

connector. Again, the line is preferably coated with an insulating layer of polyimide. A detailed description of amperometric sensor microfabrication can be found in, for example, jointly-owned U.S. Pat. No. 5,200,051, the entire contents of which are incorporated by reference.

[0120] The measured current is used by the instrument to determine the presence or absence of the suspected target nucleic acid in the original sample. This may be a qualitative result, or, where the target is present, a quantitative determination of the amount of target in the sample. An algorithm for a particular target factors the original sample volume entering the extraction chamber, the number and efficiency of amplification cycles and the efficiency of the capture reaction along with any other necessary factors to determine the original concentration of the target in the sample. Such factors are independently determined using known samples from a reference method. These methods are well known in the art.

[0121] The overall time for the assay, from sample entry into the amplification single-use device to results determined by the detection cartridge, takes between about 10 and about 30 minutes, preferably less than 20 minutes. The overall time generally depends on the specific target and the required number of amplification cycles.

[0122] A significant advantage of the disclosed device and instrument combinations is that once the sample has entered the device, all other steps are controlled by the instrument, thus eliminating possible human error in the test cycle. Consequently, the system can be used reliably by those not specifically skilled in analytical laboratory measurement. For example, a physician can use the system at the bedside or during a patient's office visit. The instrument is also portable, and can be battery-powered or solar-powered. As a result, the system can also be used at remote locations, such as, for example, in environmental monitoring and hazard assessment. An added benefit of the design of the present invention is that it also retains sample residue and amplified material within the device for safer disposal.

[0123] Various other embodiments and configuration are within the scope of the invention. For example, an instrument according to exemplary embodiments can have all the actuation and electrical connection elements in a single port with which the amplification and detection features of the cartridge mate. Alternatively, one port on an instrument can operate the amplification steps, after which the device is inserted into a second port for the detection steps. Such a second port can be on the same or a different instrument. Optionally, the transfer of amplicon from the amplification component to the detection component can be manually actuated, although such a step is preferably under instrument control. An alternative embodiment of the detection step can be based on optical detection and real-time PCR. In such an alternatively exemplary embodiment, the amplification chamber can include an optical window to permit real-time PCR measurement with optical detection. Reagents and methods for real-time PCR are well known in the art.

[0124] The examples presented herein are merely illustrative of various embodiments of the invention and are not to be construed as limiting the present invention in any way. It will be appreciated by those of ordinary skill in the art that the present invention can be embodied in various specific forms without departing from the spirit or essential characteristics thereof. The presently disclosed embodiments are considered in all respects to be illustrative and not restric-

tive. The scope of the invention is indicated by the appended claims, rather than the foregoing description, and all changes that come within the meaning and range of equivalence thereof are intended to be embraced.

[0125] All United States patents and applications, foreign patents and applications, and publications discussed above are hereby incorporated by reference herein in their entireties.

What is claimed is:

1. A single-use nucleic acid amplification device for producing an amplicon, comprising:
 - a housing; and
 - an amplification chamber, comprising:
 - an ingress with a first reversible seal;
 - an egress with a second reversible seal;
 - a sealable sample entry orifice; and
 - a first wall forming a portion of the amplification chamber,
 - wherein the first wall comprises a thermally conductive material and includes a first surface and a second surface,
 - wherein the second surface includes a heating circuit and a temperature sensor,
 - wherein the sample entry orifice permits a sample of nucleic acid to enter the amplification chamber,
 - wherein the ingress is connected to a first conduit along with a pump and a reservoir, and
 - wherein the egress is connected to a second conduit permitting egress of the amplicon from the amplification chamber.
2. The amplification device of claim 1, wherein the pump comprises a flexible diaphragm.
3. The amplification device of claim 2, wherein the flexible diaphragm is capable of engaging and being actuated by a plunger on an instrument with which the amplification device is capable of mating.
4. The amplification device of claim 1, wherein the pump comprises a pneumatic pump.
5. The amplification device of claim 1, wherein the reservoir comprises a fluid pouch.
6. The amplification device of claim 5, wherein the fluid pouch includes a fluid for performing nucleic acid amplification.
7. The amplification device of claim 1, wherein the reservoir comprises a flexible diaphragm.
8. The amplification device of claim 7, wherein the flexible diaphragm is capable of engaging and being actuated by a plunger on an instrument with which the amplification device is capable of mating.
9. The amplification device of claim 1, wherein the first wall comprises silicon.
10. The amplification device of claim 9, wherein the silicon comprises about 30 to about 50 percent of the first surface area of the amplification chamber.
11. The amplification device of claim 1, wherein the amplification chamber includes a second wall comprising a plastic material.
12. The amplification device of claim 11, wherein the second wall comprises a wall thickness in the range of about 0.2 mm to about 5 mm, and
 - wherein the second wall includes one or more additional rib supports.