

chamber 11 and also minimize bubbles being trapped in the chamber 11. It is advantageous to ensure that the chamber 11 is substantially free of bubbles, as during the heating cycle expansion of trapped bubbles can contribute significantly to an increase in pressure in the chamber 11. Such conditions result in a requirement for more robust sealing of the chamber features. Further, the trapped bubbles can impact the thermal status within the amplification chamber 11. While the device 10 is designed to withstand the additional pressure, it is desirable to avoid features that can trap or induce bubbles. Preferably, the chamber 11 and conduits 23, 26 of the device 10 include surfaces that are wettable and lack sharp angles and void spaces, as illustrated in FIG. 2. A preferred shape for the amplification chamber 11 is a rhomboid as illustrated in FIG. 2, although other suitable shapes can be used.

[0077] As illustrated in FIG. 7(d), the exterior surface 19 of the silicon wall 17 includes a heating circuit 20 that can comprise, for example, a resistive electrical path fabricated on that surface with a first and second connecting pad (38, 39) for contacting an external circuit for providing current flow through the path. The wall 17 also includes a temperature sensor 21, e.g., a thermistor, thermocouple or RTD or the like, fabricated adjacent to the heating circuit 20. There are first and second connecting pads (40, 41) for contacting an external circuit for connecting to the sensor.

[0078] It will be apparent to skilled artisans that there are several ways for getting a nucleic acid sample into the amplification chamber 11. In a preferred exemplary embodiment (FIGS. 5(a) and 5(b)), the sample entry orifice 16 is capable of mating with a sample introduction device 42 that comprises a wand 43 with a first end with an absorbent pad 44 for collecting and retaining a nucleic acid sample and a second end 45 which acts as a convenient handle. The first end is designed to pass through the sample entry orifice 16. In another exemplary embodiment (FIGS. 6(a) and 6(b)), the wand 43 also has a locking feature 46 between the first and second end for engaging and sealing the wand in the sample entry orifice. A gasket 101 provides an effective seal at the sample entry orifice 16. After inserting the wand 43 into the sample entry orifice 16, a locking mechanism 102 is pushed in place to secure wand 43 and to affect a seal with gasket 101.

[0079] In one exemplary embodiment for the sample entry orifice 16 illustrated in FIGS. 5(a) and 5(b), the engaging and sealing features are a male screw feature 61 on the wand and a female screw feature 62 on the sample entry orifice 16. In another exemplary embodiment illustrated in FIGS. 6(a) and 6(b), the engaging and sealing features are a male collar 63 locking feature on the wand and a female collar 64 locking feature on the sample entry orifice.

[0080] Regarding the sample type, the absorbent pad 44 can be used for a cheek swab to introduce buccal cells directly into the amplification chamber 11. It has been found that heat cycling of these cells is sufficient to liberate the nucleic acid for amplification. As a result, a buccal swab sample can be introduced and amplified without further sample preparation. The absorbent pad 44 can also be used to transfer nucleic acid from another separation process or device. For example, a DNA binding material can be affixed to the end 44 of the sample introduction device 42, wherein the sample is treated in a manner to come in contact with the swab end material, which is subsequently washed of inhibitory substances. The sample introduction device 42 is then

inserted into the amplification device 10 through orifice 16. The materials that can be tested could be chosen from the list of blood, urine, tissue, bone, hair, environmental sample, soil, water, and other like materials. As is apparent to those skilled in the art, many sample preparation devices and reagents are available commercially.

[0081] As will also be apparent to those skilled in the art, the device 10 uses reagents for performing amplification, including a polymerase, dNTPs, one or more primers and a buffer. These can be added externally through the sample orifice 16, or, more preferably, be present in the device 10 before use, such as being incorporated as part of the device assembly process. The reagents can be located individually or together in the amplification chamber 11, in the conduit 23 attached to the ingress 12 or in the fluid pouch 25. In a preferred exemplary embodiment, the amplification chamber 11 can include a sugar glass coating, i.e., dehydrated and glassified reagents, on at least a portion of the interior surface 18 of the silicon wall 17. The sugar glass coating can include reagents and a buffer, dNTPs (e.g., four natural deoxynucleotidyl triphosphates dATP, dCTP, dGTP and dTTP) can be used, however it is well known in the art that modified deoxynucleotidyl triphosphates can also be used), one or more primers and a polymerase (*Thermus aquaticus*, *Thermococcus* spp., and others well known in the art). Suitable sugars, either individually or in combination, can be chosen from the following: sorbitol, trehalose; arabinose; ribose; xylose; xylitol; fructose; galactose; glucose; mannose; rhamnose; sorbose; glucitol; maltose; mellibiose; sucrose; maltitol; hydrocolloids; or other sugar containing polymers including cellulose, DEAE-dextran, dextran, locust bean gum, guar gum, agar and carboxymethylcellulose.

[0082] The present device 10 enables the amplification chamber 11 to achieve a temperature increase ramp rate in the range of about 10 to about 50° C. per second, preferably about 15 to about 30° C. per second, and a temperature decrease ramp rate in the range of about 4 to about 20° C. per second, preferably about 6 to about 8° C. per second.

[0083] The method of cooling is preferably implemented where the device engages and is operated by an instrument. The instrument includes a fan 48 (see FIGS. 7(a)-(b)) for cooling the amplification chamber 11. The fan 48 is optimally positioned close to the surface of the silicon wall 17 to provide the desired angle of the air stream, as shown in FIG. 7(a). The fan 48 is activated to coincide with the desired heating and cooling cycle. Additionally or alternatively, the instrument has a heat-sink 49 capable of reversibly contacting and cooling the amplification chamber 11, as illustrated in FIG. 7(b). In a further exemplary embodiment, the silicon wall 17 includes a Peltier circuit on the exterior surface 19 adjacent to the heating circuit 20.

[0084] In certain exemplary embodiments where it is desirable to perform real-time PCR, the amplification chamber 11 includes an optical window 50, as illustrated in FIGS. 2, 3(a), 3(b), 4(a), and 4(c). The window 50 enables fluorescence detection of a signaling reagent within the chamber 11 to be measured by an optical detection component 51 (see, e.g., FIGS. 3(a) and 3(b)) in the instrument. It will be understood by those skilled in the art that the optical detection component 51 described herein can be composed of a means of generating fluorescence at one wavelength and can be composed of a filter to prevent certain wavelengths. Furthermore, the optical detection component 51 can have