

the means to detect an increase in fluorescence at a second wavelength. Alignment features on the cartridge and instrument enable proper mating of the two to ensure reliable measurement. Optical detection methods for real-time PCR are well known in the art.

[0085] Referring to FIG. 2, the reversible seal 13 or valve on the ingress 12 is preferably a flexible diaphragm that is actuated into a closed position by an applied force and is in an open position in the absence of the applied force. As illustrated in FIGS. 3(a) and 3(b), the force is preferably provided by a pin 53 in the instrument that is controlled by a motor 54. The dimensions of the conduit 23 at the ingress 12 are preferably about 0.03125" wide and 0.25" long (although the dimensions can be of any suitable width and length), and the area of the diaphragm can be 0.187 square inches (although the diaphragm can have any appropriate area). The force applied to make the seal can be in the range of about 0.25 lbs to about 5 lbs, although any suitable amount of force can be used to make the seal. Materials suitable for the diaphragm include, but are not limited to, natural rubber, latex, silicon rubber, over-molded flexible plastics (GE Plastics, GLP-division, Pittsfield, Mass.), and the like.

[0086] An alternative valve design can be based on a pylon-type structure is illustrated in FIGS. 4(a)-(b). Fluids required for the amplification reaction can be sealed into the amplification chamber 11 and sealed at the ingress 12 and egress 14 with tape or foils as depicted by 106. The sample entry port 16 can also be sealed by tape or foil. The seal is punctured when the wand 42 is pushed into the amplification chamber 11, with the fluid remaining inside the chamber 11. The amplification reaction is then allowed to proceed. After the amplification cycle, seals 106 are punctured by the barbs on the pylon-type structure 55, affected by pins 53 and 57. Air pressure generated in the previously described air bladder can be used to move fluid into the detection chamber 59, also referred to herein as a detection device and detection cartridge. Mechanical connector 114 (e.g., a pylon-type sealing mechanism or the like) can be used to control the ingress valving feature. Mechanical connector 115 (e.g., a pylon-type sealing mechanism or the like) can be used to control the egress valving feature.

[0087] Referring to FIG. 2, the reversible seal 15 or valve on the egress 14 is preferably a flexible diaphragm that is actuated into a closed position by an applied force and is in an open position in the absence of the applied force. As illustrated in FIGS. 3(a)-(b), the force is preferably provided by a pin 57 in the instrument that is controlled by a motor 58. The other general features of the egress reversible seal 15 are similar to those of the ingress reversible seal 13. Preferably, the ingress 12 and egress 14 are in opposite corners or on opposite sides of the amplification chamber 11.

[0088] Detection of the amplicon can either be by in situ detection through the window 50 in the amplification chamber 11, e.g., real-time PCR, or, more preferably, in a second custom detection device 59. Here, the second conduit 26 attached to the egress valve permits egress of the amplicon. In one exemplary embodiment, a mating feature 60 (see, e.g., FIGS. 2, 3(a), 3(b), 4(a), and 3(b)) at the end of the second conduit 26 enables engagement of the amplification device 10 with the detection device 59 for leak-proof transfer of the amplicon. In other exemplary embodiments, the amplification device 10 and the detection device 59 are

directly connected, with fluids transferring via the channel provided by the second conduit 26.

[0089] As illustrated in FIG. 2, in another exemplary embodiment, the conduit 23 connected to the ingress 12 can include a first fluid detection system 116. The first fluid detection system 116 can include a chip insert 65, preferably made of silicon, with a fluid detection sensor 66. At the ingress 12, the portion of the chip 65 is optionally coated with one or more nucleic acid amplification reagents. The fluid detection sensor 66 is used to detect that fluid has entered the amplification chamber 11. When no conductivity is detected, all (or substantially all) of the fluid has been moved into the amplification chamber 11. Similarly, a second fluid detection system 117 comprising an upstream sensor 65 (e.g., located in the conduit 26 connected to the egress 14) is used to detect that all (or substantially all) of the fluid has been removed from amplification chamber 11 after the amplification cycle.

[0090] As illustrated in FIG. 1, the instrument 111 includes a recess 67 for receiving and engaging the device 10, and also includes an electrical connector 68 for contacting the heating circuit and electrical connector 69 for contacting the temperature sensor circuits. The instrument 111 also includes mechanical connectors 25, 24, 112 and 113 that independently interact with the device 10. Mechanical connector 25 can be used to introduce fluid into the amplification chamber 11. Mechanical connector 24 can be used with an air bladder to control fluid movement in the device 10. Mechanical connector 112 can be used to control the ingress valving feature. Mechanical connector 113 can be used to control the egress valving feature.

[0091] Mechanical connectors 25, 24, 112, and 113 have similar features. Each of the mechanical connectors 25, 24, 112, and 113 has a motor system 74, 30, 54 and 58, respectively. In addition, each of the connectors also has a pin feature 33, 29, 53 and 57, respectively. As illustrated in FIG. 8, the detection device 59 connected to the amplification device 10 with attached wand 42 is inserted into instrument 111.

[0092] Assembly of the preferred exemplary embodiment reflects the need to provide a simple and reliable manufacturing method for achieving large annualized production of amplification devices, e.g., in the many millions. An assembly process for a preferred embodiment can be as follows: an injection molded plastic component with fluid paths is used as a base element into which a fluid pouch and silicon chips are added. Double sided adhesive tape is applied to the base holding the chips and pouch in place, then a second plastic cover component is applied to the tape and sealed. These types of processes are amenable to automated manufacture.

[0093] In one specific additional exemplary embodiment illustrated in FIG. 12, the wall 17 can be held firmly in contact with the plastic component 22 and tape 37 by one or more holding means 200, such as, for example, a snap-closure feature or the like that enables the chip to be engaged but not retracted. Such a structure has the added advantage of providing further assurance that the chamber 11 does not leak during thermocycling. Various suitable configurations of the holding means 200 can be used to firmly hold the wall 17 in contact with the plastic component 22 and tape 37. For example, an alternative structure for the holding means 200 is illustrated in FIG. 13.

[0094] In the present invention, where electrochemical detection is preferred, the main objective of the nucleic acid