

an air-tight manner by a flexible latex sheet. While the pump is preferably actuated automatically by an instrument, it can also be actuated manually.

[0070] As illustrated in FIGS. 3(a)-(b), the fluid pouch 25 preferably contains a fluid 104 for performing nucleic acid amplification. The volume of fluid in the pouch 25 is preferably in the range of about 5 to about 100 uL. Like the pump 24, the pouch 25 includes a flexible diaphragm 32 capable of manual actuation or engaging and being actuated by a plunger 33 on an instrument 74 with which it is capable of mating. The pouch 25 is punctured by a barb 105 when the pouch with fluid 104 is forced against the barb 105. The fluid pouch 25 can contain a fluid for performing a nucleic acid amplification with one or more reagents including, deionized water, a buffer material, dNTPs, one or more primers and a polymerase. The polymerase can be in an inactive form bound to an antibody (e.g. anti-polymerase antibody) for stabilization, as is known in the art. After an initial heat cycle to denature the antibody, the enzyme becomes active. As will be apparent to those skilled in the art, the pouch 25 should be made of a material selected for biocompatibility of exposed surfaces, chemical/UV resistance, sterility, sealability, reliable fluid release and low wet retention, as well as other like factors. This is preferably by a form-fill-and-seal method using plastic coated metal foils of the following type, PRIMACOR™ (Dow Chemical Company, Midland, Mich.) coated aluminum foil. Alternatively other plastic-coated foils can be used. Such plastic-coated foils are widely commercially available.

[0071] As illustrated in FIGS. 7(a)-(d), while one wall 17 of the amplification chamber 11 is preferably silicon, other materials can also be used as described above. Such materials are selected to be thermally conductive materials and also support fabricated structures on the exterior surface, in addition to providing biocompatibility of exposed surfaces with amplification reagents and providing for sterility.

[0072] The other walls 34, 35 of the amplification chamber 11 are preferably made of plastic, such as, for example, polycarbonate (lexan), acetal (delrin), polyester, polypropylene, acrylics, and ABS and other like materials. While plastics are moldable to desired geometries, they generally have poor thermal conduction properties. Accordingly, the design of the plastic parts of the chamber wall substantially reduce the thermal mass in order to improve efficiency of operation, i.e., the thermocycling efficiency. An alternative is to use plastic materials that have been modified to improve their conductive properties. Such products are known in the art and are available from various companies including, for example, Cool Polymers Inc. (Warwick, R.I.), LNP (KONDUIT™) (offered by GE Plastics, Pittsfield, Mass.), and PolyOne Inc. (Avon Lake, Ohio). In one exemplary embodiment, the entire or substantially entire amplification chamber 11 can be made of a conductive polymer (e.g., COOLPOLY™ D-Series made by Cool Polymers Inc.), in one or more parts. According to such an exemplary embodiment, the heater and temperature sensor components can be screen printed onto the plastic surface, or formed as a flexible plastic circuit and bonded to the conductive plastic component. Circuitry made on flexible plastic sheets is well known in the art and made by companies including Flextronics Inc. (Singapore).

[0073] In a preferred exemplary embodiment, while the plastic portion of the wall of the amplification chamber 11 can have a thickness in the range of about 0.1 to about 5 mm,

it is preferably about 0.25 to about 0.5 mm. Such a preferable thickness meets the minimum requirements of physical integrity and supporting sealing of the closed chamber at elevated temperature, e.g., near-boiling point in PCR amplification, and the associated increase in pressure. Preferably, one or more additional rib supports 36 are provided to confer improved rigidity to this component.

[0074] To provide leak-proof bonding between the silicon wall 17 and the plastic wall 35, a double sided adhesive tape gasket 37 of FIG. 1 and FIGS. 7(a)-(b) can be used. The double-sided adhesive tape gasket 37 is preferably selected to be biocompatible and adhere over a temperature range of about -60° C. to around 150° C. In other words, it should seal sufficiently well such that the material inside the chamber 11, during a PCR or other amplification reaction, is retained and does not leak out. This tape must also preferably have heat curing requirements within the range compatible with the plastic. Furthermore, the tape gasket 37 can include design features where it seals to the plastic, but preferably leaves a space that is in contact with the fluid, much like a washer or O-ring. A preferred adhesive tape material is 9244 tape supplied by 3M Corporation (St. Paul, Minn.), although other suitable adhesive tape materials can be used. For example, the 9244 tape accommodates adhesion between materials with different coefficients of expansion, e.g., silicon and plastic, and seals over the desired operating temperature range. It also withstands pressure changes and is biocompatible. This tape can also be pre-cut and placed on rolls for automated manufacturing. Alternatives to tape gasket materials include, for example, Dow Corning (Midland, Mich.) sealant 3145 RTV. A further alternative can be to glue the silicon to the plastic to form the seal, with suitable glues including, but not limited to, HERNON 126 (offered by HERNON Manufacturing, Sanford, Fla.), 3M bonding films and LOCTITE™ glues (offered by Henkel Corp., Rocky Hill, Conn.).

[0075] With regard to the proportion of the area of the amplification chamber wall 17 that is formed by silicon, it is preferably in the range of about 30 to about 50%. In a preferred exemplary embodiment, as illustrated in FIG. 2, it is about 31%. The objective is to maximize the heating and cooling surface area of the chamber wall 17, while keeping the chamber volume relatively low. In a preferred exemplary embodiment, the internal volume of the chamber 11 is in the range of about 5 uL to about 50 uL, preferably about 15 to about 25 uL. In the exemplary embodiment illustrated in FIG. 2, the silicon wall 17 has a chamber surface area of approximately 40 mm², with a depth of approximately 0.375 mm, giving a chamber volume of approximately 15 mm³. The total chamber surface area is approximately 90 mm², i.e., approximately 40 mm² each for the top and bottom walls plus approximately 10 mm² for the side walls. Preferably, the amplification chamber surface area is in the range of about 50 to about 200 mm², and the volume is in the range of about 5 to about 30 mm³. The sealable sample entry orifice 16 increases the amplification chamber volume by approximately 5 uL.

[0076] With respect to the shape of the amplification chamber 11, it is preferably substantially rectangular with a low height, as shown in FIGS. 3-7, but can also be rectangular with rounded corners and also edges. Other useful shapes include a cylindrical structure and a shape that is roughly oval in cross-section. The objective of the design is to provide for fluid mobility in and out of the amplification