

acid-bead complex through a substantially immiscible liquid layer to separate the nucleic acid from the aqueous mixture.

[0043] Another embodiment of the present invention is directed to a method of transferring nucleic acid, comprising: contacting nucleic acid at a first location with one or more beads to form a nucleic acid-bead complex in a liquid, and transporting the nucleic acid-bead complex to a second location separated from the first location by an intermediary layer, wherein said intermediary layer is substantially immiscible with the liquid.

[0044] In yet another embodiment, a nucleic acid separation method is provided, comprising: introducing a sample comprising biological cells through a first layer to a second layer comprising magnetic beads, wherein said first layer is substantially contiguous with said second layer; incubating the sample for sufficient time to permit lysis of the cells and form a nucleic acid-bead complex in said second layer; and applying a magnetic field in proximity to the complex sufficient to move said complex from said second layer through said first layer, thereby effectively filtering said complex.

[0045] In a further embodiment of the present invention, a method for extracting and amplifying nucleic acid is provided, comprising: introducing a sample comprising biological cells through a first layer to a second layer comprising magnetic beads, wherein said first layer is substantially contiguous with said second layer; incubating the sample for sufficient time to permit lysis of the cells and form a nucleic acid-bead complex in said second layer; applying a magnetic field in proximity to the complex sufficient to move said complex from said second layer through said first layer, thereby substantially removing said complex from said second and first layers; and introducing said complex to a vessel containing a polymerase chain reaction (PCR) cocktail, wherein at least a portion of the nucleic acid elutes off said magnetic beads during a first heating cycle of the PCR.

[0046] Another embodiment of the present invention is directed to a method for extracting nucleic acid from a biological sample, comprising: applying a biological sample comprising cells containing nucleic acid to a biochemically inert filter positioned between a first location and a second location in a conduit; retaining the sample on the filter for sufficient time to permit extraction of nucleic acid onto the filter; applying a wash fluid through the conduit, whereby said wash fluid substantially removes from the filter matter capable of interfering with a nucleic acid amplification reaction, while substantially retaining the extracted nucleic acid on the filter; and applying an aqueous solution to the filter at a temperature above about 75° C. to elute the extracted nucleic acid from the filter.

[0047] The present invention particularly addresses expanding opportunities for point-of-care diagnostic testing, i.e. testing that is rapid, inexpensive and convenient using small volumes of accessible bodily fluids such as, for example, blood or buccal cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] FIG. 1 shows nucleic acid purification in a tube using a lytic buffer layer, a wax layer and magnetic beads.

[0049] FIG. 2 shows a polyacrylamide gel of PCR products with and without beads and with and without blood, and also purified DNA controls.

[0050] FIG. 3 shows a polyacrylamide gel of PCR products with beads and blood.

[0051] FIG. 4(a)-(d) show different perspectives of the filter holder.

[0052] FIG. 5 shows PCR amplification of a buccal swab sample isolated from a filter.

[0053] FIG. 6 shows a topological representation of the integrated single-use device and its interaction with the instrument.

[0054] FIG. 7(a) shows a schematic of the PCR amplification method, FIG. 7(b) shows a schematic of PCR amplification without a self-annealing primer and FIG. 7(c) shows a schematic of PCR amplification with a self-annealing primer.

[0055] FIG. 8(a) shows a typical chronoamperometry output for PCR plus conjugate and conjugate alone, and FIG. 8(b) shows a typical chronoamperometry output for control plus conjugate and conjugate alone.

[0056] FIG. 9(a) shows chronoamperometry of different amplicon concentrations and FIG. 9(b) shows a plot of the steady-state current signal versus amplicon number.

[0057] FIG. 10 shows a schematic for rolling circle amplification (RCA).

[0058] FIG. 11 shows a schematic for strand displacement amplification (SDA).

[0059] FIG. 12(a)-(b) show two perspectives of an electrophoresis component for integration into a single-use device for nucleic acid testing.

[0060] FIG. 13(a)-(g) show an electrophoretic separation using a component for integration into a single-use device for nucleic acid testing.

[0061] FIG. 14 shows an electrophoretic separation of a primer and an amplicon using a component (as shown in FIG. 13) for integration into a single-use device for nucleic acid testing, confirmed by a second electrophoresis gel.

[0062] FIG. 15 shows an oligonucleotide primer lacking CLAM-like features.

[0063] FIG. 16(a) shows the CLAMI primer and FIG. 16(b) shows the CLAM2 primer.

[0064] FIG. 17(a) shows an optical detection-based single-use cartridge where an optical sensor is integrated into the device and FIG. 17(b) shows an optical single-use cartridge where the sensing region is a cuvette feature permitting detection with a light source and detector integrated into the instrument.

[0065] FIG. 18 shows an extraction device containing a filter region integrated into a single use cartridge for nucleic acid testing.

[0066] FIG. 19 shows a two-part cartridge with a separate extraction component that can mate with the amplification and detection component.

[0067] FIG. 20 shows a two-part cartridge with a separate detection component that can mate with the extraction and amplification component.

[0068] FIG. 21(a) shows a cartridge and instrument separately and FIG. 21(b) shows the cartridge inserted into the instrument.

[0069] FIG. 22 shows examples of optical detection chemistries.

[0070] FIG. 23 shows an extraction and amplification component where a silicon chip provides one of the walls forming the extraction and amplification chambers.

[0071] FIG. 24 shows a single-use device with electrophoretic separation of unused primers after amplification.

[0072] FIG. 25(a)-(b) show a cleavage reaction creating a "trigger event" for further amplification and detection.