and assaying the nucleic acids isolated by the integrated sample preparation system for *Candidatus Liberibacter* the etiologic agent of citrus greening.

#### DETAILED DESCRIPTION OF THE INVENTION

Embodiments of the present invention comprise a disposable platform which integrates instrumentation independent means of conducting all requisite steps of a nucleic acid molecular assay and complements current immuno-lateral flow rapid assays with a new generation of nucleic acid tests offering more informative and sensitive analyses. Embodiments of the present invention facilitate the broader use of rapid nucleic acid testing in small clinics and austere settings where infectious disease, biothreat agent, agriculture and environmental testing are the most likely to have the greatest impact. Certain embodiments of the present invention are completely self-contained and disposable which enables "surge capacity" in times of increased demand by allowing parallel tests to be run without instrumentation-imposed bottlenecks. Additionally, in those application areas where a low cost disposable cartridge coupled with an inexpensive battery-powered or AC adapter energized docking station is preferable, an embodiment of the invention where a simple docking station is employed further reduces test costs by placing reusable components in a reusable yet inexpensive base. The platform technology disclosed herein offers sensitivity similar to laboratory nucleic acid amplification-based methods, minimal user intervention and training requirements, sequence specificity imparted by both amplification and detection, multiplex capacity, stable reagents, compatibility with low-cost large-scale manufacturing, battery

operation to allow use in austere settings, and a flexible platform technology allowing the incorporation of additional or alternative biomarkers without device redesign.

Embodiments of the present invention provide systems and methods for low-cost, point-of-use nucleic acid detection and identification suitable to perform analyses in locations remote from a laboratory environment where testing would ordinarily be performed. Advantageously, nucleic acid amplification reaction volumes can be in the same volume range commonly used in traditional laboratory testing (e.g. 5-100  $\mu$ L). The reaction conducted in embodiments of the present invention is thus directly comparable to accepted laboratory assays, and allows the accommodation of the same specimen volumes typically employed in traditional molecular testing.

Embodiments of the present invention may be used to detect the presence of a target nucleic acid sequence or sequences in a sample. Target sequences may be DNA such as chromosomal DNA or extra-chromosomal DNA (e.g. mitochondrial DNA, chloroplast DNA, plasmid DNA etc) or RNA (e.g. rRNA, mRNA, small RNAs and viral RNA). Similarly, embodiments of the invention may be used to identify nucleic acid polymorphisms including single nucleotide polymorphisms, deletions, insertions, inversions and sequence duplications. Further, embodiments of the invention may be used to detect gene regulation events such as gene up- and down-regulation at the level of transcription. Thus, embodiments of the invention may be employed for such applications as: 1) the detection and identification of pathogen nucleic acids in agricultural, clinical, food, environmental and veterinary samples; 2) detection of genetic biomarkers of disease; and 3) the diagnosis of disease or a metabolic state through the detection of relevant biomarkers of the disease or metabolic state, such as gene regulation events (mRNA up- or down regulation or the induction of small RNAs or other nucleic acid molecules generated or repressed during a disease or metabolic state) in response to the presence of a pathogen, toxin, other etiologic agent, environmental stimulus or metabolic state.

Embodiments of the present invention comprise a means of target nucleic acid sample preparation, amplification, and detection upon addition of a nucleic acid sample, comprising all aspects of fluid control, temperature control, and reagent mixing.

In some embodiments of the invention, the device provides a means of performing nucleic acid testing using a portable power supply such as a battery, and is fully disposable. In other embodiments of the invention, a disposable nucleic acid test cartridge works in conjunction with a

simple reusable electronic component which does not perform all of the functions of typical laboratory instrumentation.

Embodiments of the present invention provide for a nucleic acid amplification and detection device comprising, but not limited to, a housing, a circuit board, and a fluidic or microfluidic layer. In certain embodiments, the circuit board may contain a variety of surface-mount components such as resistors, thermistors, light-emitting diodes (LEDs), photo-diodes, and microcontrollers. The fluidic or microfluidic layer is responsible for moving aqueous reaction volumes and may be made from a variety of plastics and by a variety of manufacturing techniques including bonding, fusing or lamination of laser cut, water-jet cut or injection molded pieces. The fluidics and circuit board components are held together and their thermal coupling may be enhanced by heat conducting materials or compounds. The housing preferably serves in part as a cosmetic and protective sheath, hiding the delicate components of the microfluidic and circuit board layers, and may also serve to facilitate sample input, buffer release, nucleic acid elution and the initiation of processes required for device functionality. For example, the housing may incorporate a sample input port, a button or similar mechanical feature to allow user activation, buffer release, sample flow initiation and/or nucleic acid elution.

In some embodiments of the invention, the fluidic or microfluidic layer preferably comprises four chambers, including a sample input chamber, an amplification chamber, a nucleic acid labeling chamber, and a detection chamber. The solution input chamber preferably comprises a sample input orifice where a nucleic acid containing sample may be added, and an egress conduit leading to the amplification chamber. The sample input chamber may also comprise lyophilized reagents that may include suitable buffers, salt, deoxyribonucleotides, ribonucleotides, oligonucleotide primers, and enzymes such as DNA polymerase and reverse transcriptase. Such lyophilized reagents are preferably solubilized upon addition of the nucleic acid sample. The amplification chamber is preferably situated in register and thermal contact with heater elements on the circuit board. Similarly, electronic components present on the circuit board are placed in physical contact or proximity to vents or valve structures in the fluidic layer to enable electronic control. The circuit board physical layout is designed to provide registration with elements of the fluidic or microfluidic layer such that resistive heating elements of the circuit board for amplification

and/or fluid flow control are situated to form contacts with elements of the fluidic component with which they interact.

Other embodiments of the invention comprise a nucleic acid amplification and detection device that is integrated with a sample preparation device. Embodiments including the sample  
5 preparation device provide a means for the communication of fluids between sample preparation subsystem output ports or valves and the input port or ports of the fluidic or microfluidic components of the device.

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to  
10 which this invention pertains. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodologies by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and *Current Protocols in Molecular*  
15 *Biology* (Ausbel et al., eds., John Wiley & Sons, Inc. 2001). As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

As used throughout the specification and claims, the terms 'target nucleic acid' or 'template nucleic acid' mean a single-stranded or double-stranded DNA or RNA fragment or  
20 sequence that is intended to be detected.

As used throughout the specification and claims, the terms 'microparticle' or 'detection particle' mean any compound used to label nucleic acid product generated during an amplification reaction, including fluorescent dyes specific for duplex nucleic acid, fluorescently modified oligonucleotides, and oligonucleotide-conjugated quantum dots or solid-phase elements such as a  
25 polystyrene, latex or paramagnetic particles or microspheres.

As used throughout the specification and claims, the term 'chamber' means a fluidic compartment where fluid resides for some period of time before being directed to another chamber. For example, a chamber may be the sample chamber, amplification chamber, labeling chamber, or the detection chamber.

As used throughout the specification and claims, the term 'pocket' means a compartment overlaid onto a resistor or other mechanism that serves as a venting mechanism. For example, unlike fluidic chambers as described above, a pocket created in the fluidic layer may have one open face that aligns with a resistor on the PCA. This open face is preferably covered by a thin  
5 membrane to create a sealed cavity that is easily ruptured by energizing the underlying resistor.

As used throughout the specification and claims, the term 'channel' means a narrow conduit within the fluidic assembly which typically connects two or more chambers and/or pockets or combinations thereof, including, for example, an inlet, outlet, or a vent channel. In the case of an inlet or outlet channel, fluid sample migrates through the channel. In the case of a vent channel,  
10 the conduit remains clear of fluid and connects a fluidic chamber to a vent pocket.

As used throughout the specification and claims, the term "external instrument" means a reusable instrument that heats and/or cools a disposable assay, and/or performs a mechanical action on a disposable assay, including but not limited to piercing buffer packets and/or pumping or otherwise actively providing a transport force for fluids.

15 Embodiments of the present invention are devices for low-cost, point-of-use nucleic acid testing suitable to perform analyses in locations remote from a laboratory environment where testing would ordinarily be performed. Certain devices comprise fluidic and electronic components or layers, optionally encased by a protective housing. In embodiments of the present invention, the fluidic layer is composed of plastic and is a series of chambers and pockets connected by narrow  
20 channels in which chambers are oriented vertically with respect to one another during operation. The fluidic layer is overlaid or otherwise placed in physical contact with electronic components such as a printed circuit board containing off-the-shelf surface mount devices (SMDs) and controlled via a microcontroller. In some embodiments of the device, the entire assembly is disposable. In other embodiments, the fluidic and physically bonded electronic layers are  
25 disposable, while a small inexpensive controlling unit is reusable. In another embodiment, the fluidic layer is disposable, and a small controlling base unit is reusable. For all embodiments, the present invention may be integrated with a nucleic acid sample preparation device such as that described in International Publication No. WO 2009/137059 A1, entitled "Highly Simplified Lateral Flow-Based Nucleic Acid Sample Preparation and Passive Fluid Flow Control" (incorporated  
30 herein by reference), and/or use methods described therein.

Embodiments of the present invention comprise an integrated nucleic acid testing device that can be manufactured inexpensively with established manufacturing processes. The invention provides molecular test data while retaining the simplicity from the end-user perspective of widely accepted hand-held immunoassays, overcoming the challenges of regulating fluid temperatures  
5 within the device, transporting small sample volumes in sequential steps, reagent mixing, and detecting nucleic acids. Embodiments of the present invention are uniquely adapted to utilize off-the-shelf electronic elements that may be constructed by standard assembly techniques, and requires no moving parts. Furthermore, the fluid layer design enables the use of readily available plastics and manufacturing techniques. The result is an inexpensive, disposable, and reliable  
10 device capable of nucleic acid isolation, amplification, and detection without the need for a dedicated laboratory infrastructure.

Existing nucleic acid testing devices generally use sophisticated heating elements such as deposited film heaters and Peltier devices that add significant cost and/or require specialized manufacturing methods. In embodiments of the invention, heating of the reaction solution is  
15 preferably accomplished by use of simple resistive surface-mount devices that may be purchased for pennies or less and are assembled and tested by common manufacturing standards. By layering fluidic chambers over these resistive elements and associated sensor elements, the fluid temperature of the reaction solutions may be conveniently regulated. The broad use of SMD resistors in the electronics industry ensures that the present invention is amenable to well  
20 established quality control methods. Many nucleic acid amplification techniques, such as PCR, require not only rapid heating of the reaction solution but rapid cooling as well. Reaction chambers in the present invention are preferably heated on one side and the ambient temperature across the opposite face is used to help reduce fluid temperature. In addition, vertical orientation of  
embodiments of the device allows for more rapid cooling by passive convection than if the device  
25 was oriented horizontally, thus, reducing the thermal cycle period without the use of costly fans or Peltier devices.

Fluid control is another challenge associated with low-cost nucleic acid test device designs. Devices known in the art generally employ electromechanical, electrokinetic, or piezoelectric pumping mechanisms to manipulate fluids during device operation. These pumping  
30 elements increase both device complexity and cost. Similarly, valves making use of elaborate



micromechanical designs or moving parts can increase fabrication costs and reduce reliability due to complications such as moving part failure or bio-fouling. Unlike previously described nucleic acid testing devices, embodiments of the present invention utilize hydrostatic pressure under microcontroller control together with capillary forces and surface tension to manipulate fluid volumes. The vertical orientation of some embodiments of the present invention allows for the reaction solution to cascade from chamber to chamber under microcontroller control to accommodate required manipulations of the assay. Fluid may be held in individual reaction chambers through a balance of channel size and surface tension, where surface tension prohibits fluid advancement by gas displacement. Sample advances to the lower chamber preferably only after activation of a simple venting mechanism under microcontroller control. Once open, the vent allows fluid to move from a first chamber to a second chamber by means of providing a path for displaced air to escape from the second chamber as fluid enters. Each chamber within the fluidic layer preferably connects to a sealed vent pocket through a narrow vent channel. The vent pocket is preferably sealed on one face with a thin plastic membrane that is easily ruptured by heating a small surface mount resistor underlying the membrane. Once the vent of a lower chamber is opened, fluid advancement proceeds, even under low hydrostatic pressures.

As more specifically described below, the fluidic or microfluidic valve mechanism used in some embodiments of the present invention preferably employs a heating element in thermal and (optional) physical contact with a heat labile seal to enable electronic control of fluid movement by means of venting a chamber of lower elevation to allow a fluid from a chamber of higher elevation to flow into the lower chamber. In one embodiment, a surface mount resistor is mounted on a printed circuit board, using widely used and well-established electronics manufacturing methods, and placed in physical contact with a channel seal composed of heat labile material. When energized the surface mount resistor generates sufficient heat to rupture the seal, which results in the venting of the chamber to lower pressure, such as ambient pressure, thus allowing the movement of fluid from a chamber of higher elevation to a chamber of lower elevation. A direct seal between higher and lower elevation chambers is preferably not employed. The channel and seal may be located remotely from the fluid chambers, thus facilitating fluidic device layout in configurations efficient for manufacture. The seal material may comprise any material that can seal the vent channel and be ruptured from heating as described, for example a thin plastic sheet. This

approach to fluid movement control in the apparatus benefits from low materials costs, suitability for manufacture using established lamination and electronics manufacturing techniques while providing the capacity to move fluids through a series of chambers under the control of electronic control circuits such as microprocessors or microcontrollers. The use of vents, a heat labile  
5 material to seal the vents (and not to seal the fluid chambers or fluid microchannels themselves) and an electronic means of breaking said seal with heat provides a means of controlling fluid flow through the device to enable movement of fluid at predetermined times or following the completion of specific events (for example, attaining a temperature, a temperature change or a series of temperature changes, or the completion of an incubation time or times or other events).

10 In addition, the vent approach has a number of advantages over sealing the fluid chambers themselves. Vent pockets can be located anywhere on the fluidics layout and simply communicate with the chamber they regulate via a vent channel. From a manufacturing standpoint, vent pockets can be localized so that only a single sealing membrane for all vent pockets (which may comprise a vent pocket manifold) is affixed to the fluidic layer, preferably by  
15 well established methods such as adhesives, heat lamination, ultrasonic welding, laser welding etc. In contrast, directly sealing a fluid chamber requires that the seal material be placed at different locations corresponding to each chamber location, which is more difficult to manufacture. This presents a more challenging scenario during manufacture compared to a single vent pocket manifold sealed by a single membrane.

20 In addition, sealing material located at the chamber will likely come into contact with the solution held in the chamber. This requires the use of a material that (i) does not interfere with the reactions conducted in the chamber, and (ii) is not affected by the solution. Given the sensitivity of the biochemical reactions to chemical inhibitors and the elevated temperatures used for both amplification and labeling prior to detection, the list of acceptable materials becomes limited.

25 Furthermore, the physical proximity of heat sensitive material directly associated with reaction chambers used to conduct reactions at elevated temperatures presents a significant challenge to ensure thermal isolation of the valve sealing material from the elevated temperatures employed during the reactions, in addition to preventing the solution from heating up when the sealing material is melted. To avoid valve failure, the heat sensitive material must be remotely located  
30 relative to the heat source or the heat sensitive material must be activated at temperatures well

above the highest temperature employed in the reactions. Remotely locating seals directly associated with chambers in a miniaturized device imposes constraints on fluidics layouts that impede the use of compact physical designs. And the use of higher temperatures to trigger valves located at the reaction chamber site can be deleterious to biochemical components that lose stability slightly above the employed reaction temperatures. Finally, if chambers are directly sealed, melted sealing material can remain in the channels between chambers, occluding flow. The viscosity of the sealing material may require more pressure in the fluid column than is obtained in a miniaturized gravity driven apparatus.

In embodiments of the present invention, reagent mixing requires no more complexity than other systems. Reagents necessary for nucleic acid amplification such as buffers, salts, deoxyribonucleotides, oligonucleotide primers, and enzymes are preferably stably incorporated by use of lyophilized pellets or cakes. These lyophilized reagents, sealed in a fluidic chamber, may be readily solubilized upon contact with aqueous solution. In the case that additional mixing is required, the vertical orientation of embodiments of the present invention offers opportunities for novel methods of mixing solutions. By utilizing heaters underlying fluidic chambers, gas may be heated, delivering bubbles to the reaction solution in the chamber above when the solution contains thermally-sensitive components. Alternatively, heaters may be used to directly heat a solution to the point that boiling occurs, in the case that the solution contains no thermally-sensitive components. The occurrence of air bubbles is often undesirable in previously disclosed fluidic and microfluidic devices, as they may accumulate in fluidic chambers and channels and displace reaction solutions or impede fluid movement within the device. The vertical design of embodiments of the invention presented herein allows bubbles to rise to the fluid surface, resulting in only minimal and transient fluid displacement, effectively ameliorating any disadvantageous impacts of bubbles on the fluidic or microfluidic system. Mixing by boiling is also convenient with this vertical design, as fluid displaced during the process simply returns to the original fluidic chamber by gravity after the heating elements are turned off.

In embodiments of the invention, a colorimetric detection strip is used to detect amplified nucleic acids. Lateral flow assays are commonly used in immuno-assay tests due to their ease of use, reliability, and low cost. The prior art contains descriptions of the use of lateral flow strips for the detection of nucleic acids using porous materials as a sample receiving zone which is at or

near a labeling zone also comprised of a porous material and placed at or near one end of the lateral flow assay device. In these prior inventions labeling moieties are in the labeling zone. The use of porous materials to comprise the sample receiving zone and the labeling zone results in the retention of some sample solution as well as detection particles in the porous materials. Although labeling zones comprising porous materials having reversibly immobilized moieties required for detection may be used in embodiments of the present invention, embodiments of the present invention preferably utilize detection particles or moieties held in a region of the device distinct from the sample receiving zone of the lateral flow strip and comprising nonporous materials with low fluid retention characteristics. This approach allows nucleic acid target containing samples to be labeled prior to introduction to the porous components of the sample receiving end of the lateral flow component of the device and thereby eliminates the retention and/or loss of sample material and detection particles in a porous labeling zone. This method further enables the use of various treatments of the sample in the presence of detection moieties, such as treatment with high temperatures, to accomplish denaturation of a double-stranded target or secondary structures within a single-stranded target without concern for the impacts of temperature on porous sample receiving or labeling zone materials or the lateral flow detection strip materials. Additionally, the use of a labeling zone not in lateral flow contact with the sample receiving zone but subject to the control of fluidic components such as vents or valves allows target and label to remain in contact for periods of time controlled by fluid flow control systems. Thus embodiments of the present invention can be different than traditional lateral flow test strips wherein sample and detection particle interaction times and conditions are determined by the capillary transport properties of the materials. By incorporating the detection particles in a temperature-regulated chamber, denaturation of duplex nucleic acid is possible allowing for efficient hybridization-based detection. In alternative embodiments, fluorescence is used to detect nucleic acid amplification using a combination of LEDs, photodiodes, and optical filters. These optical detection systems can be used to perform real-time nucleic acid detection and quantification during amplification and end-point detection after amplification.

Embodiments of the invention comprise a low cost, point-of-use system is provided wherein a nucleic acid sample may be selectively amplified and detected. Further embodiments include integration with a nucleic acid sample preparation device such as that described in

International Publication No. WO 2009/137059 A1, entitled "Highly Simplified Lateral Flow-Based Nucleic Acid Sample Preparation and Passive Fluid Flow Control". An embodiment of the device preferably comprises both a plastic fluidic component and printed circuit assembly (PCA), and is optionally encased in a housing that protects the active components. Temperature regulation, fluid  
5 and reagent mixing are preferably coordinated by a microcontroller. The reaction cassette is preferably oriented and run vertically so that hydrostatic pressure, capillary forces and surface tension, in conjunction with microcontroller triggered vents, control fluid movement within the device.

Referring to the representative schematic in FIG. 1, a nucleic acid sample is added to  
10 sample chamber **10**. The nucleic acid sample may derive from an online (*i.e.* integrated nucleic acid preparation sub-system), a separate nucleic acid preparation process (such as one of many commercially available methods, *e.g.* spin-columns) followed by addition of the purified nucleic acid to the device by pipette, or an unprocessed nucleic acid containing sample. Preferably already present in the sample chamber, or alternatively added later, is a reagent mix, which may  
15 be in liquid or dry form, containing all components necessary for the amplification reaction, such as buffering agents, salts, dNTPs, rNTPs, oligonucleotide primers, and/or enzymes. In some embodiments the reagent mix is lyophilized to form lyophilized reagents **20**. Introduction of the sample to the sample chamber causes reagents and samples to commingle such that the reagents act upon the sample. An optional bubble-mixing step to further mix the sample with the reagents or  
20 resuspend the reagents may optionally be performed. Fluid is then preferably directed through inlet channel **40** to one or more amplification chamber(s) **30** that reside below the sample chamber when the device is in the vertical orientation. Outlet channel **45** connects amplification chamber **30** to a subsequent chamber. To facilitate multiplexed tests, wherein multiple amplicons are generated, multiplexed amplification can be accomplished by deposition of multiple primer sets  
25 within the amplification chambers. Additionally, circuit board and fluidic designs in which multiple amplification and detection chambers are incorporated into the device support multiple parallel amplification reactions that may be single-plex or multiplex reactions. This approach reduces or eliminates the complications known to those skilled in the art that result from multiplexed amplification using multiple pairs of primers in the same reaction. Moreover, the use of multiple  
30 amplification reaction chambers allows simultaneous amplification under different temperature

regimens to accommodate requirements for optimal amplification, such as different melting or annealing temperatures required for different target and/or primer sequences.

Fluid movement from a first chamber to a second chamber of the device is preferably accomplished by the opening of a vent connected to the second chamber as shown in FIGS. 1-2.

5 One embodiment of the vent comprises two components, vent pocket **50**, one face of which comprises a membrane such as polyolefin and is in contact with a resistor mounted to the printed circuit board assembly (PCA) **75**, and vent channel **60** that connects vent pocket **50** to the microfluidic compartment under its control. When fluid is first added to the system at the sample chamber, the vent, connected to the downstream chamber, is sealed and fluid will not pass  
10 through the channel connecting the two chambers. A microcontroller is responsible for sending electrical current to a heating element, such as resistor **70**, located at or near the membrane that comprises one face of vent pocket **50**. Heat produced by the energized resistor disrupts thin membrane **80**, thus opening the vent. Once open, the vent allows fluid to drop from the first chamber to the second chamber by means of providing a path for displaced air to escape from the  
15 second chamber as fluid enters. Other embodiments of the vent pocket may comprise seals other than a heat-sensitive membrane, and may utilize other methods of breaking the seals, such as puncturing, tearing, or dissolving.

The amplification chamber is preferably in contact with heater elements to provide a means for the temperature regulation necessary to support nucleic acid amplification. In some  
20 embodiments of the invention the amplification chamber may contain oligonucleotides on at least a portion of the interior surface. As shown in FIGS. 1-3, an embodiment of the device comprises inlet channel **30** leading from sample chamber **10** to amplification chamber **30**, outlet channel **45** preferably leading from amplification chamber **30** to labeling chamber **90**, and vent channel **60** leading to vent pocket **50** as described above. At the interface between the amplification chamber  
25 wall **95** and heater element(s) **100** it may be advantageous to place a thermally conductive material such as a thermal grease or compound. A microcontroller modulates current to the preferably resistive heating element(s), preferably by means of metal oxide semiconductor field effect transistors (MOSFETs), based upon data collected from temperature sensor **110**, preferably using simple on/off or proportional integral derivative (PID) temperature control methods or other  
30 algorithmic temperature control known to those skilled in the art.

Existing systems employ active heating and cooling devices located in a reusable instrument to accomplish temperature control for nucleic acid amplification taking place in a disposable cartridge, which necessarily requires an instrument of sufficient precision to be capable of reliably forming reproducible thermal contact with a removable disposable cartridge. This results in increased instrument cost and complexity, as well as reduced reliability of the thermal interface between the temperature control subsystem of the instrument and the fluidic subsystem of the disposable cartridge. Unlike these systems, embodiments of the present invention preferably comprise resistive heating elements for temperature control placed on the disposable portion of the apparatus, such as those illustrated in FIG. 11 and as described above. Placing the heating elements and corresponding temperature sensor(s) on the disposable component enables the manufacture of highly reproducible thermal coupling between the temperature control subsystem and the amplification and detection chambers to which they interface. This approach enables a highly reliable means of coupling the fluidic subsystem to the electronic thermal control subsystem by forming the thermally conductive interface during manufacture. The resulting superior thermal contact between the electronic temperature control components and the fluidic subsystem results in rapid temperature equilibration, and therefore rapid assays.

Embodiments of the amplification chamber preferably comprise materials capable of withstanding repeated heating and cooling to temperatures in the range of approximately 30 °C to approximately 110 °C. Even more preferably, the amplification chamber comprises materials capable of withstanding repeated heating and cooling to temperatures in the range of approximately 30 °C to approximately 110 °C at a rate of temperature change on the order of approximately 10 °C to approximately 50 °C per second. The amplification chamber is preferably capable of maintaining solutions therein at temperatures suitable for either thermal cycling (FIG. 4A-D) or isothermal amplification protocols (FIG. 4E), depending on the programming of the microcontroller. In some nucleic acid amplification applications, it is desirable to provide an initial incubation at an elevated temperature, for example a temperature between approximately 37 °C and approximately 110 °C for a period of 1 second to 5 minutes, to denature the target nucleic acid. Subsequently, the reaction solution is varied in temperature between at least two temperatures including, but not limited to, a temperature that results in nucleic acid duplex denaturation and a temperature suitable to primer annealing by hybridization to the target and

extension of the primer through polymerase catalyzed nucleic acid polymerization. The duration of incubations at each requisite temperature in a thermal cycling regimen may vary with the sequence composition of the target nucleic acid and the composition of the reaction mix, but is preferably between approximately 0.1 seconds and approximately 20 seconds. Repeated heating and cooling is typically performed for approximately 20 cycles to approximately 50 cycles. In 5 embodiments involving isothermal amplification methods, the temperature of the reaction solution is maintained at a constant temperature (in some cases following an initial incubation at an elevated temperature) for between approximately 3 minutes and approximately 90 minutes depending on the amplification technique used. Once the amplification reaction is complete, the 10 amplification reaction solution is transported, by opening the vent that is in communication with the labeling chamber, to the labeling chamber that is located below the amplification chamber.

In some embodiments, additional biochemical reactions may be conducted in the amplification chamber prior to, during, or after the amplification reaction. Such processes may include but are not limited to reverse transcription wherein RNA is transcribed into cDNA, 15 multiplexing wherein multiple primer pairs simultaneously amplify multiple target nucleic acids, and real time amplification wherein amplification products are detected during the amplification reaction process. In the case of the latter, the amplification chamber may not contain a valve or outlet channel, and the amplification chamber would preferably comprise an optical window or otherwise configured to enable interrogation of amplicon concentration during the amplification reaction 20 process. In one real time amplification embodiment, either fluorescently labeled oligonucleotides complementary to the target nucleic acid or fluorescent dyes specific for duplex DNA are monitored for fluorescence intensity by means of an excitation light source such as LEDs or diode laser(s) and a detector such as a photodiode, and appropriate optical components including but not limited to optical filters.

25 In alternative embodiments of the invention, fluid movement is facilitated using resistive heating to expand gasses within the device chambers (FIG. 5). For example, by heating amplification chamber **30**, gas within the chamber expands and will escape through the vented channel, in this case inlet channel **40**, as bubbles **120**. In some embodiments of the invention, such heating of a downstream chamber may be used to generate bubbles sufficient to mix 30 reagents present in the fluid volume of an upstream chamber, such as sample chamber **10**. Once



the heating element is turned off, the gas within amplification chamber **30** will cool and contract, drawing fluid **125** from sample chamber **10** above into amplification chamber **30**. By repeating the process several times, the entire fluid volume may be directed from one chamber to another. In alternative embodiments of the invention, such a mechanism may be used in conjunction with a resistor vent mechanism to displace fluid volumes.

Embodiments of the labeling chamber preferably provide for the specific labeling of amplified target nucleic acids generated in the amplification chamber and works in conjunction with the detection chamber to provide the analytical results of the test. As shown in FIG. 6A, labeling chamber **90** may contain detection particles **130** that are dried, lyophilized, or present on at least a portion of the interior surface as a dried mixture of detection particles in a carrier such as a polysaccharide, detergent, protein or other compound known to those skilled in the art to facilitate resuspension of the detection particles. Labeling chamber preferably is connected to inlet channel **135** leading from amplification chamber **30**, outlet channel **140** leading to the detection chamber, and vent channel **150** leading to a vent pocket as described above. Inlet channel **135** is typically the same channel as outlet channel **45** of amplification chamber **30**. At the interface with the PCA, a thin layer of thermally conductive material such as thermal grease is preferably disposed between one face of the labeling chamber and a resistive heating element.

Suitable detection particles include but are not limited to fluorescent dyes specific for duplex nucleic acid, fluorescently modified oligonucleotides, or oligonucleotide-conjugated dyed microparticles or colloidal gold. Detection of amplicon involves a 'detection oligonucleotide' or other 'detection probe' that is complementary or otherwise able to bind specifically to the amplicon to be detected. Conjugation of a detection oligonucleotide to a microparticle may occur by use of streptavidin coated particles and biotinylated oligonucleotides, or by carbodiimide chemistry whereby carboxylated particles are activated in the presence of carbodiimide and react specifically with primary amines present on the detection oligonucleotide. Conjugation of the detection oligonucleotide to the detectable moiety may occur internally or at the 5' end or the 3' end. Detection oligonucleotides may be attached directly to the microparticle, or more preferably through a spacer moiety such as ethyleneglycol or polynucleotides.

In the case of a duplex DNA amplification product, heating the reaction solution following introduction to the detection chamber facilitates detection. Melting the duplex DNA and then

cooling in the presence of detection oligonucleotide results in the sequence-specific labeling of the amplified target nucleic acid. The resistive element underlying the labeling chamber may be used to heat the fluid volume for approximately 1 to approximately 120 seconds to initiate duplex DNA melting. As the solution is allowed to cool to room temperature, the amplified target nucleic acid  
5 may specifically hybridize to detection microparticles. The reaction volume is then preferably directed to a region of the detection chamber below the labeling chamber by opening the vent of the detection chamber.

For efficient labeling to occur, the solubilized detection particles are preferably well mixed with the reaction solution. In embodiments of the invention, a second mixing method involving  
10 resistive heaters may be employed during labeling to both denature double-stranded nucleic acid target and sufficiently mix detection microparticles in the reaction solution. Heating of the solution in the labeling chamber to above the boiling point may be used to induce turbulence and mixing in solution. Rising bubbles nucleated at the bottom and sides of the chamber by a textured feature such as laser etched line **132**, shown in FIG. 6B (or a series of such lines), preferably effectively  
15 stirs the solution. This effect has been demonstrated to work at many altitudes, independent of corresponding boiling temperature variations. Any solution displaced into upper chambers by boiling preferably flows downstream back into the labeling chamber during subsequent cooling. In some embodiments of the invention, regions of the inner face or the labeling chamber walls may be textured or otherwise treated to localize nucleate boiling to a specific chamber wall or face. In  
20 other embodiments, one or more boiling chips may be placed in the labeling chamber to localize nucleate boiling to a specific point(s).

Embodiments of the detection chamber of the present invention provide for the specific detection of amplified target nucleic acids that have been labeled in the labeling chamber. In certain embodiments of the invention, detection is accomplished by capillary wicking of solution  
25 containing labeled amplicon through an absorbent strip comprised of a porous material (such as cellulose, nitrocellulose, polyethersulfone, polyvinylidene fluoride, nylon, charge-modified nylon, or polytetrafluoroethylene) patterned with lines, dots or other visually discernable elements comprising a binding moiety capable of specifically binding to the labeled amplicon either directly or indirectly. In some embodiments, the absorbent strip component of the device comprises up to  
30 three porous substrates in physical contact: a surfactant pad comprising amphipathic reagents to

enhance wicking, a detection zone comprising a porous material (such as cellulose, nitrocellulose, polyethersulfone, polyvinylidene fluoride, nylon, charge-modified nylon, or polytetrafluoroethylene) to which at least one binding moiety capable of selectively binding labeled amplicon is immobilized, and/or an absorbent pad to provide additional absorbent capacity. Unlike previously  
5 described lateral flow detection devices, detection particles are preferably not incorporated within the lateral flow porous materials in the detection chamber, but are instead held upstream in the labeling chamber where manipulations to substantially enhance the formation of binding complexes between amplicon and detection particles, such as heating/boiling, may be conducted prior to the introduction of the resultant labeled nucleic acids to the porous components of the  
10 device.

A 'capture oligonucleotide' or 'capture probe' is preferably immobilized to the detection strip element of the device by any of a variety of means known to those skilled in the art, such as UV irradiation. The capture probe is designed to capture the labeled nucleic acid as solution containing the labeled nucleic acid wicks through the capture zone resulting in an increased  
15 concentration of label at the site of capture probe immobilization, thus producing a detectable signal indicative of the presence of the labeled target nucleic acid amplicon(s). A single detection strip may be patterned with one or multiple capture probes to enable multiplexed detection of multiple amplicons, determination of amplicon sequence, and assay quality control (positive and negative controls).

20

#### Fluidic Subassembly Layer

Components of embodiments of the fluidic subassembly preferably comprise plastic, such as acrylic, polycarbonate, PETG, polystyrene, polyester, polypropylene, and/or other like materials. These materials are readily available and able to be manufactured by standard methods. As  
25 illustrated in FIGS. 3 and 7, fluidic subassemblies comprise both chambers and channels. Fluidic chambers are comprised of walls, two faces **160**, and connect to one or more channels such as an inlet, an outlet, or a vent. Channels can connect two fluidic chambers, and are comprised of walls and two faces. Fluidic chamber design preferably maximizes the surface area to volume ratio to facilitate heating and cooling. The internal volume of the chamber is preferably between  
30 approximately 1  $\mu$ L and approximately 50  $\mu$ L. The area of chamber face **160** in contact with

solution preferably corresponds with the area to which heating elements are interfaced to ensure uniform fluid temperature during heating. The shape of the fluidic chambers may be selected to mate with heating elements and to provide favorable geometries for solution ingress and egress. In some embodiments, the volume of the chamber may be larger than the fluid volume in order to  
5 provide a space for bubbles that appear during the course of device operation. Fluidic chambers may have enlarged extensions leading to vent channels, to ensure that fluid does not encroach upon the channel by capillary action or otherwise block the venting mechanism. Portions of those chambers to which vent channels communicate may optionally include one or more non-wetting or hydrophobic faces to further reduce fluid invasion into the vent channel.

10 In some embodiments, each fluidic subassembly comprises three laminated plastic sheets, where one component **200** forms the walls of fluidic chambers and two other face components **210**, **220** are laminated to the first to form the faces. Face component **210** may optionally comprise holes **212** for viewing LED indicators **214**. Face component **220** preferably comprises lyophilized reagents **20**, detection particles **130**, and detection strip assembly **230**, and  
15 preferably interfaces with PCB **75** via adhesive shim **222**, which may include membrane with adhesive border **224**. In alternative embodiments, each fluidic subassembly may comprise two plastic components, where one component forms the walls and one face, and the other component is laminated to the first to seal the chamber and form the second face. In embodiments of the present invention, plastic components of the fluidic subassembly may be manufactured by means  
20 of industrial laser- or water-jet cutting, punch or stamp processes, and injection molding.

In some embodiments of the invention the thickness of the fluidic chambers and channel walls are in the range of approximately 0.025 mm to approximately 1 mm, and preferably in the range of approximately 0.1 mm to approximately 0.5 mm. This thickness preferably meets requirements of both structural integrity of the fluidic layer and to support sealing of the closed  
25 chamber under high temperatures and associated pressures. The thickness of channel walls, particularly vent channel walls, are preferably less than that of the chambers and in the range of approximately 0.025 mm to approximately 0.25 mm. The width of inlet and outlet channels is preferably chosen to enhance capillarity. A shallow vent channel imparts improved rigidity to the fluidic layer with no adverse effect on venting to atmospheric pressure. Plastic forming faces of the  
30 fluidic layer is preferably thinner than that forming the walls in order to maximize heat transfer.

Optional thermal breaks **170** cut through some components of the fluidic layer and surround the amplification and detection chambers, contributing to the thermal isolation of the temperature-controlled chambers.

Plastics used in the assembly of the fluidic layer, such as acrylic and polyester, preferably  
5 comprise hydrophobic materials. In embodiments of the invention, components of the fluidic layer may be treated to enhance wettability (*i.e.* decrease hydrophobicity). Such treatments ensure proper fluid control in conjunction with fluidic channel dimensions. In some embodiments, a biocompatible surfactant such as Triton X-100 may be applied to uncoated materials. Plasma discharge treatment is another optional treatment to alter the hydrophobicity of fluid contacting  
10 surfaces.

In some embodiments of the invention, double-sided adhesive film may be used to seal the various components of the fluidic layer. Adhesive film, such as that comprising adhesive shim **222** or membrane **224**, is applied to sides of the interior component in the case of a three component fluidic layer, or to one side in the case of a two component fluidic layer. Before face  
15 component **220** is added to the other layers, additional components of the fluidic layer such as detection strip assembly **230**, detection particles **130** and lyophilized reagents **20** may be incorporated. In some embodiments, the components may be laminated by applying pressure to ensure good adhesion. Adhesives known or found to negatively impact performance of nucleic acid amplification reactions must be avoided. Acrylic- or silicon-based adhesives have been  
20 successfully used in the invention. One preferred adhesive film is SI7876 supplied by Advanced Adhesives Research. Other adhesives may be used if found to be chemically compatible with employed buffers, plastics and reaction chemistries while providing robust sealing over the temperatures encountered during device operation.

Referring to FIG. 2 and 3, vent pockets are preferably differentiated from other chambers  
25 in their construction. After construction of the fluidic layer as described above, vent pockets possess an open face on the side of the fluidic layer that will meet with the PCA layer **75**. To form the vent pocket, an additional plastic component is laminated to seal the chamber, preferably comprising a thin, heat labile membrane **80** with one adhesive face for application to the fluidic layer side adjacent to vent resistor **70** of the PCA. Membrane **80** comprises polyolefin of between  
30 approximately 5  $\mu\text{m}$  and approximately 200  $\mu\text{m}$  thickness, although other similar films may be

used. This thin membrane is well suited to both seal the vent pocket and allow for easy perforation and, thus, venting to the atmosphere when current is passed through the vent resistor generating a rapid temperature increase.

5

#### Additional Components of the Fluidic Layer

As described above, several additional components are preferably incorporated within the fluidic layer of the present invention before final lamination and sealing. Reagents including  
10 buffers, salts, dNTPs, oligonucleotide primers, and enzymes such as DNA polymerase and reverse transcriptase may be lyophilized, or freeze-dried, into pellets or cakes prior device assembly. Reagent lyophilization is well known in the art and involves dehydration of frozen reagent aliquots by sublimation under an applied vacuum. By adding specific formulations of lyoprotectants such as sugars (di- and polysaccharides) and polyalcohols to the reagents prior to  
15 freezing, the activity of enzymes may be preserved and the rate of rehydration may be increased. Lyophilized reagent pellets or cakes are manufactured by standard methods and, once formed, are reasonably durable and may be easily placed into specific chambers of the fluidic layer prior to laminating the final face.

In some embodiments of the invention, detection microparticles are another additional  
20 component of the fluidic layer. In some embodiments, these microparticles may be lyophilized as described for the reaction reagents above. In other embodiments, microparticles in liquid buffer may be directly applied to an interior face of a fluidic chamber and dried before sealing. The liquid buffer containing the microparticles preferably also comprises sugars or polyalcohols that aid in rehydration. Incorporation of microparticles in aqueous buffer directly into the fluidic layer prior to  
25 drying may simplify and reduce the final cost of manufacturing, and may require heating or nucleate boiling as described above to both adequately mix the microparticles with the reaction solution, and to denature double-stranded nucleic acid product for hybridization to the detection particles.

In some embodiments of the present invention, a lateral flow detection strip assembly is  
30 also incorporated into the fluidic layer. The detection strip preferably comprises a membrane

assembly comprised of at least one porous component and optionally may comprise an absorbent pad, a detection membrane, a surfactant pad, and a backing film. The detection membrane is made of nitrocellulose, cellulose, polyethersulfone, polyvinylidene fluoride, nylon, charge-modified nylon, or polytetrafluoroethylene and may be backed with a plastic film. As described above, capture probe may be deposited and irreversibly immobilized on the detection membrane in lines, spots, or any pattern that can be visualized by the unaided human eye. Deposited oligonucleotides may be permanently immobilized by UV-irradiation of the detection membrane following capture probe deposition. The surfactant pad may comprise a porous substrate, preferably with minimal nucleic acid binding and fluid retention properties, that permits unobstructed migration of the nucleic acid product and detection microparticles. The surfactant pad may comprise materials such as glass fiber, cellulose, or polyester. In embodiments of the invention, formulations including at least one amphipathic reagent are dried on the surfactant pad to allow uniform migration of sample through the detection membrane. The absorbent pad may comprise any absorbent material, and helps to induce sample wicking through the detection membrane assembly. Using an adhesive backing film, such as a double-sided adhesive film as a base, the detection membrane component is assembled by first placing the detection membrane, followed by optional absorbent pad and/or surfactant pad in physical contact with the detection membrane with between approximately 1 mm and approximately 2 mm overlap.

#### 20 Electronic Subassembly Layer

In some embodiments, the printed circuit board (PCB) comprises a standard 0.062 inch thick FR4 copper clad laminate material, although other standard board materials and thicknesses may be used. Electronic components such as resistors, thermistors, LEDs, and the microcontroller preferably comprise off-the-shelf surface mount devices (SMDs) and are placed according to industry standard methodology.

In alternative embodiments, the PCA could be integrated with the cassette wall and comprise a flexible plastic circuit. Flex circuit materials such as PET and polyimide may be used. The use of flexible plastic circuitry is well known in the art. In another embodiment, heating elements and temperature sensors may be screen printed onto the plastic fluidic layer with technology developed by companies such as Soligie, Inc.

In some embodiments of the invention, the PCB thickness as well as the amount and placement of copper in regions surrounding the resistive heaters are tailored for thermal management of the reaction solution in the fluidic layer. This can be accomplished by use of standard manufacturing techniques already mentioned.

5 Example resistor heater assemblies are shown in FIG. 2 and FIG. 3. In some embodiments of the invention, the resistor is a thick film 2512 package, although other resistors may be used. Heating chambers in the fluidic layer are preferentially of dimensions similar to those of the resistor to ensure uniform heating throughout the chamber. A single resistor of this size is sufficient to heat approximately 15  $\mu$ L of solution, assuming a fluidic layer thickness of 0.5 mm.

10 The schematic in FIG. 3 shows two resistors **100** forming a heater sufficient to heat approximately 30  $\mu$ L of solution, assuming a fluidic layer thickness of 0.5 mm. In this case, the resistors are preferably 40 ohm each and arranged in a parallel configuration.

In some embodiments of the invention, temperature sensor **110** preferably comprises a, thermistor, such as a 0402 NTC device, which has a height similar to that of the 2512 resistor

15 package. The thermistor is preferably aligned either adjacent to or in between the resistor heaters in the case of a one resistor or two resistor set-up, respectively; see for example FIG. 8. By closely aligning these electronic elements, only a very thin air gap results between them. Furthermore, application of a thermal compound before assembling the fluidic with the electronic layer ensures good thermal contact between the fluidic layer, resistor, and thermistor.

20 In some embodiments of the invention, vent resistor **70** is a thick film 0805 package, although similar resistors may be used.

In some embodiments of the invention, the microcontroller is an AVR Atmega32. The microcontroller is preferably matched to the complexity of the fluidic system. For example, with multiplexing, the number of individual vents and heaters is commensurate with the number of

25 microcontroller I/O lines. Memory size can be chosen to accommodate program size.

In certain embodiments of the invention, N-channel MOSFETs in the SOT-23 package operating in an ON-OFF mode are used to modulate current load to vent and heater resistors. Modulation signals are sent via the microcontroller. In alternative embodiments, a pulse-width-modulation scheme and/or other control algorithms could be used for more advanced thermal



management of fluidics. This would typically be handled by the microcontroller and may require additional hardware and/or software features known to those skilled in the art.

#### Final Device Assembly

5           The final assembly of fluidic, electronic, and housing components into a finished device preferably begins by lamination of the fluidic layer(s) **250** and electronic layer **75** to ensure good thermal contact between PCA heating elements and chambers and/or pockets present in the fluidic layer. As shown in FIG. 9, adhesive shim **222** both binds the two layers together and ensures level contact between the flat fluidic layer and the topographically raised electronic  
10 components present on the PCA. Thermal compound or grease may be placed on heating elements before lamination to further improve thermal contact. After assembly of the fluidic and electronic layers, a protective plastic housing may be affixed to result in the final device.

          Depending on the application, different embodiments of the invention may be of the most utility. Some embodiments comprises a device in which a small controlling base unit operates a  
15 smaller disposable unit containing the nucleic acid amplification and detection systems. This particular embodiment helps to reduce cost of an individual diagnostic test. A representative device designed for this purpose is shown in FIG. 10. As described above, the electronic functions of such a device is preferably split into two separate subassemblies. Disposable subassembly **260** comprises pin connector **270** or other similar electronic connector and the low-cost electronic  
20 components such as amplification chamber heating elements **100**, labeling chamber heating elements **265**, vent heating elements **70**, temperature sensors such as thermistors, and optional LED indicators **214**, including those components which directly interact with the fluidic system components. Connector **270** preferably provides current to the resistive heaters along with a power and signal line to the thermistor(s). Reusable subassembly or base unit **280** preferably  
25 incorporates reusable components such as the microcontroller, MOSFETs, switches, power supply or a power jack **275** and/or battery, optional cooling fan, optional user interface, and connector **272** compatible with connector **270** of disposable subassembly **260**. When the subassemblies are mated via connectors **270** and **272**, base unit **280** preferably supports disposable subassembly **260** in a substantially vertical or near-vertical orientation. Although a substantially vertical  
30 orientation is preferable in some of the embodiments described herein, similar results may be

obtained if the device is operated at a tilt, especially if certain pathways are coated to reduce the wetting angle of solutions used.

Another embodiment comprises a device in which the entire assembly is disposable, as shown in FIG. 11. In this embodiment, there is only a single electronic assembly that is powered by 5 9-volt battery **305** preferably attached to the backside of the device via terminals **307**, as shown in FIG. 11C. Microcontroller **300**, power conditioning circuitry **302**, and MOSFETs **310** are preferably also located on the back of the device, shown in FIG. 11B, whereas the opposing side, which is in contact with the fluidic layer and shown in FIG. 11A, comprises amplification chamber heating elements **100**, labeling chamber heating elements **265**, vent heating elements **70**, and temperature 10 sensors. The device depicted in FIG.11 is designed to incorporate chambers and other components required to conduct two reactions amplification and labeling reactions in parallel for multiplexing applications. This particular embodiment is ideal for applications where testing is performed in remote locations. The device may alternatively be powered by a wall adaptor or another battery or batteries with sufficient capacity.

15 In order to provide a complete sample-to-result molecular test, either of the above embodiments of the invention may be interfaced to a sample preparation system **320** that provides nucleic acids as output to sample chamber **10** via channel **325**. This has been demonstrated using the sample preparation technology described in International Publication No. WO 2009/137059 A1, entitled "Highly Simplified Lateral Flow-Based Nucleic Acid Sample Preparation and Passive Fluid 20 Flow Control". An embodiment of the resulting integrated device is illustrated in FIG. 12.

#### Fluidic Subassembly With Multiple Wall Components

In some embodiments, such as that illustrated in FIG. 7, the fluidic subassembly may comprise three laminated plastic sheets, where one sheet forms the walls of fluidic chambers and 25 the other two components are laminated to the first to form the faces. In alternative embodiments, the fluidic subassembly may comprise two plastic components, where one component forms the walls and one face, and the other component is laminated to the first to seal the chamber and form the second face. In embodiments of the present invention, plastic components of the fluidic subassembly may be manufactured by means of industrial laser- or water-jet cutting, punch or 30 stamp processes, and injection molding. In other alternative embodiments the fluidic subassembly

may comprise laminated layers such that the detection chamber is situated in a separate layer of the device so that it is disposed in front of a layer comprising the amplification and labeling chambers. This physical configuration reduces the width of the device while also imparting additional functionality. Specifically, this embodiment places a detection strip in the detection chamber such that it is situated over the labeling chamber, allowing the heater elements underlying the labeling chamber to be used (during the detection step of an assay) for the control of temperature in the detection chamber. Control of detection chamber temperature enables the use of elevated temperatures during hybridization to the detection strip. Temperature mediated modulation of hybridization stringency during hybridization based detection can be used to achieve enhanced hybridization specificity which is of utility, for example, in the discrimination of closely related nucleic acid sequences (e.g. single nucleotide polymorphisms).

FIG.13 shows the components of a multilayer fluidic cassette assembly **290** of an embodiment of the present invention such as that described immediately above. First wall component **300** comprises detection chamber **302** for accommodating detection strip **305** and portion **304** of the sample chamber. Second wall component **310** comprises another portion **306** of the sample chamber, amplification chamber **314**, labeling chamber **316**, vent pockets **318**, and corresponding channels. Three face components **330**, **335**, **340** form the chambers, pockets and channels. Face component **335** acts as the rear face of wall component **300** and the front face of wall component **310** and comprises opening **303** for forming the sample chamber and opening **345** for the solution comprising labeled target nucleic acids to transfer from labeling chamber **316** to detection chamber **302**. The component layers are preferably bonded together with a silicone transfer adhesive. Interior surfaces are preferably treated to control wetting. Reagents, the lateral flow assembly comprising detection strip **305**, and heat-fusible thermoplastic vent valves **320** are preferably added during fabrication. An adhesive membrane is preferably sealed over the vent pockets.

FIG. 14 shows an exploded view of a disposable assay cartridge **350** that incorporates the fluidic layer **290** of FIG. 13. Assay cartridge **350** comprises front shell, micro-fluidic cassette assembly **290**, bonding tape **360**, circuit board **370**, and rear shell **380**. FIG. 15 is an illustration of disposable PCA/fluidic assembly **350** in place in docking station **400**. Sample is added to the sample chamber via sample port **390**. The docking station preferably contains the control

electronics and power supply and may include buttons to initiate electronic processes required for the assay.

EXAMPLE 1: Method of Amplification and Detection of a Target Nucleic Acid for the Diagnosis of

5 Candidatus Liberibacter Infection in Citrus

An embodiment of the invention wherein a disposable component interfaces with a reusable dock was employed to test citrus leaf tissue for the presence of *Candidatus Liberibacter asiaticus*, the etiologic agent of citrus greening.

A partially disposable device as described above was constructed. The reusable unit  
10 comprised a standard 1.5 oz copper-clad PCB. Circuit components included an ATmega328 microcontroller, 0.5 Amp N-channel MOSFETs, SMD resistors, and power conditioning components. A stereolithography (SLA) formed plastic shell covered the board and tactile switches. A female pin connector was mounted to the top surface to allow for a vertical connection to the disposable PCA. The disposable PCA comprised a similar PCB along with thick-film  
15 resistors, 0402 thermistor, and 0603 LEDs. A right angle male pin connector was placed one end of the board to allow for vertical orientation when inserted into the female socket of the reusable unit.

The fluidic layer comprised two face components, a wall component, and a thin membrane. Face components were made from 0.004" polyester (PET). The wall components were  
20 made from 0.5 mm acrylic that was laminated with 0.002" silicone transfer film from Advanced Adhesives, Inc. The vent membrane was made with 0.0005" polyolefin with 0.004" solvent resistant acrylic adhesive from 3M, Inc. Individual components were cut to shape using a Universal Laser Systems, Inc. VersaLaser 3.5 laser cutter. Prior to assembly, all laser cut plastic fluidic components, except the membrane component, were placed in a sonicator bath containing 100  
25 mM sodium hydroxide and 0.1% sodium dodecyl sulfate, and sonicated for 30 minutes to remove any debris, contaminating nucleic acids, or nucleases. The cleaned plastic components were finally washed with nuclease-free water. The wall and face components (PCA-oriented) were first laminated by applying 5000 psi pressure. Detection oligonucleotide conjugated polystyrene beads in 500 mM sucrose were deposited into the labeling chamber and dried under vacuum. After  
30 drying, a piece of double sided tape was placed into the detection chamber and the detection

membrane component was assembled using a nitrocellulose membrane strip, an Accuflow-P surfactant pad, and blotting paper to serve as an absorbent pad. In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. Finally, the fluidic layer was sealed with the other face component, and the vent membrane component was laminated to seal the vent pockets. Silicone thermal compound (Radio Shack, Inc.) was lightly applied to amplification and labeling resistors, and the fluidic and electronic layers were laminated using an adhesive shim.

After completion of device assembly, 28  $\mu\text{L}$  of a reaction mixture was added to the sample chamber. Depending on the experiment, enzymes required for amplification were either added to this reaction mixture in liquid form (FIG. 16A), or present in a lyophilized cake incorporated into the sample chamber of the fluidic layer (FIG. 16B). In both cases, the nucleic acid template used was extracted from infected plant tissue using a QIAshredder and spin column kit (Qiagen, Inc.). The primers *hyvl\_For* and *hyvl\_Rev* were used to amplify a 139 bp nucleic acid sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus Liberibacter asiaticus*. Proprietary amplification reaction chemistry was performed using a premade amplification buffer (10X) comprising 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate, 100 mM potassium chloride, and 0.25% Triton X-100. Each twenty  $\mu\text{L}$  of reaction solution contained:

9.4  $\mu\text{L}$  water  
2.0  $\mu\text{L}$  10x amplification Buffer  
2.0  $\mu\text{L}$  DMSO  
0.4  $\mu\text{L}$  potassium chloride (2 M)  
0.5  $\mu\text{L}$  magnesium chloride (100 mM)  
0.5  $\mu\text{L}$  dithiothreitol (100 mM)  
0.5  $\mu\text{L}$  dNTPs (10 mM)  
2.0  $\mu\text{L}$  Primer set *hyvl\_For* and *hyvl\_Rev* (8  $\mu\text{M}$  each)  
0.5  $\mu\text{L}$  VentR (exo-) DNA Polymerase (2 U/  $\mu\text{L}$ )\*  
0.2  $\mu\text{L}$  Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu\text{g}/\text{mL}$ )\*  
2.0  $\mu\text{L}$  of DNA extracted from healthy or *C. Liberibacter* infected tissue (17.2 ng/  $\mu\text{L}$ )

\*either included in solution, or in the case that lyophilized enzyme was used, substituted by water

Venting of the amplification chamber and initiation of the amplification and detection program was accomplished by pressing a tactile switch on the reusable unit that serves as a start button. After venting, the reaction solution entered the amplification chamber where the solution was heated to 85 °C for 2 minutes, followed by 40 cycles of: 76 °C for 10 seconds and 60 °C for 25  
5 seconds. After thermal cycling was complete, the reaction was allowed to flow into the labeling chamber by microcontroller initiated venting. The labeling chamber contained blue dyed polystyrene detection microspheres dried to one interior face of the labeling chamber in the presence of 500 mM sucrose. The detection oligonucleotide conjugated to the dyed microspheres was complementary to the sense strand of the nucleic acid amplification product. The labeling  
10 chamber was heated to 105 °C for 2 minutes and then maintained at 90 °C for 30 seconds to induce boiling and thorough mixing of the polystyrene beads and denature the double-stranded DNA product. After heating, reaction solution in the labeling chamber was allowed to cool for two minutes. The detection chamber was vented, causing the solution to flow from the labeling chamber to the detection chamber and onto the detection strip assembly. Three capture lines were  
15 immobilized on the lateral flow membrane; from the bottom of the device they were: a negative control oligonucleotide not complementary to any assayed targets; capture probe complementary to the amplification product; and a positive control oligonucleotide complementary to the detection probe. As can clearly be seen in FIG. 16, the invention successfully amplified and detected the target nucleic acid with no detectable cross-hybridization to the negative control line.

20 The sequences of the amplification primers used were:

hyvI\_For

ggccgtttta acacaaaaga tgaatatcat agatggggta gtcaa (SEQ ID NO 1)

hyvI\_Rev

cggccatttt agataaatca attgttcta gtttagatac atcaattgt t (SEQ ID NO 2)

25

The sequences for capture and detection oligonucleotides used were:

Capture

tcgttgagt agctagatcc nnnnnnnnnn nt (SEQ ID NO 3)

Detection

30 /5AmMC12/ aattgatgga tgacgtgata gtttagcacc aacatctt/3Phos/ (SEQ ID NO 4)

A more complete description of the amplification process may be found in commonly owned U.S. Provisional Patent Application Serial No. 61/477,437, entitled " Oscillating Amplification Reaction for Nucleic Acids", incorporated herein by reference. This process enables the use of larger solution volumes with higher sensitivities, and doesn't require active cooling to perform thermal cycling. Because the process requires only passive cooling, a narrow cycling temperature range, and isn't substantially affected by looser temperature tolerances than those typically used in PCR, simple resistive heating elements may be used, thus enabling the device to be compact and inexpensive. Furthermore, superior thermal coupling is achievable because the amplification chamber is preferably flat on the side adjacent to the heating resistor, thus providing good thermal contact. This thermal interface may be enhanced by the use of a thermally conductive adhesive compound.

EXAMPLE 2: Method of Isolation, Amplification, and Detection of a Target Nucleic Acid for the Diagnosis of *Candidatus Liberibacter* Infection in Citrus

A partially disposable device as described above was constructed. The reusable unit comprised a standard 1.5 oz copper-clad PCB. Circuit components included an ATmega328 microcontroller, 0.5 Amp N-channel MOSFETs, SMD resistors, and power conditioning components. A stereolithography (SLA) formed plastic shell covers the board and tactile switches. A female pin connector was mounted to the top surface to allow for a vertical connection to the disposable PCA. The disposable PCA comprised a similar PCB along with thick-film resistors, 0402 thermistor, and 0603 LEDs. A right angle male pin connector was placed at one end of the board to provide vertical orientation when inserted into the female socket of the reusable unit.

The fluidic layer comprised two face components, a wall component, and a thin membrane. Face components comprised 0.004" polyester. The wall components comprised 0.5 mm acrylic that was laminated with 0.002" silicone transfer film from Advanced Adhesives, Inc. To accommodate the integration of the fluidic layer with the sample preparation sub-system, the wall and face components were fabricated to provide an opening and channel situated such that, when laminated to the sample preparation sub-system, purified nucleic acids would be communicated into the sample chamber of the invention during the elution phase of the sample preparation

process. The vent membrane was made with 0.0005" polyolefin with 0.004" solvent resistant acrylic adhesive from 3M, Inc. Individual components were cut to shape using a Universal Laser Systems, Inc. VersaLaser 3.5 laser cutter. Prior to assembly, all laser cut plastic fluidic components, except the membrane component, were placed in a sonicator bath containing 100 mM sodium hydroxide and 0.1% sodiumdodecyl sulfate, and sonicated for 30 minutes to remove any debris, contaminating nucleic acids, or nucleases. The cleaned plastic components were finally washed with nuclease-free water. The wall and face components (PCA-oriented) were first laminated by applying 5000 psi pressure. Detection oligonucleotide conjugated polystyrene beads in 500 mM sucrose were deposited into the labeling chamber and dried under vacuum. After drying, a piece of double sided tape was placed into the detection chamber and the detection membrane component was assembled using a nitrocellulose membrane strip, an Accuflow-P surfactant pad, and blotting paper to serve as an absorbent pad. In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. Finally, the fluidic layer was sealed with the other face component, and the vent membrane component was laminated to seal the vent pockets. Silicone thermal compound (Radio Shack, Inc.) was lightly applied to amplification and labeling resistors, and the fluidic and electronic layers were laminated using an adhesive shim.

The sample preparation sub-system, to which the fluidic layer of the invention was interfaced, was fabricated from laser cut acrylic laminated to form buffer reservoirs and physical supports for absorbent material components of the sub-system. A passive buffer exchange structure was cut in a geometry described in International Publication No. WO 2009/137059 A1, entitled "Highly Simplified Lateral Flow-Based Nucleic Acid Sample Preparation and Passive Fluid Flow Control". Nonwoven nylon was used as the buffer exchanger material. Whatman GF/B glass fiber filter was employed as the nucleic acid affinity matrix. Cotton gauze was used as an absorbent pad to provide an absorbent sink of suitable capacity.

Plant tissue, specifically four 1.5 mm biopsy punches collected from citrus leaf midrib near the petiole, was briefly ground in a microcentrifuge tube in 150  $\mu$ L of extract buffer (4M guanidinium thiocyanate, 25 mM tris, pH 6.4). The resulting crude extract was introduced to the sample reservoir of the sample preparation sub-system immediately following the addition of 200  $\mu$ L of wash buffer 1 (2M guanidinium thiocyanate, 30% ethanol, 25 mM tris, pH 7.4) and 800  $\mu$ L



wash buffer 2 (400 mM NaCl, 50% ethanol, 50 mM tris, pH 6.4) to their respective reservoirs. 15 minutes following the addition of sample, the nucleic acid binding matrix of the sample preparation component was “punched” into the underlying elution chamber and nucleic acids were eluted with 50  $\mu$ L of reaction buffer. Punch-through and reaction buffer injection was accomplished by pushing a 1 cc tuberculin syringe (without needle) through the hole overlying the affinity matrix to displace the matrix into the underlying elution chamber. The elution chamber was connected to the sample chamber of the invention by a channel in the specially designed fluidic layer. Depressing the syringe plunger resulted in elution of captured nucleic acid, which flowed through said channel into the sample chamber.

10 With the exception of the enzymes, the elution buffer contained all reagents necessary for target amplification by a proprietary amplification technique, including the primers *hyvl\_For* and *hyvl\_Rev*, which selectively amplify a 139 bp sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus Liberibacter asiaticus*. Amplification buffer (10X) was premade and contained 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate, 100 mM potassium chloride, and 0.25% Triton X-100. Twenty  $\mu$ L of elution buffer contained:

12.1  $\mu$ L water  
2.0  $\mu$ L 10x amplification Buffer  
2.0  $\mu$ L DMSO  
0.4  $\mu$ L potassium chloride (2 M)  
20 0.5  $\mu$ L magnesium chloride (100 mM)  
0.5  $\mu$ L dithiothreitol (100 mM)  
0.5  $\mu$ L dNTPs (10 mM)  
2.0  $\mu$ L Primer set *hyvl\_For* and *hyvl\_Rev* (8  $\mu$ M each)

Prior to energizing the device, the following enzymes were added to the eluted nucleic acid sample in the sample chamber and briefly mixed using a gel-loading pipette tip.

25 1.0  $\mu$ L VentR (exo-) DNA Polymerase (2 U/  $\mu$ L)  
0.4  $\mu$ L Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu$ g/mL)  
Venting of the amplification chamber and initiation of the amplification and detection program was accomplished by pressing a tactile switch on the reusable unit that serves as a start  
30 button. After venting, the reaction solution entered the amplification chamber where the solution

was heated to 85 °C for 2 minutes, followed by 40 cycles of: 76 °C for 10 seconds and 60 °C for 25 seconds. After thermal cycling was complete, the reaction was allowed to flow into the labeling chamber by microcontroller initiated venting. The labeling chamber contained blue dyed polystyrene detection microspheres dried to one interior face of the labeling chamber in the presence of 500 mM sucrose. The detection oligonucleotide conjugated to the dyed microspheres was complementary to the sense strand of the nucleic acid amplification product. The labeling chamber was heated to 105 °C for 2 minutes and then maintained at 90 °C for 30 seconds to induce boiling and thorough mixing of the polystyrene beads and denature the double-stranded DNA product. After heating, reaction solution in the labeling chamber was allowed to cool for two minutes. The detection chamber was vented, causing the solution to flow from the labeling chamber to the detection chamber and onto the detection strip assembly. Three capture lines were immobilized on the lateral flow membrane, from the bottom of the device they were: A negative control oligonucleotide not complementary to any assayed targets; capture probe complementary to the amplification product; and a positive control oligonucleotide complementary to the detection probe. As can clearly be seen in FIG. 14, the fully integrated device resulted in successful nucleic acid isolation, amplification and detection of the target nucleic acid.

EXAMPLE 3: Method of Amplification and Detection of a Target Nucleic Acid for the Diagnosis of *Candidatus Liberibacter* Infection in Citrus

An embodiment of the invention wherein a disposable component interfaces with a reusable dock was employed to test crude citrus leaf tissue extracts for the presence of *Candidatus Liberibacter asiaticus*, the etiologic agent of citrus greening without a preceding nucleic acid isolation step.

A partially disposable device as described above was constructed. The reusable unit comprised a standard 1.5 oz copper-clad PCB. Circuit components included an ATmega328 microcontroller, 0.5 Amp N-channel MOSFETs, SMD resistors, and power conditioning components. A stereolithography (SLA) formed plastic shell covered the board and tactile switches. A female pin connector was mounted to the top surface to allow for a vertical connection to the disposable PCA. The disposable PCA comprised a similar PCB along with thick-film resistors, 0402 thermistor, and 0603 LEDs. A right angle male pin connector was placed one end

of the board to allow for vertical orientation when inserted into the female socket of the reusable unit.

The fluidic layer comprised two face components, a wall component, and a thin membrane. Face components were made from 0.004" polyester. The wall components were made from 0.5 mm acrylic that was laminated with 0.002" silicone transfer film from Advanced Adhesives, Inc. The vent membrane comprised 0.0005" polyolefin with 0.004" solvent resistant acrylic adhesive from 3M, Inc. Individual components were cut to shape using a Universal Laser Systems, Inc. VersaLaser 3.5 laser cutter. Prior to assembly, all laser cut plastic fluidic components, except the membrane component, were placed in a sonicator bath containing 100 mM sodium hydroxide and 0.1% sodiumdodecyl sulfate, and sonicated for 30 minutes to remove any debris, contaminating nucleic acids, or nucleases. The cleaned plastic components were finally washed with nuclease-free water. The wall and face components (PCA-oriented) were first laminated by applying 5000 psi pressure. Detection oligonucleotide conjugated polystyrene beads in 500 mM sucrose were deposited into the labeling chamber and dried under vacuum. After drying, a piece of double sided tape was placed into the detection chamber and the detection membrane component was assembled using a nitrocellulose membrane strip, an Accuflow-P surfactant pad, and blotting paper to serve as an absorbent pad. In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. Finally, the fluidic layer was sealed with the other face component, and the vent membrane component was laminated to seal the vent pockets. Silicone thermal compound (Radio Shack, Inc.) was lightly applied to amplification and labeling resistors, and the fluidic and electronic layers were laminated using an adhesive shim.

After completion of device assembly, 40  $\mu$ L of a reaction mixture was added to the sample chamber. Depending on the experiment, enzymes required for amplification were either present in this reaction mixture in liquid form, or present in a lyophilized cake incorporated into the sample chamber of the fluidic layer. In both cases, the assayed specimen was comprised of 4  $\mu$ L of crude citrus tissue extract prepared by crushing 5 biopsy punches of 1.5 mm diameter in 500  $\mu$ L of nuclease free water. The primers *hyvI\_For* and *hyvI\_Rev* were used to amplify a 139 bp nucleic acid sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus Liberibacter asiaticus*. Proprietary amplification reaction chemistry was performed. Amplification buffer (10X)

was premade and contained 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate, 100 mM potassium chloride, and 0.25% Triton X-100. Each forty  $\mu$ L of reaction solution contained:

- 18.8  $\mu$ L water
- 4.0  $\mu$ L 10x amplification buffer
- 5 4.0  $\mu$ L DMSO
- 0.8  $\mu$ L potassium chloride (2 M)
- 1.0  $\mu$ L magnesium chloride (100 mM)
- 1.0  $\mu$ L dithiothreitol (100 mM)
- 1.0  $\mu$ L dNTPs (10 mM)
- 10 4.0  $\mu$ L Primer set *hyvI*\_For and *hyvI*\_Rev (8  $\mu$ M each)
- 1.0  $\mu$ L VentR (exo-) DNA Polymerase (2 U/  $\mu$ L)\*
- 0.4  $\mu$ L Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu$ g/mL)\*
- 4.0  $\mu$ L of citrus tissue extract generated by briefly crushing 5 biopsy punches (1.5 mm diameter obtained from citrus leaf petiole or the midrib proximal to the petiole of either healthy or
- 15 *C. Liberibacter* infected citrus trees) or a ~4 mm length of petiole tissue in 500  $\mu$ L of nuclease free water

\*either included in solution, or in the case that lyophilized enzyme was used, substituted by water

- Venting of the amplification chamber and initiation of the amplification and detection
- 20 program was accomplished by pressing a tactile switch on the reusable unit that serves as a start button. After venting, the reaction solution entered the amplification chamber where the solution was heated to 85 °C for 2 minutes, followed by 40 cycles of: 76 °C for 10 seconds and 60 °C for 25 seconds. After thermal cycling was complete, the reaction was allowed to flow into the labeling chamber by microcontroller-initiated venting. The labeling chamber contained blue dyed
  - 25 polystyrene detection microspheres dried to one interior face of the labeling chamber in the presence of 500 mM sucrose. The detection oligonucleotide conjugated to the dyed microspheres was complementary to the sense strand of the nucleic acid amplification product. The labeling chamber was heated to 105 °C for 2 minutes and then maintained at 90 °C for 30 seconds to induce boiling and thorough mixing of the polystyrene beads and denature the double-stranded
  - 30 DNA product. After heating, reaction solution in the labeling chamber was allowed to cool for two

minutes. The detection chamber was vented, causing the solution to flow from the labeling chamber to the detection chamber and onto the detection strip assembly. Three capture lines were immobilized on the lateral flow membrane, from the bottom of the device they were: A negative control oligonucleotide not complementary to any assayed targets; capture probe complementary to the amplification product; and a positive control oligonucleotide complementary to the detection probe. The invention successfully amplified and detected the target nucleic acid with no detectable cross-hybridization to the negative control lines of the detection strip.

10 EXAMPLE 4: Method of Amplification and Detection of a Target Nucleic Acid for the Detection of *Candidatus Liberibacter* in the Asian Citrus Psyllid *Diaphorina citri* Kuwayama

An embodiment of the invention wherein a disposable component interfaces with a reusable dock was fabricated as described in Example 3 and employed to test crude whole insect extracts prepared from *Diaphorina citri* Kuwayama for the presence of *Candidatus Liberibacter asiaticus*, the etiologic agent of citrus greening without a preceding nucleic acid isolation step.

15 In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. After completion of device assembly, 40  $\mu$ L of a reaction mixture was added to the sample chamber. Depending on the experiment, enzymes required for amplification were either present in this reaction mixture in liquid form, or present in a lyophilized cake incorporated into the sample chamber of the fluidic layer. In both cases, the sample was  
20 comprised of 4  $\mu$ L of a solution prepared by crushing 5 whole live *Diaphorina citri* Kuwayama in 500  $\mu$ L of nuclease free water. The primers *hyvI*\_For and *hyvI*\_Rev were used to amplify a 139 bp nucleic acid sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus Liberibacter asiaticus*. Proprietary amplification reaction chemistry was performed. Amplification buffer (10X) was premade and contained 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate,  
25 100 mM potassium chloride, and 0.25% Triton X-100. Each forty  $\mu$ L of reaction solution contained:

18.8  $\mu$ L water  
4.0  $\mu$ L 10x amplification buffer  
4.0  $\mu$ L DMSO  
30 0.8  $\mu$ L potassium chloride (2 M)

- 1.0  $\mu$ L magnesium chloride (100 mM)
- 1.0  $\mu$ L dithiothreitol (100 mM)
- 1.0  $\mu$ L dNTPs (10 mM)
- 4.0  $\mu$ L Primer set *hyvl\_For* and *hyvl\_Rev* (8  $\mu$ M each)
- 5 1.0  $\mu$ L VentR (exo-) DNA Polymerase (2 U/  $\mu$ L)\*
- 0.4  $\mu$ L Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu$ g/mL)\*
- 4.0  $\mu$ L of whole *Diaphorina citri* Kuwayama extract generated by briefly crushing 5 whole insects in 500  $\mu$ L of nuclease free water

\*either included in solution, or in the case that lyophilized enzyme was used,  
10 substituted by water

Venting of the amplification chamber and initiation of the amplification and detection program was accomplished by pressing a tactile switch on the reusable unit that serves as a start button. After venting, the reaction solution entered the amplification chamber where the solution was heated to 85 °C for 2 minutes, followed by 40 cycles of: 76 °C for 10 seconds and 60 °C for 25  
15 seconds. After thermal cycling was complete, the reaction was allowed to flow into the labeling chamber by microcontroller-initiated venting. The labeling chamber contained blue dyed polystyrene detection microspheres dried to one interior face of the labeling chamber in the presence of 500 mM sucrose. The detection oligonucleotide conjugated to the dyed microspheres was complementary to the sense strand of the nucleic acid amplification product. The labeling  
20 chamber was heated to 105 °C for 2 minutes and then maintained at 90 °C for 30 seconds to induce boiling and thorough mixing of the polystyrene beads and denature the double-stranded DNA product. After heating, reaction solution in the labeling chamber was allowed to cool for two minutes. The detection chamber was vented, causing the solution to flow from the labeling  
25 chamber to the detection chamber and onto the detection strip assembly. Three capture lines were immobilized on the lateral flow membrane, from the bottom of the device they were: A negative control oligonucleotide not complementary to any assayed targets; capture probe complementary to the amplification product; and a positive control oligonucleotide complementary to the detection probe. The invention successfully amplified and detected the target nucleic acid with no detectable cross-hybridization to the negative control lines of the detection strip.

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EXAMPLE 5: Method of Amplification and Detection of a Target Nucleic Acid for the Detection of Candidatus Liberibacter in periwinkle (*Catharanthus roseus*)

An embodiment of the invention wherein a disposable component interfaces with a reusable dock was fabricated as described in Example 3 and employed to test crude periwinkle (*Catharanthus roseus*) tissue extracts for the presence of *Candidatus* Liberibacter asiaticus, the etiologic agent of citrus greening without a preceding nucleic acid isolation step.

In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. After completion of device assembly, 40  $\mu$ L of a reaction mixture was added to the sample chamber. Depending on the experiment, enzymes required for amplification were either present in this reaction mixture in liquid form, or present in a lyophilized cake incorporated into the sample chamber of the fluidic layer. In both cases, the sample was comprised of 4  $\mu$ L of a solution prepared by crushing 5 biopsy punches of 1.5 mm diameter each in 500  $\mu$ L of nuclease free water. Biopsy punches were obtained from either *Candidatus* Liberibacter asiaticus infected or uninfected periwinkle (*Catharanthus roseus*). The primers hyvl\_For and hyvl\_Rev were used to amplify a 139 bp nucleic acid sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus* Liberibacter asiaticus. Proprietary amplification reaction chemistry was performed. Amplification buffer (10X) was premade and contained 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate, 100 mM potassium chloride, and 0.25% Triton X-100. Each forty  $\mu$ L of reaction solution contained:

20           18.8  $\mu$ L water  
              4.0  $\mu$ L 10x amplification buffer  
              4.0  $\mu$ L DMSO  
              0.8  $\mu$ L potassium chloride (2 M)  
              1.0  $\mu$ L magnesium chloride (100 mM)  
25           1.0  $\mu$ L dithiothreitol (100 mM)  
              1.0  $\mu$ L dNTPs (10 mM)  
              4.0  $\mu$ L Primer set hyvl\_For and hyvl\_Rev (8  $\mu$ M each)  
              1.0  $\mu$ L VentR (exo-) DNA Polymerase (2 U/  $\mu$ L)\*  
              0.4  $\mu$ L Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu$ g/mL)\*

4.0  $\mu$ L of periwinkle tissue extract generated by briefly crushing 5 biopsy punches of 1.5 mm diameter (taken from the petiole of a periwinkle leaf) in 500  $\mu$ L of nuclease free water

\*either included in solution, or in the case that lyophilized enzyme was used, substituted by water

5 Venting of the amplification chamber and initiation of the amplification and detection program was accomplished by pressing a tactile switch on the reusable unit that serves as a start button. After venting, the reaction solution entered the amplification chamber where the solution was heated to 85 °C for 2 minutes, followed by 40 cycles of: 76 °C for 10 seconds and 60 °C for 25 seconds. After thermal cycling was complete, the reaction was allowed to flow into the labeling  
10 chamber by microcontroller-initiated venting. The labeling chamber contained blue dyed polystyrene detection microspheres dried to one interior face of the labeling chamber in the presence of 500 mM sucrose. The detection oligonucleotide conjugated to the dyed microspheres was complementary to the sense strand of the nucleic acid amplification product. The labeling chamber was heated to 105 °C for 2 minutes and then maintained at 90 °C for 30 seconds to  
15 induce boiling and thorough mixing of the polystyrene beads and denature the double-stranded DNA product. After heating, reaction solution in the labeling chamber was allowed to cool for two minutes. The detection chamber was vented, causing the solution to flow from the labeling chamber to the detection chamber and onto the detection strip assembly. Three capture lines were immobilized on the lateral flow membrane, from the bottom of the device they were: A negative  
20 control oligonucleotide not complementary to any assayed targets; capture probe complementary to the amplification product; and a positive control oligonucleotide complementary to the detection probe. The invention successfully amplified and detected the target nucleic acid with no detectable cross-hybridization to the negative control lines of the detection strip.

25 EXAMPLE 6: Method of Amplification and Detection of a Target Nucleic Acid for the Detection of *Candidatus Liberibacter asiaticus* in dodder (*Cuscuta pentagona*)

An embodiment of the invention wherein a disposable component interfaces with a reusable dock was fabricated as described in Example 3 and employed to test crude periwinkle (*Catharanthus roseus*) tissue extracts for the presence of *Candidatus Liberibacter asiaticus*, the  
30 etiologic agent of citrus greening without a preceding nucleic acid isolation step.



In some cases, a lyophilized bead composed of reaction enzymes and excipients, was added to the sample chamber. After completion of device assembly, 40  $\mu\text{L}$  of a reaction mixture was added to the sample chamber. Depending on the experiment, enzymes required for amplification were either present in this reaction mixture in liquid form, or present in a lyophilized cake incorporated into the sample chamber of the fluidic layer. In both cases, the sample was comprised of 4  $\mu\text{L}$  of a solution prepared by crushing a 1 cm length of dodder (*Cuscuta pentagona*) vine in 500  $\mu\text{L}$  of nuclease free water. The primers *hyvl\_For* and *hyvl\_Rev* were used to amplify a 139 bp nucleic acid sequence diagnostic for the presence of plant pathogenic bacteria *Candidatus Liberibacter asiaticus*. Proprietary amplification reaction chemistry was performed.

Amplification buffer (10X) was premade and contained 400 mM Tris-HCl (pH 8.4), 10 mM ammonium sulfate, 100 mM potassium chloride, and 0.25% Triton X-100. Each forty  $\mu\text{L}$  of reaction solution contained:

18.8  $\mu\text{L}$  water  
4.0  $\mu\text{L}$  10x amplification buffer  
4.0  $\mu\text{L}$  DMSO  
0.8  $\mu\text{L}$  potassium chloride (2 M)  
1.0  $\mu\text{L}$  magnesium chloride (100 mM)  
1.0  $\mu\text{L}$  dithiothreitol (100 mM)  
1.0  $\mu\text{L}$  dNTPs (10 mM)  
4.0  $\mu\text{L}$  Primer set *hyvl\_For* and *hyvl\_Rev* (8  $\mu\text{M}$  each)  
1.0  $\mu\text{L}$  VentR (exo-) DNA Polymerase (2 U/  $\mu\text{L}$ )\*  
0.4  $\mu\text{L}$  Et SSB, Extreme Thermostable Single Stranded Binding Protein (500  $\mu\text{g}/\text{mL}$ )\*  
4.0  $\mu\text{L}$  of *Cuscuta pentagona* extract generated by briefly crushing a 1 cm length of vine in 500  $\mu\text{L}$  of nuclease free water.

25

Although the invention has been described in detail with particular reference to the described embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover all such modifications and equivalents. The entire disclosures of all patents and publications cited above are hereby incorporated by reference.

30

CLAIMS

What is claimed is:

- 5           1.       A disposable platform for detecting a target nucleic acid, the disposable platform comprising:
- a sample chamber for receiving a sample comprising the target nucleic acid;
- an amplification chamber connected via a first channel to said sample chamber and connected via a second channel to a first vent pocket;
- 10               a labeling chamber connected via a third channel to said amplification chamber and connected via a fourth channel to a second vent pocket;
- a detection subsystem connected to said labeling chamber via a fifth channel and connected via a sixth channel to a third vent pocket;
- a plurality of resistive heating elements; and
- 15               one or more temperature measuring devices;
- wherein said vent pockets are each sealed from the atmosphere by a heat labile membrane located in a vicinity of one of said resistive heating elements.
- 20           2.       The disposable platform of claim 1 further comprising a sample preparation stage comprising an output in direct fluid connection with an input of said sample chamber.
- 25           3.       The disposable platform of claim 1 wherein dimensions of a substantially flat surface of said amplification chamber are approximately the same as dimensions of a substantially flat surface of a resistive heating element in thermal contact with said amplification chamber.
4.       The disposable platform of claim 1 wherein said amplification chamber is not cooled by an active cooling device.

5. The disposable platform of claim 1 wherein said amplification chamber contains an amplification solution, said sample chamber comprises a liquid amplification reagent mix or a lyophilized amplification reagent mix, and/or said labeling chamber comprises detection particles.

5 6. The disposable platform of claim 1 wherein said labeling chamber is heatable using one of said resistive heating elements.

7. The disposable platform of claim 1 wherein said detection subsystem comprises a lateral flow strip that does not comprise detection particles.

10

8. The disposable platform of claim 1 wherein said chambers, said channels, and said vent pockets are located on a fluid assembly layer and said electronic elements are located on a separate layer comprising a printed circuit board, said separate layer bonded to said fluid assembly layer.

15

9. The disposable platform of claim 1 wherein said detection subsystem is located on said fluid assembly layer or on a second fluid assembly layer.

10. The disposable platform of claim 1 wherein a volume of at least one of said  
20 chambers is between approximately 1 microliter and approximately 50 microliters.

11. The disposable platform of claim 1 further comprising a connector for docking said  
disposable platform with a base unit that is not an external instrument and that maintains the  
disposable platform in a vertical or tilted orientation.

25

12. A method for detecting a target nucleic acid, the method consisting of:
- disposing a sample comprising the target nucleic acid in a sample chamber of a disposable platform;
  - orienting the disposable platform vertically or at a tilt;
  - 5 reacting the sample with a liquid or previously lyophilized amplification reagent mix;
  - opening a first vent pocket connected to an amplification chamber to atmosphere, thereby enabling the reacted sample to flow into the amplification chamber;
  - amplifying the target nucleic acid in the amplification chamber;
  - 10 opening a second vent pocket connected to a labeling chamber to atmosphere, thereby enabling the amplified target nucleic acid to flow into the labeling chamber;
  - labeling the amplified target nucleic acid using detection particles in the labeling chamber;
  - opening a third vent pocket connected to a detection subsystem to atmosphere, thereby enabling the labeled target nucleic acid to flow into the detection subsystem;
  - 15 and
  - detecting the amplified target nucleic acid.

13. The method of claim 12 wherein the amplifying step comprises amplifying the target nucleic acid using a resistive heating element located within the disposable platform in a vicinity of the amplification chamber.
- 20

14. The method of claim 12 further comprising passively cooling the amplification chamber.
- 25

15. The method of claim 12 further comprising heating the labeling chamber during the labeling step using a resistive heating element located within the disposable platform in a vicinity of the labeling chamber.

16. The method of claim 12 wherein the detection subsystem does not comprise detection particles.

17. The method of claim 12 further comprising controlling operation of the disposable  
5 platform by using a docking station which is not an external instrument.

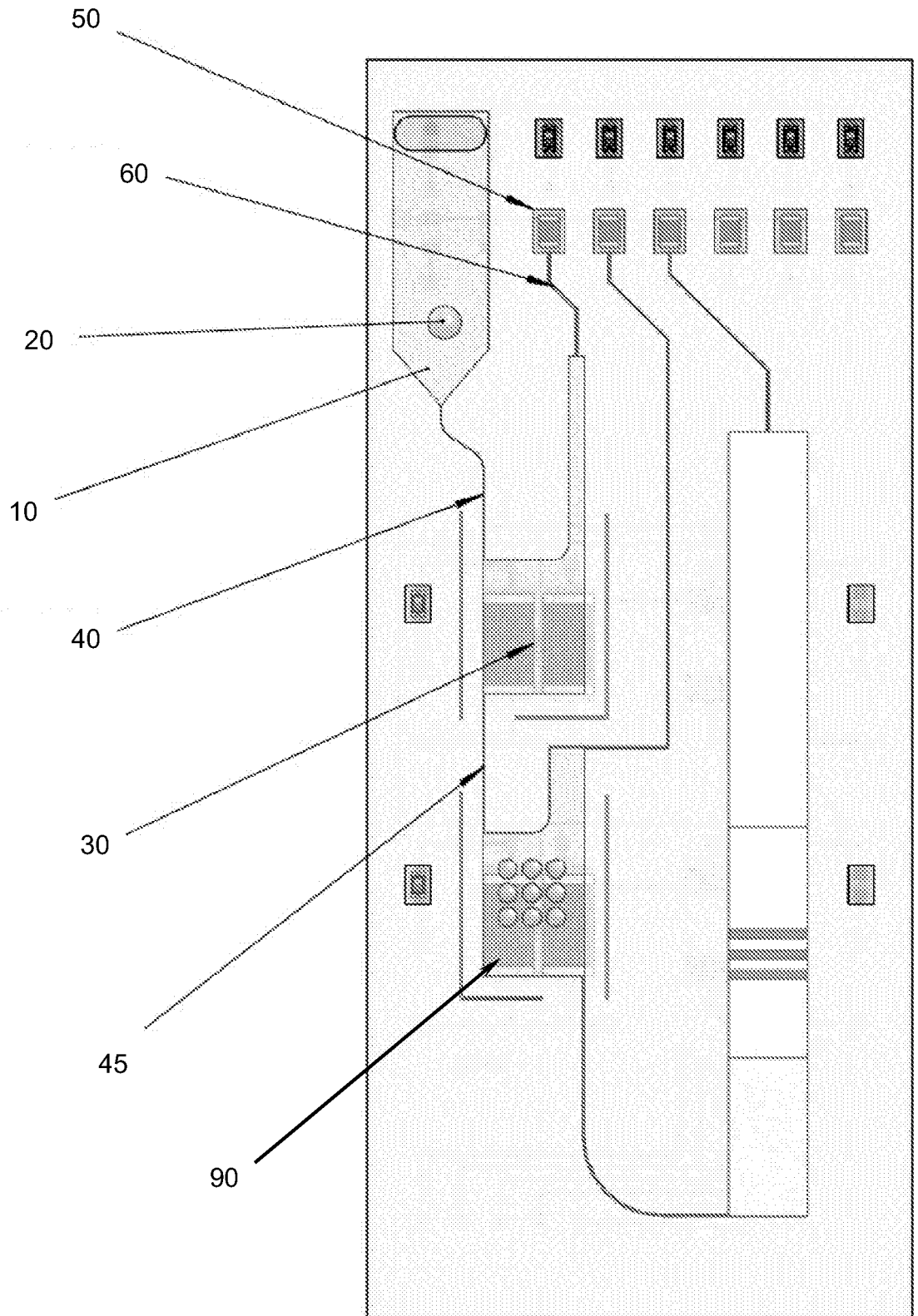


FIG. 1

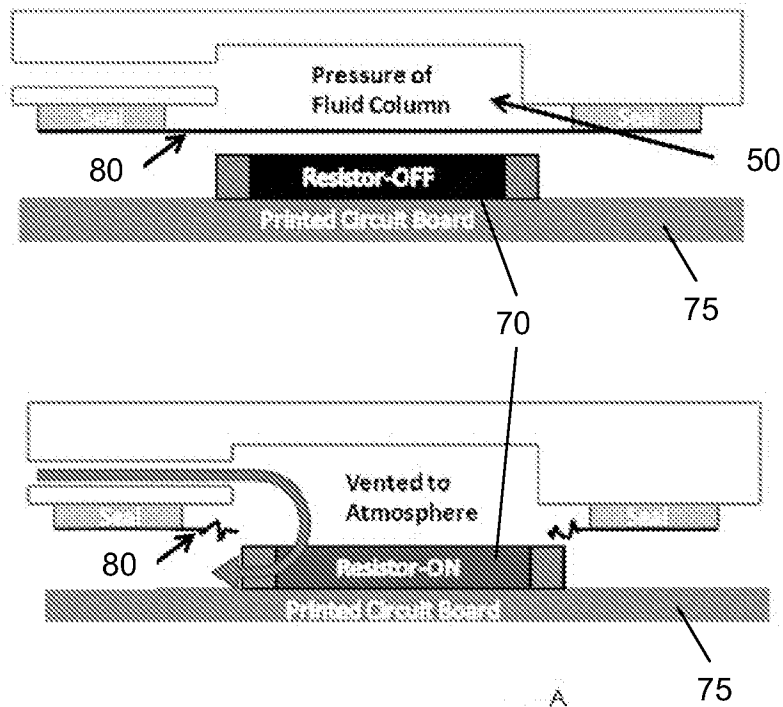


FIG. 2A

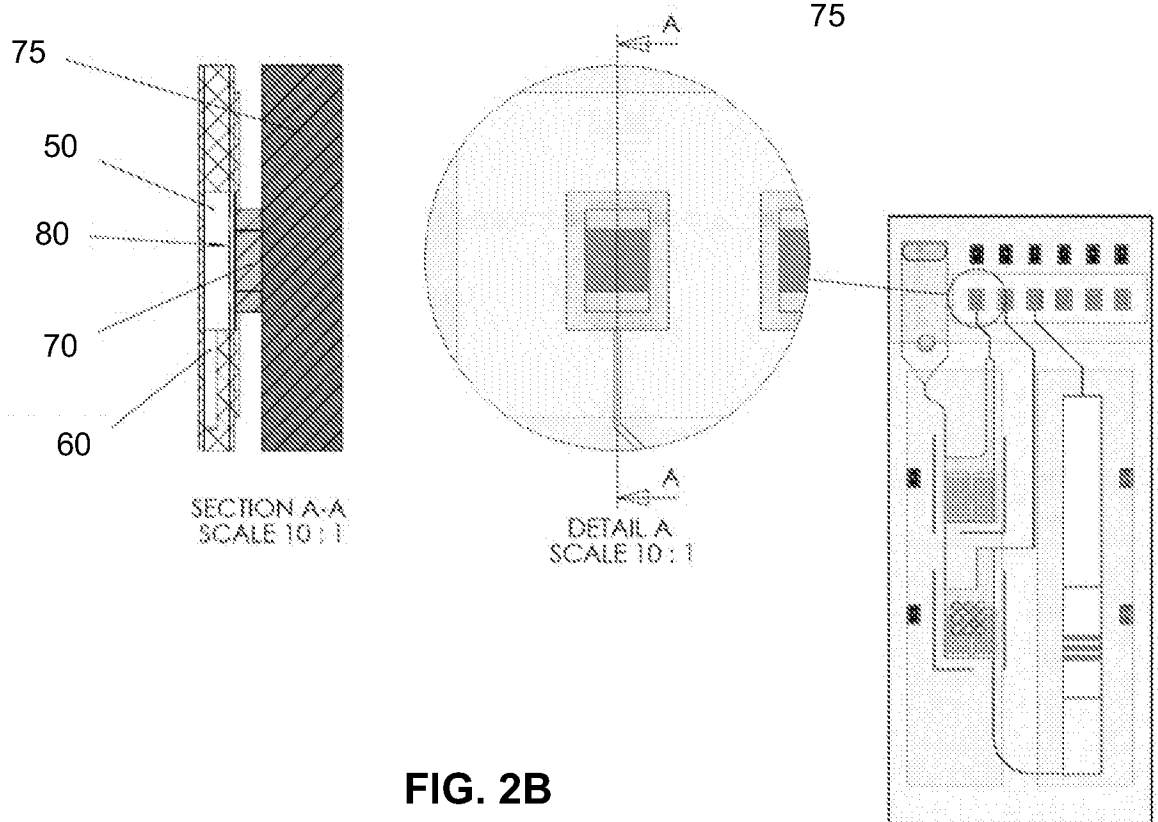


FIG. 2B

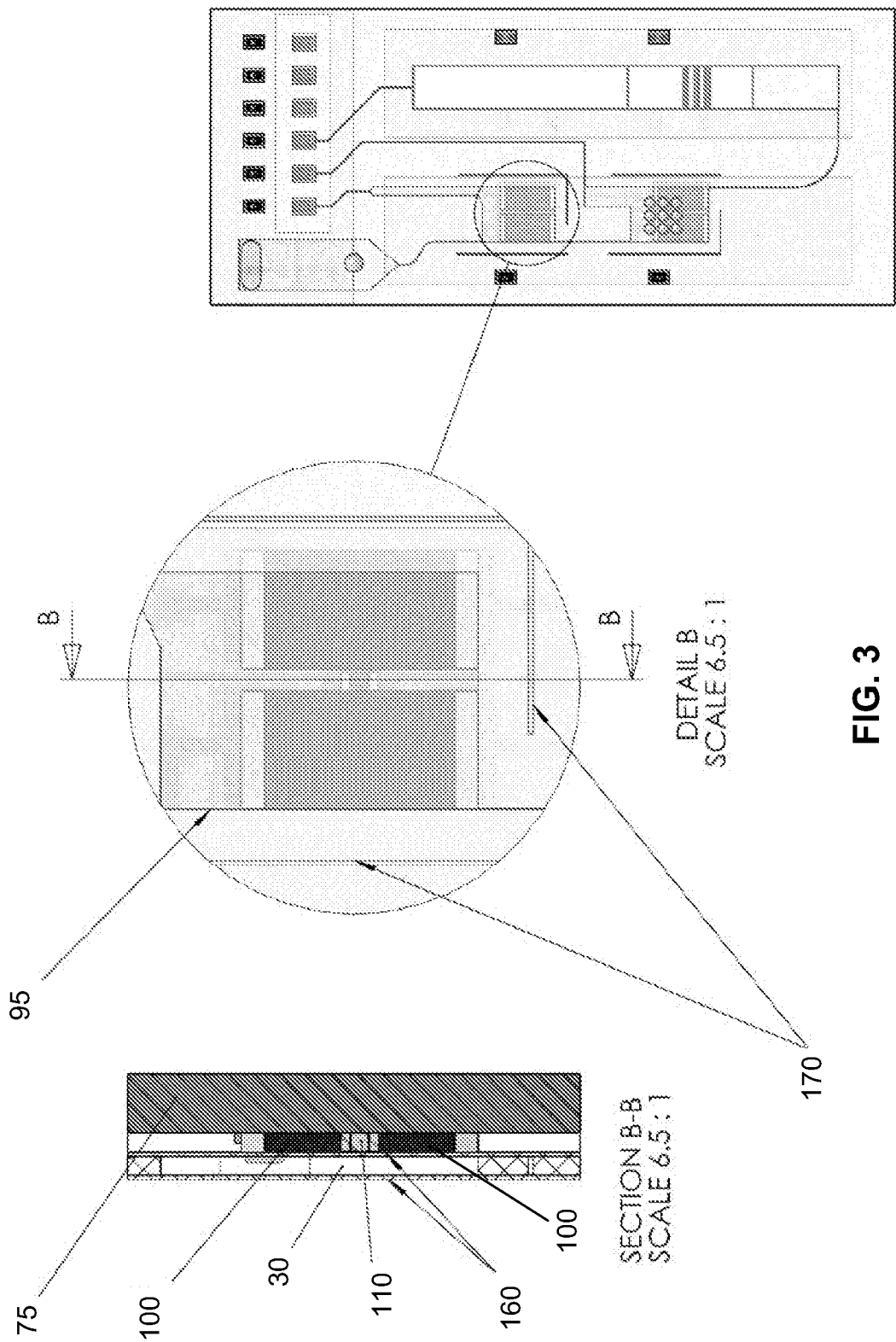


FIG. 3



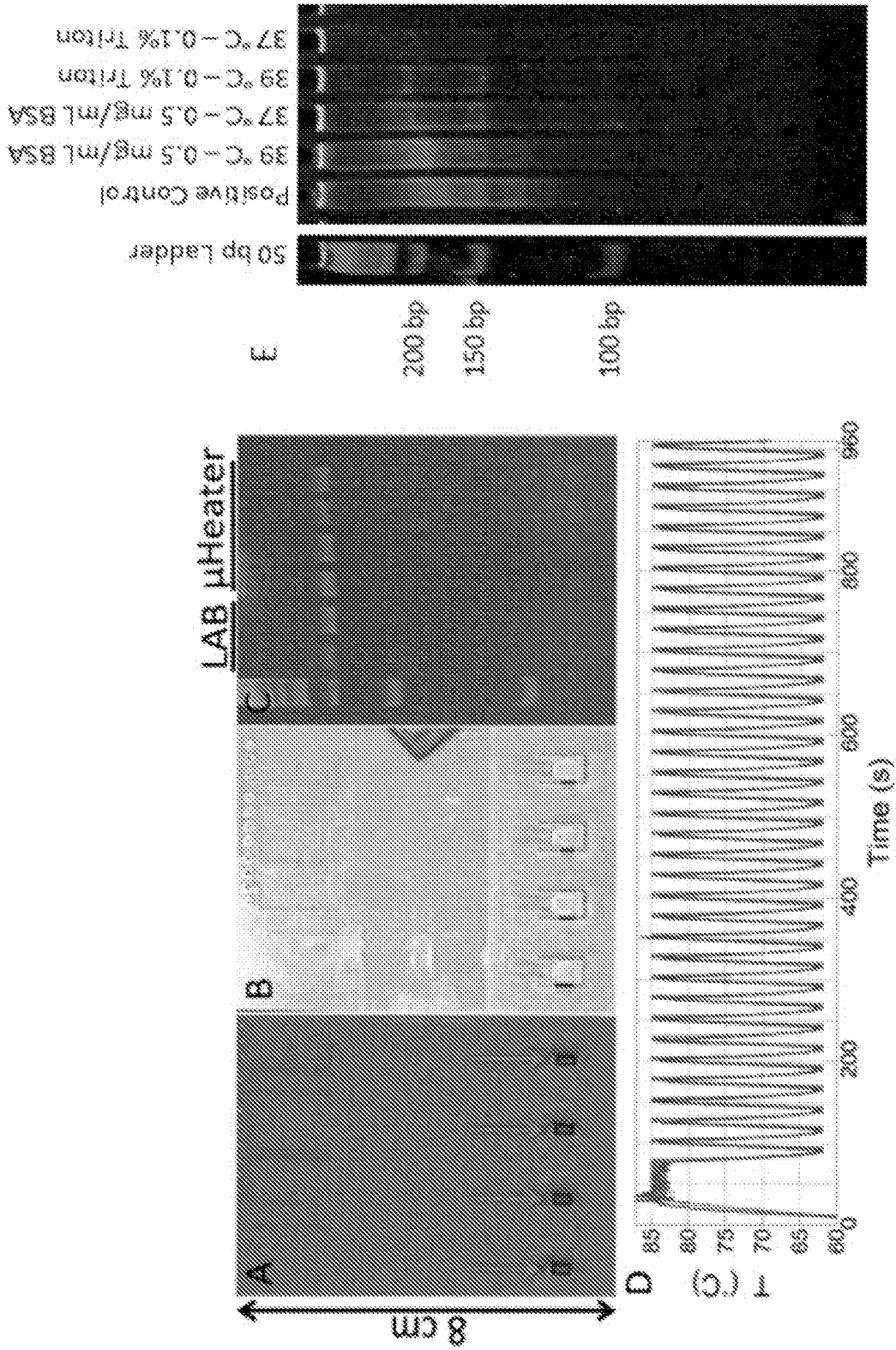


FIG. 4

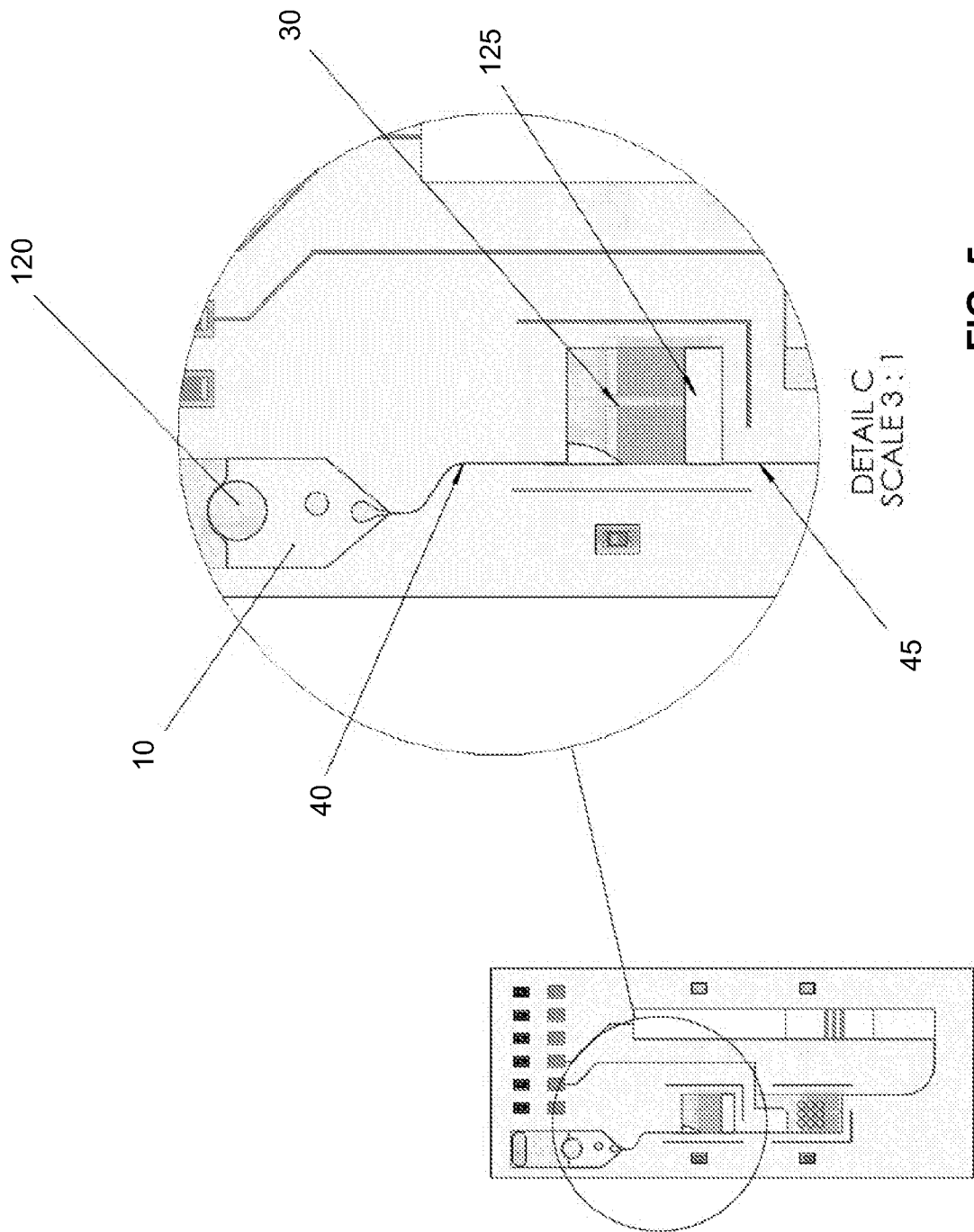
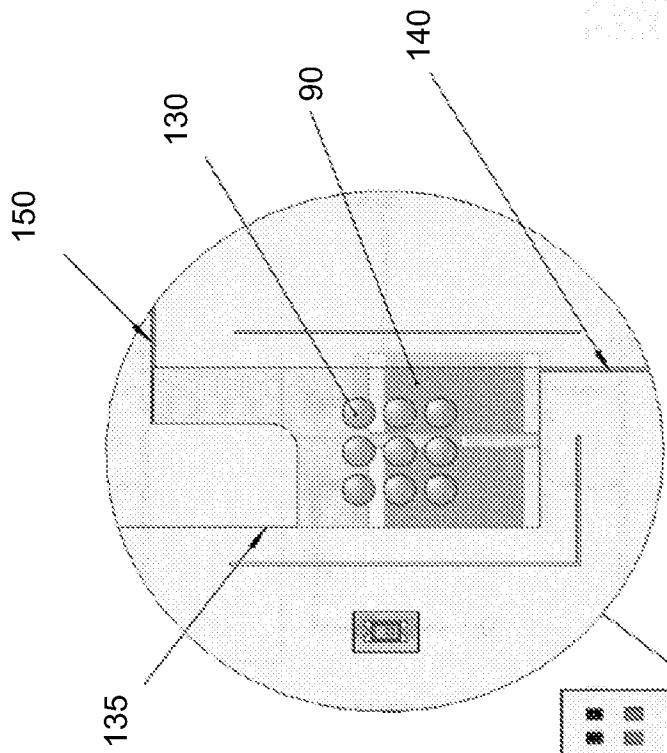


FIG. 5



DETAIL D  
SCALE 4:1

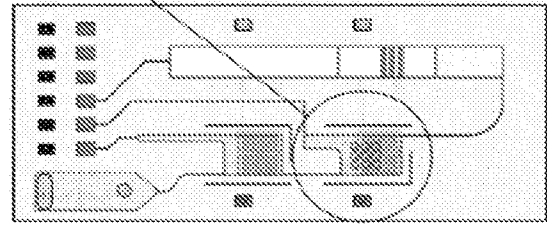
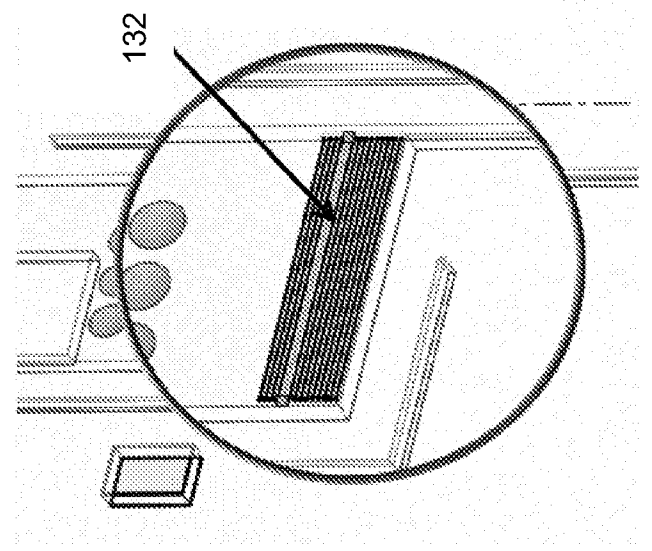


FIG. 6A

FIG. 6B



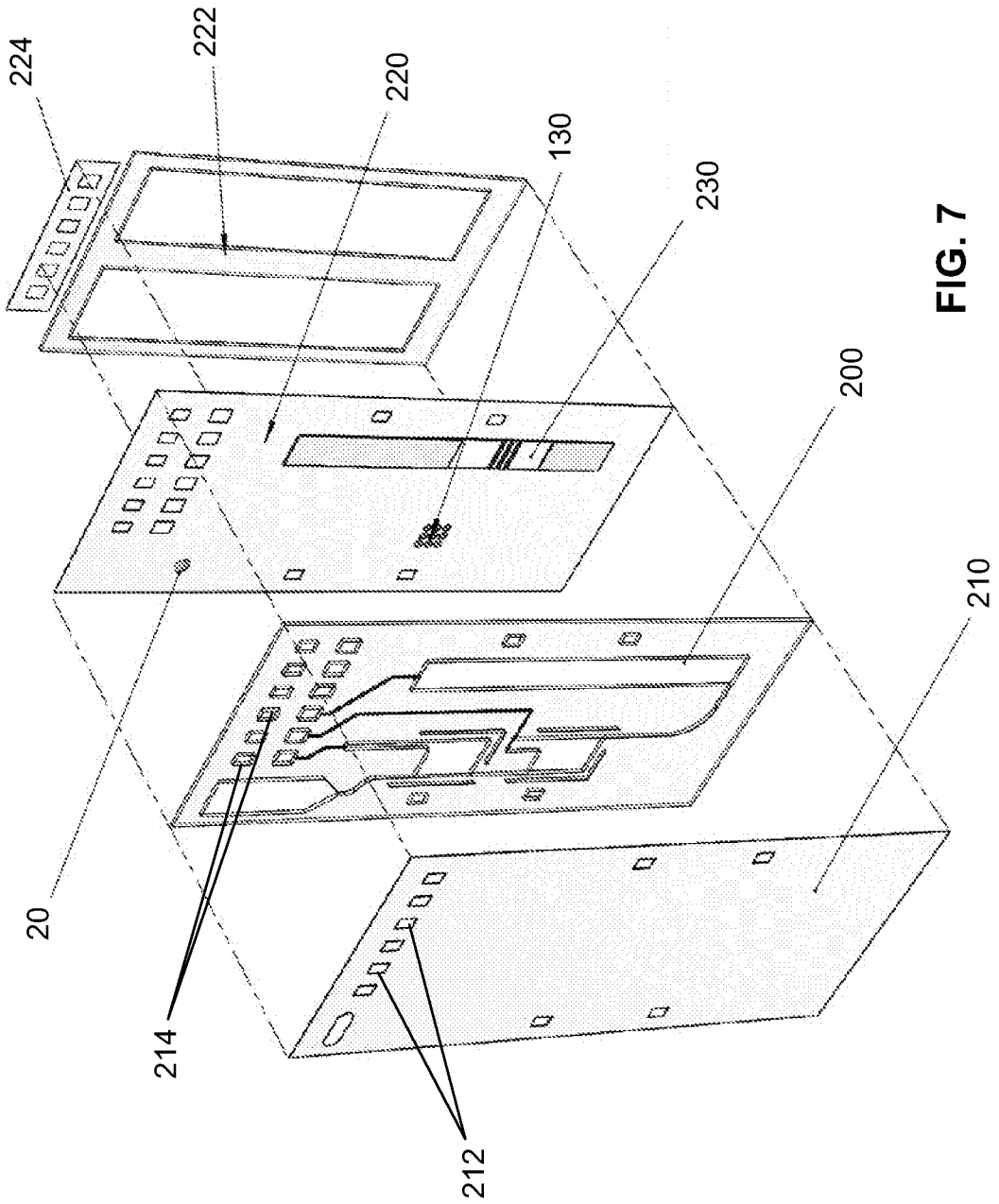


FIG. 7

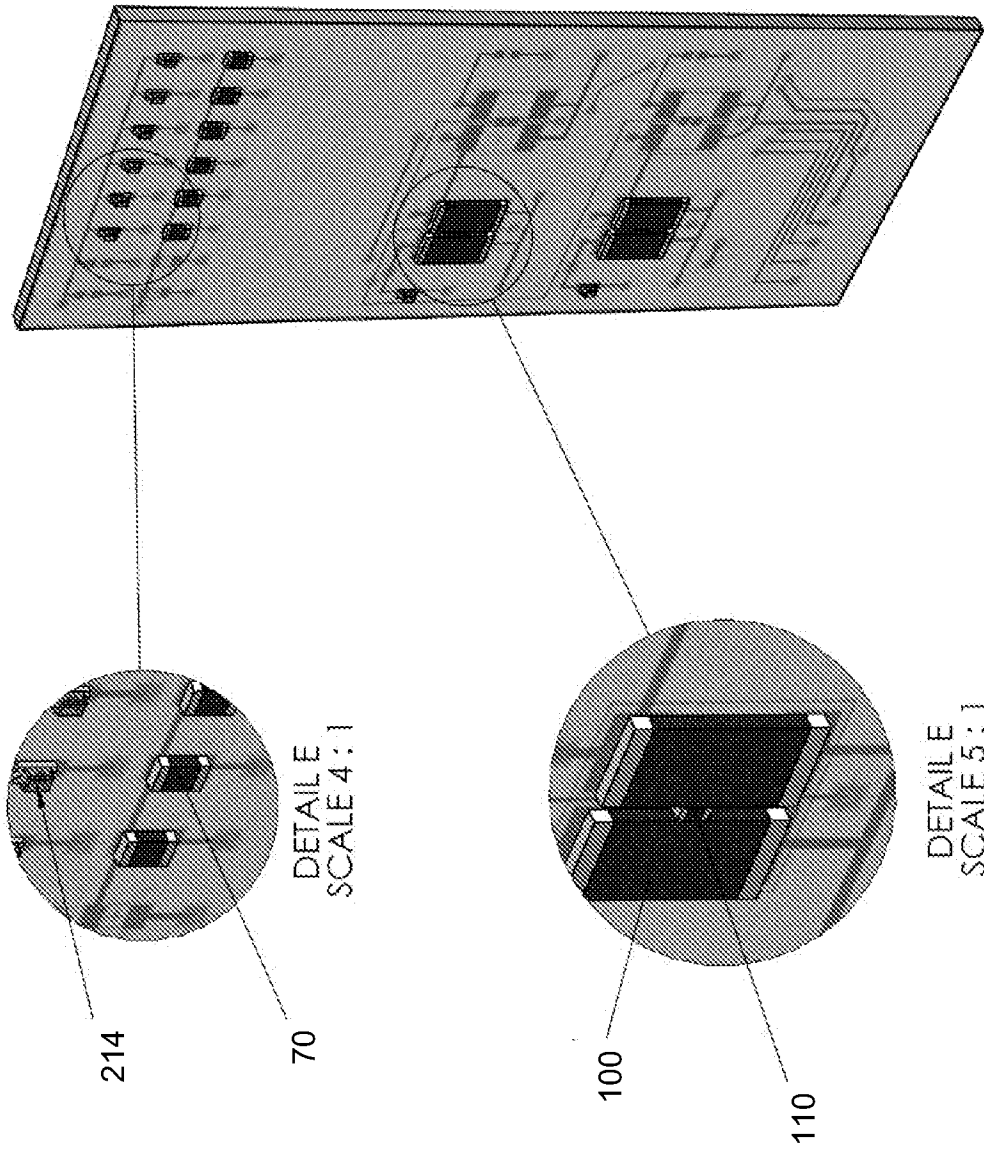
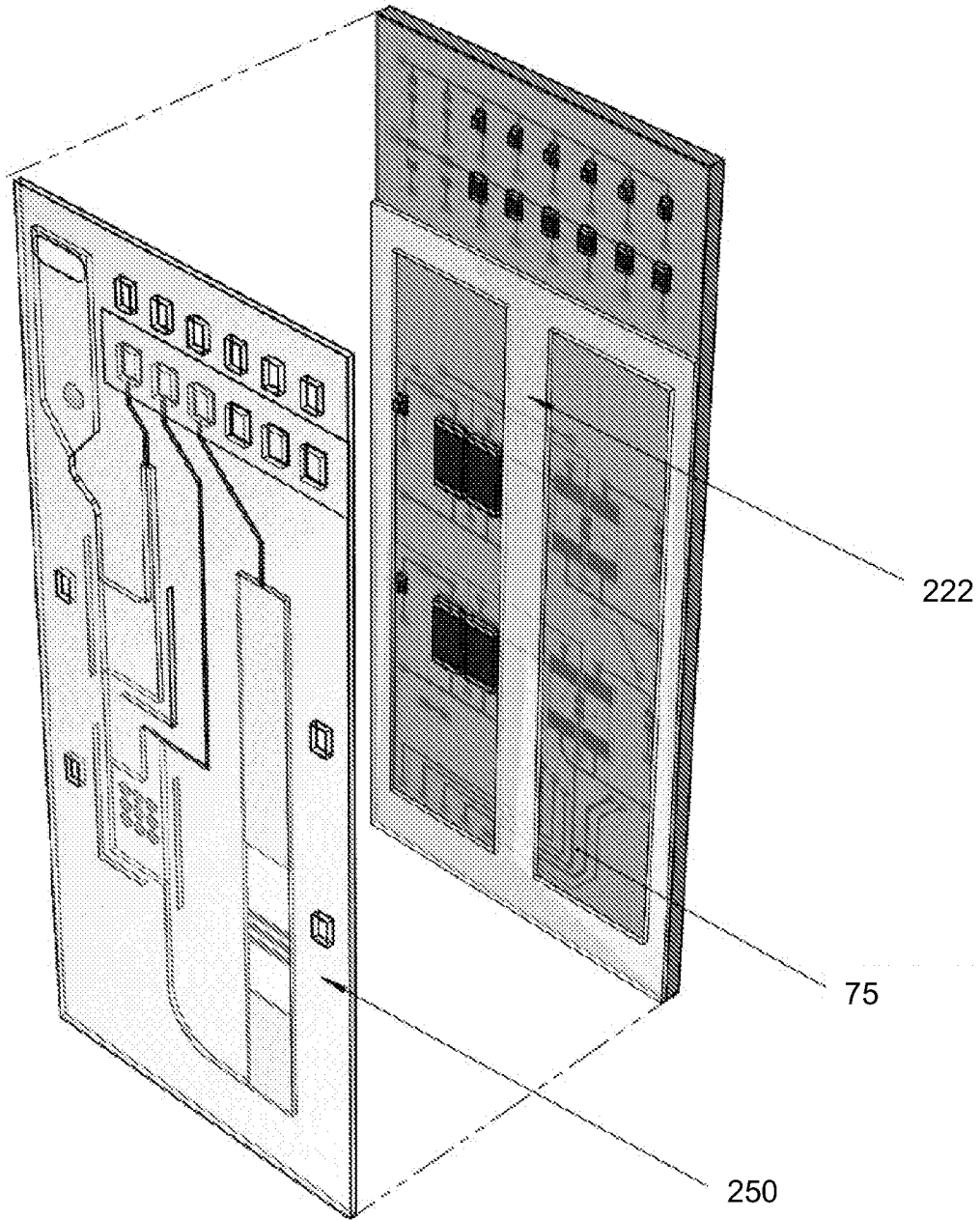


FIG. 8



**FIG. 9**

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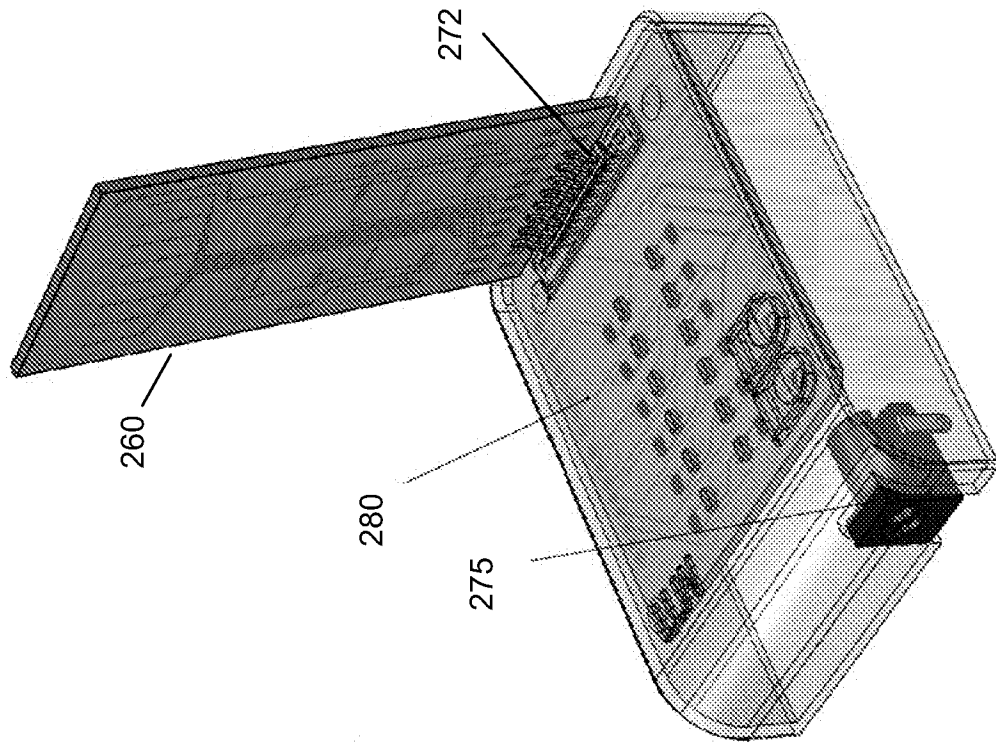


FIG. 10B

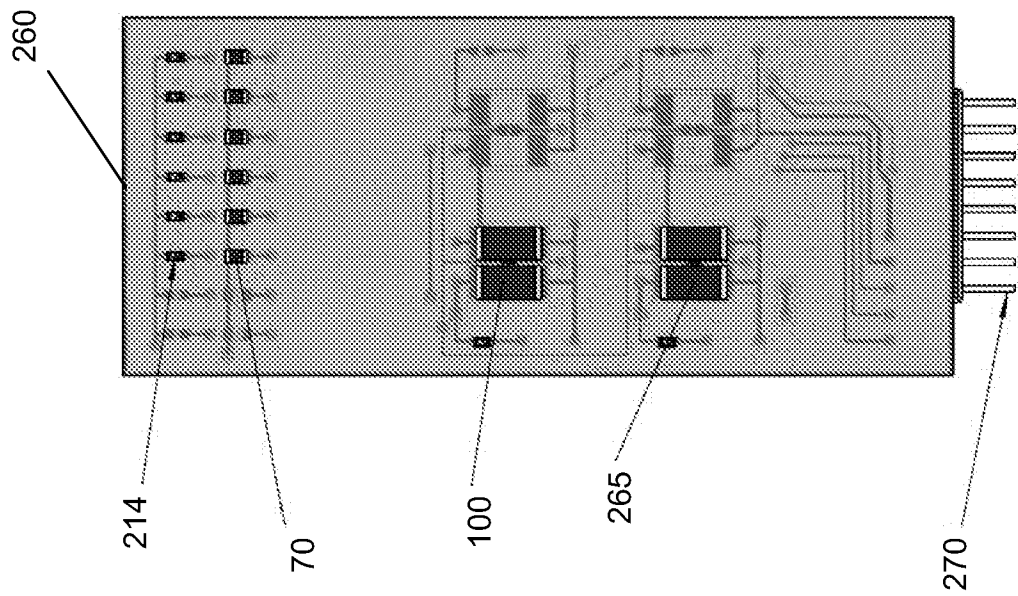


FIG. 10A

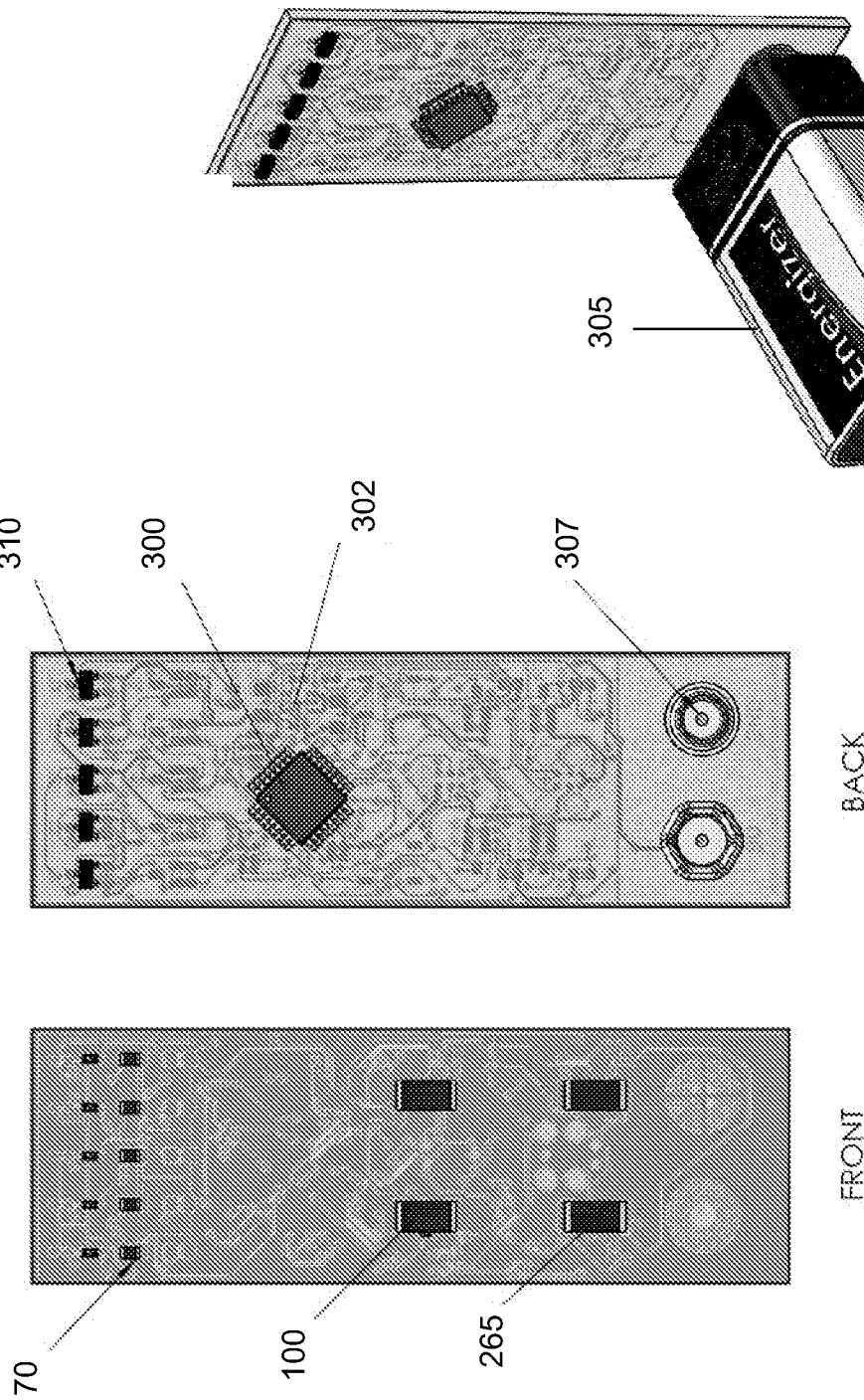


FIG. 11B

FIG. 11A

FIG. 11C



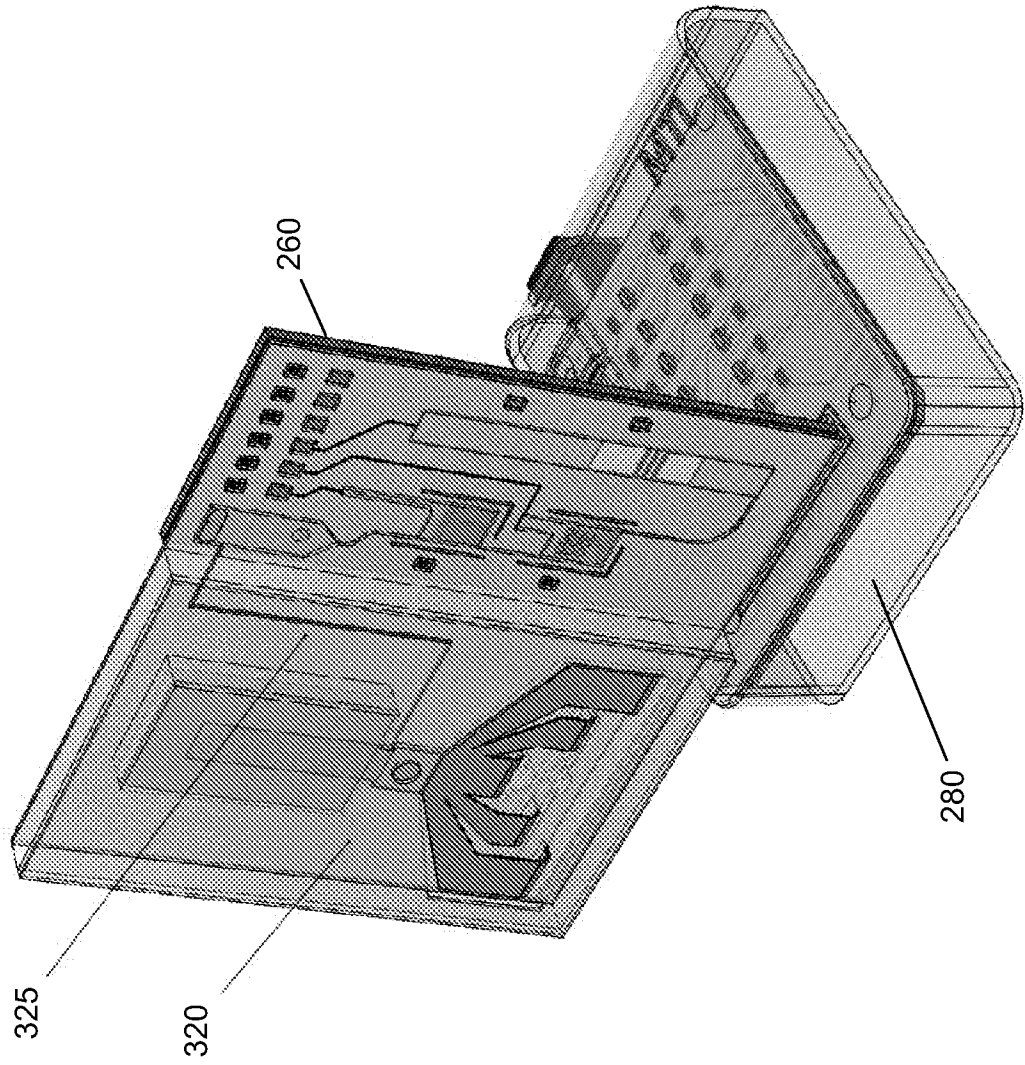


FIG. 12



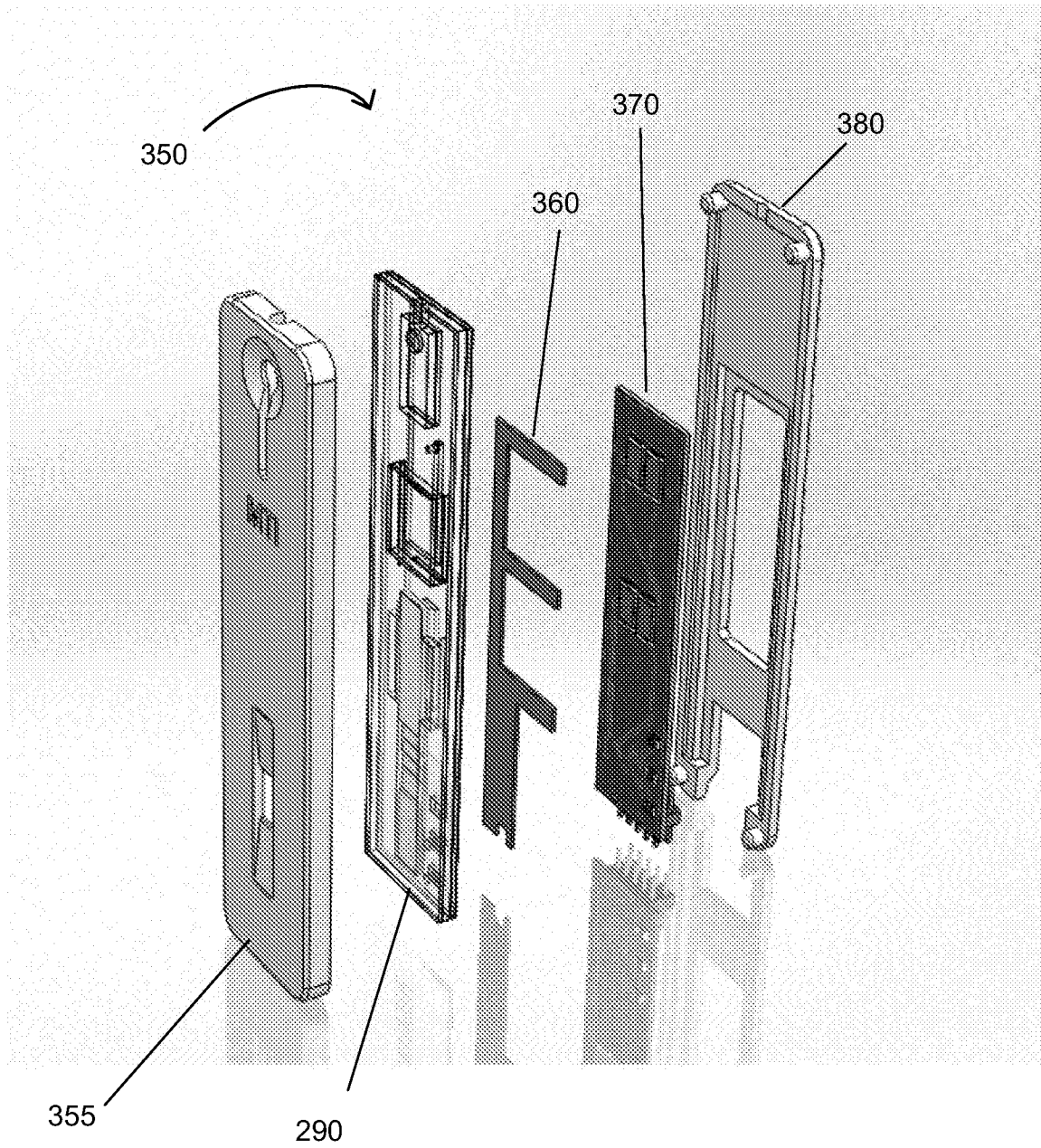
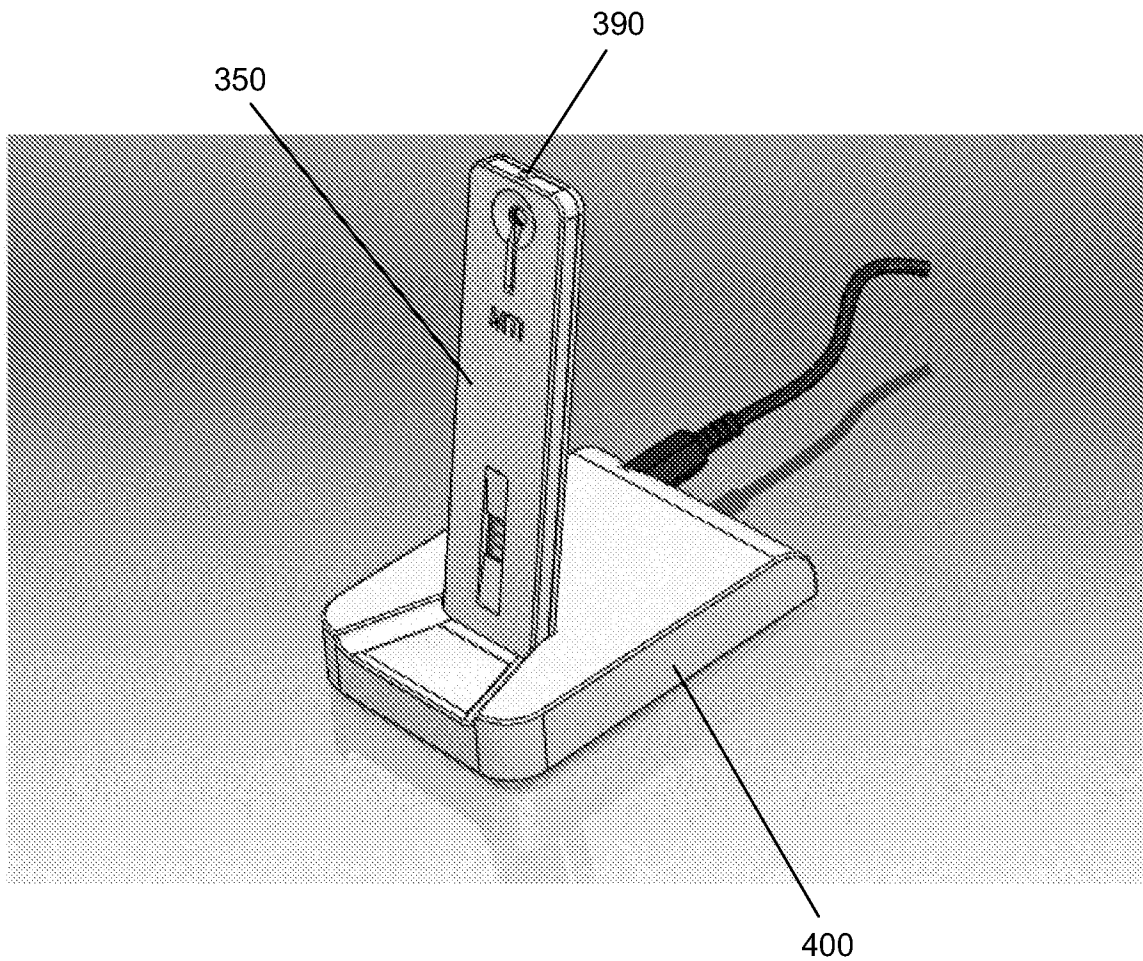


FIG. 14



**FIG. 15**

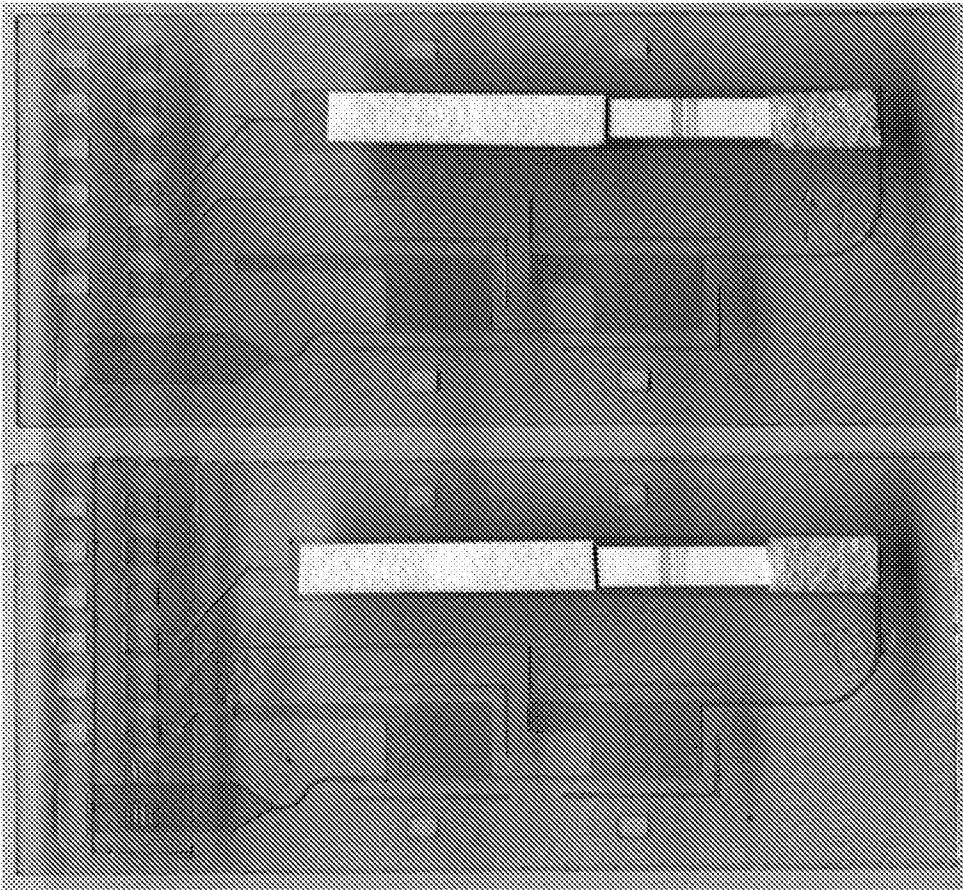


FIG. 16B

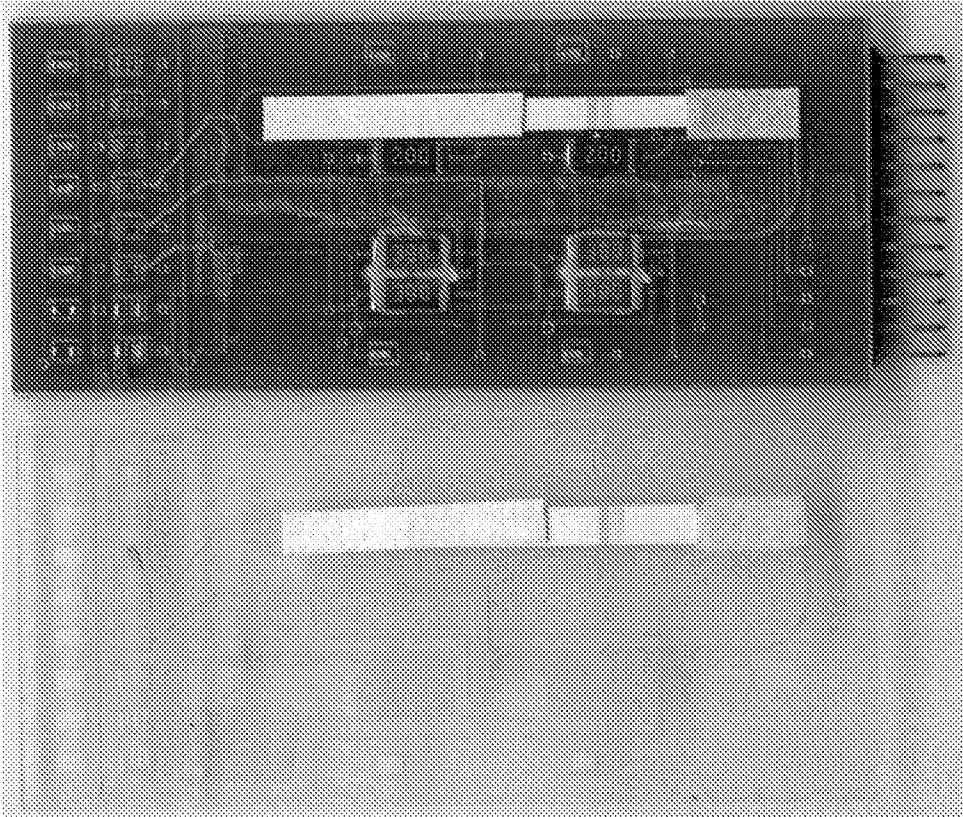


FIG. 16A

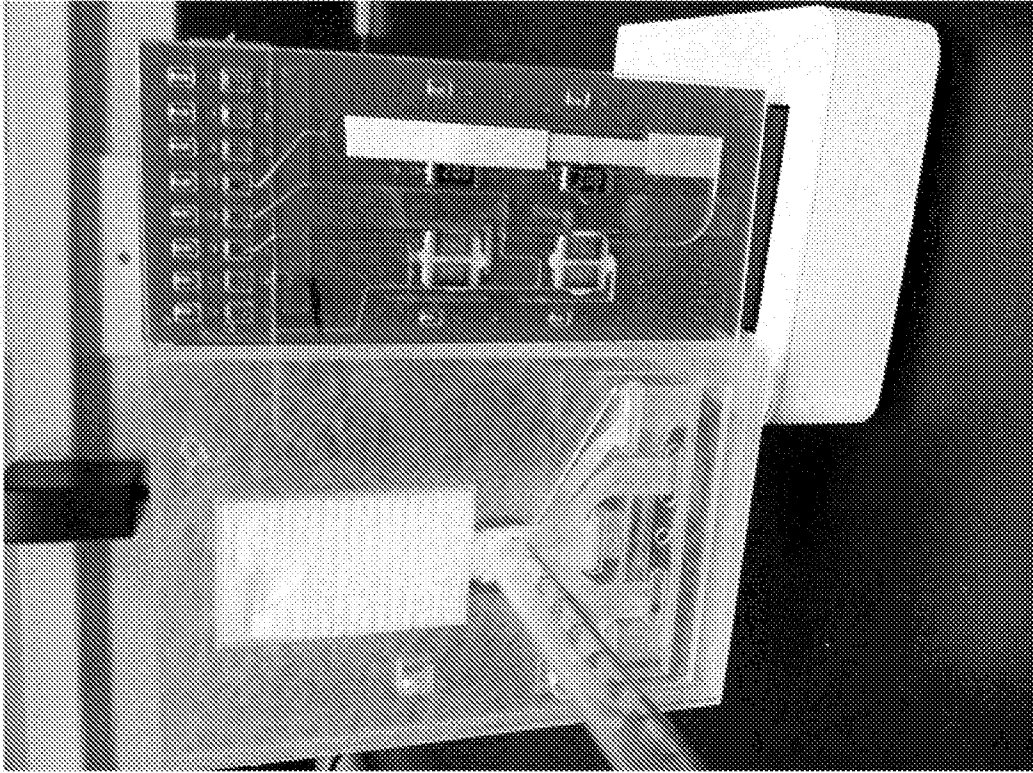


FIG. 17B

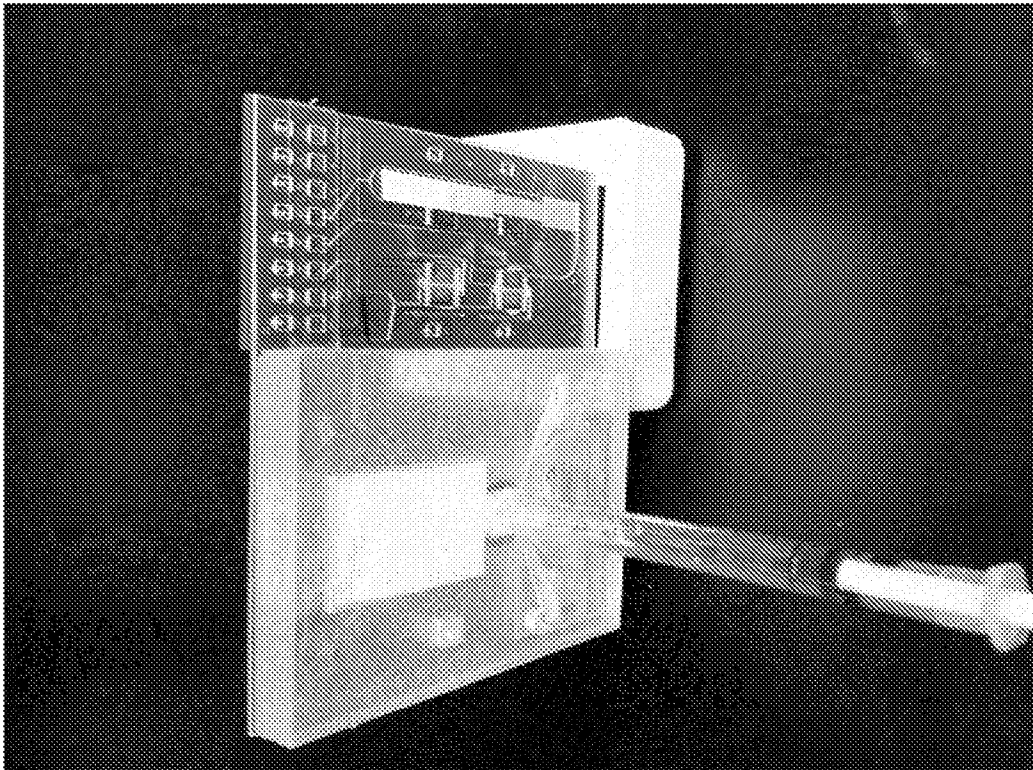


FIG. 17A