

may reside in this chamber and element **108** omitted. In another alternative where amplification reagents are best dry-stored, chamber **108** may contain diluents and the reagents coated onto the wall of the amplification chamber.

[**0166**] The amplification reagents as described above can provide for various amplification methodologies, e.g. rolling circle and ligase chain reaction. In the preferred embodiment the reagents incorporate a detectable moiety into an amplified target by means of PCR. Optionally, an applied magnetic field may be used to provide mixing of the beads in the amplification chamber. This is in the same manner as described for the extraction chamber.

[**0167**] The amplification chamber also has a heating element **109** and a temperature sensing thermistor **110** for controlling the temperature of the amplification chamber and thus effecting conditions suitable for amplification of the target nucleic acid. In the preferred embodiment the amplification chamber is cycled between 680 C and 900 C for thirty cycles. The time duration at each temperature is more than 5 and less than 30 seconds respectively. While the main part of the housing **100** is made of plastic, at least one wall of the amplification chamber is made of an inert material with superior thermal conduction properties, preferably silicon. The reverse side of the silicon has a resistive path **111** and two electrical contact pads **112** and **113** which constitute the heating element **109**. An electric current passing through the resistive path causes heating of the silicon chip and thus the contents of the amplification chamber. The reverse side of the silicon also has a thermistor **110** wired by leads **114** to two electrical contact pads **115** and **116**. The output of the thermistor is used by the instrument to control the current passing through the resistive path and thus the temperature of the amplification chamber.

[**0168**] The single-use device **100** may also optionally include closure element **117** to seal the entry port. This can be a plastic snap-closure element of the type described in jointly owned U.S. Pat. No. 5,096,669 or the slide closure of jointly owned pending U.S. application Ser. No. 10/658,528.

[**0169**] The amplification chamber may also be sealed at the ingress and egress by **118** and **119** respectively. This is desirable for ensuring reagents remain in the chamber during temperature cycling. For example, element **118** and **119** may be deformable rubber seals. Actuation can be by pin elements **209** and **210** in the instrument, which move through opening **120** and **121** in the housing to contact **118** and **119** and cause sealing. Pin elements **209** and **210** may be actuated independently or together by the instrument.

[**0170**] The egress of the amplification chamber is attached to a second conduit **125** containing a sensing region **126** comprising an immobilized capture oligonucleotide **127** and a sensor **128**. The housing **100** contains a second pump means **129** attached to the amplification chamber for moving the amplified target to said sensing region. The pump means comprising an air-filled chamber **130** with a diaphragm **131**. The instrument **200** contains an actuating means **122** for applying a force to element **131** to pneumatically displace air from chamber **130** and thus displace the amplified target towards the sensing region.

[**0171**] When the amplified target arrives in the detector region it can bind to the capture oligonucleotide and be retained. The detection region also contains a dry reagent layer coated onto the wall **151**. In the preferred embodiment, the moiety associated with the primer (which becomes part of the amplicon) is biotin and the dry reagent **151** is streptavidin-

labeled alkaline phosphatase. Dissolution of the reagent with the amplicon causes it to bind to the biotin via the well known biotin-avidin interaction. In operation this step generally takes from about 5 to about 15 minutes. In alternative embodiments the moiety can be 5' FAM or 15 5'-biotin and the dry reagent anti-FITC-ALP (alkaline phosphatase) or streptavidin-glucose oxidase conjugate.

[**0172**] A third conduit **132** is attached to the second conduit **125** between the egress of the amplification chamber and the sensing region. It has a chamber **133** with a detection reagent **134**. Optionally, the reagent is contained in a flexible sealed foil pouch **135** and in operation the instrument contains an actuating means **213** which can provide force to the pouch and cause it to rupture by being pressed against a rupturing feature **136**, preferably a sharp plastic point molded into the housing. This caused the detection reagent to move out through the third conduit and into the second conduit. This displaces and washes away any uncaptured amplified target and other material from the sensing region while permitting amplified target to remain bound to the capture oligonucleotide. The housing **200** also contains a waste chamber **137** attached to the second conduit for receiving the displaced material.

[**0173**] In the final step, the detection reagent reacts with the moiety **138** incorporated into said amplified target **139** to generate a signal at the sensor **140**. In the preferred embodiment where the moiety is biotin and is bound to streptavidin-labeled alkaline phosphatase, the detection reagent is p-aminophenol phosphate which is hydrolysed to form p-aminophenol by the enzyme. This is then electrochemically oxidized at the electrode surface of an amperometric sensor to generate a current proportional to the amount of moiety that is present, as illustrated in figures showing chronoamperometry (current versus time plots).

[**0174**] The instrument, **200** in FIGS. 6 and **650** in FIG. 21, used to operate the integrated single use device is shown interacting with the test device in FIG. 21. It includes a port **654** for receiving the single-use device **100** and **651**. The instrument has a keypad **652** for user entries and a display **653**. One or more locating features **202** for locating the device with respect to the instrument to provide for the desired interaction of electrical connecting elements and actuating elements are provided. The instrument contains an electromagnet **203** adjacent to the location of the beads **104** in chamber **103**. The electromagnet may be used to move the beads from the extraction chamber to the amplification chamber and to promote mixing of the beads within each chamber. The instrument includes an actuating means **204** adjacent to the location of the amplification reagent holding chamber **108** which can provide pressure to the chamber and cause the reagent to be displaced into the amplification chamber. The instrument also has a pair of electrical contacts **205** and **206** for contacting element **112** and **113** and a power source for passing a current through **111**. It also includes a pair of electrical contacts **207** and **208** for contacting element **115** and **116** for contacting the thermistor **110**. Furthermore, the instrument includes suitable electrical circuitry and an embedded algorithm for controlling the temperature of the amplification chamber through these means.

[**0175**] The instrument includes actuation pin elements **209** and **210**, which move through opening **120** and **121** in the housing to contact and close **118** and **119** to seal the amplification chamber. Suitable electromechanical features are