

contains a molecule that the moiety, e.g. alkaline phosphatase, causes to generate an optically detectable signal, e.g. fluorescence. Such molecules are well known in the art. In all other respects the operation of the single-use device is the same as in the electrochemical detection mode.

Detailed Description of Nucleic Acid Testing Cycle with Single-Use Device

[0185] The preferred embodiment of an assay cycle using the single-use device **100** in conjunction with the instrument **200** is as follows. An approximately 10 uL blood sample is added to the entry port **101** and is drawn by capillary action into the extraction chamber **102**. An entry port closure element **117** is then used to seal the entry port. Reagents **103** comprising a chaotropic agent, lithium dodecylsulfate and dithiothreitol and a chelating agent, ethylene diamine tetraacetic acid, which are coated on the wall of the chamber dissolve into the blood sample and cause lysis of the cells and permit nucleic acid from within the cells to be liberated and to be adsorbed onto the carboxylate coating on the magnetic beads **104**. A magnetic field can be used to agitate the beads to promote mixing within the chamber and speed up the rate of extraction. This step of the extraction process generally takes about 0.3 to less than 1 minute. Where the magnetic field is deployed, this is under the automatic control of the instrument and is determined by an embedded algorithm that controls the test cycle. Once this step is complete, the instrument deploys a magnetic field which holds the magnetic particles to the side of the extraction chamber. Wash fluid from the wash fluid chamber **122** is then pneumatically forced into the extraction chamber and flushes the contents into the wash fluid waste chamber **123**. Note that the wash fluid waste chamber has a vent **146** and that during this step the instrument seals the ingress **118** to the amplification chamber, thus waste fluid is directed into the waste chamber rather than entering conduit **106**. This step takes about 30 seconds. The wash fluid in the preferred embodiment is deionized water and the volume of wash fluid that passes through the extraction chamber is 20 to 30 uL. Note also that the silicon chip that forms one wall of the amplification chamber also forms one wall of the extraction chamber, as shown in FIG. **23**, thus the extraction process can be performed at a controlled temperature. In the preferred embodiment nucleic acid extraction from blood occurs at room temperature.

[0186] In the next step, the instrument opens the ingress seal **118** and releases the magnetic particles from the wall of the extraction chamber and draws them through the wax layer at the boundary of the extraction chamber and conduit leading to the amplification chamber. The instrument ensures that the temperature of the extraction chamber is sufficient for the wax to be in liquid form and permit the magnetic particles to pass through. In the preferred embodiment the wax is paraffin and the controlled temperature is at between 45 to 700 C. As discussed previously passage of the particles through the wax minimizes interferents of PCR amplification, which can include hemoglobin. The particles are then drawn into the amplification chamber. In the preferred embodiment the amplification chamber has a volume 10 of 10 to 20 uL. As shown in FIG. **23** the chamber **606** is "U" shaped having a total length of 8 mm, width of 8 mm and height of 0.25 mm. Other features of the element **609** shown in FIG. **23** are chambers **600** and **602**, ports **603**, **604** and **607**, conduits **601** and **608**, and heater **605**.

[0187] The next step of the process involves the instrument pneumatically displacing the PCR amplification reagent from

its chamber into the amplification chamber. The PCR amplification reagents comprise DNA polymerase, a buffer and a modified primer. The primer comprises a sequence of bases complementary to a first region of the target nucleic acid, a polymerase blocking region, a single stranded hybridization region attached to the polymerase blocking region with an attached detectable moiety, which is biotin. In the preferred embodiment the buffer consists of 22 U/ml *Thermococcus* species KOD, thermostable polymerase complexed with anti-KOD antibodies, 66 mM Tris-SO₄ (pH 8.4), 30.8 mM (NH₄)₂SO₄, 11 mM KCl, 1.1 mM MgSO₄, 330 uM dNTPs, as well as proteins and stabilizers (Invitrogen Life Technologies AccuPrime Pfx SuperMix manual, Cat. No. 12344040), but alternatively could be 20 mM Tris-HCL (pH 8.8), 2 mM MgSO₄, 10 mM KCl, 10 25 mM (NH₄)₂SO₄, 0.1% Triton-X-100, 0.1 mg/ml nuclease-free BSA as described in the Stratagen Pfu DNA polymerase Instruction Manual Cat#600135 Revision\$064003d).

[0188] In the next step the instrument seals the two sealing elements in the device, **118** and **119**, to retain the beads and reagent in the amplification chamber and the cycles the temperature thirty times between 95° C. and 99° C., and a hybridization step at 68 C with durations at each temperature of 2 seconds and 12 seconds respectively. The overall amplification time is about 12 minutes. Once this step is completed, the amplified target is then transferred from the amplification chamber and into the conduit that leads to the detection region of the device. In one embodiment, at the end of the PCR reaction gaskets sealing the PCR chip entry and exit ports are lifted off of both the entry and exit ports. An air bladder is depressed in the cartridge, creating a positive air pressure in the entry port gasket, forcing the liquid out of the exit port gasket, moving the liquid towards the final detection region of the chip. Here, a set of conductivity bars are used for monitoring the movement of liquid to the detection region.

[0189] In the preferred embodiment the clam-like primers are used, thus in the unheated conduit that leads to the detection region, these primers re-anneal to themselves and are effectively removed from the assay as interferents. In an alternative embodiment, where electrophoresis is used to separate out unwanted primer the elements described in FIG. **12** and FIG. **13** are combined into the single-use device as shown in FIG. **24**. This separation process is described above. In the single-use device with electrophoretic separation, the instrument makes electrical connection to the electrophoresis electrodes **74**, **75** and **76** (see FIG. **13**), and **652**, **653** and **654** (see FIG. **24**). In the device the time for this step is typically less than 1 to 2 minutes, depending on the sizes of primer and amplicon. In another alternative embodiment where enzymatic removal of unused primer is employed, the enzymatic mixture is applied to a portion of the wall **150** of the conduit leading from the amplification chamber to the detection region. This material dissolves onto the liquid containing the amplicon and converts the primer to a non-interfering form as described above. The dry reagent mixture on the wall is preferably the enzyme in a support matrix comprising trehalose or ficoll, which promotes rapid dissolution. The time taken for the enzymatic step is typically about six minutes and is dependent on the amount of enzyme, temperature, type of primer being removed. In another embodiment, post-hybridization of the amplicons with a first capture oligonucleotide, which removes the detection region of the amplicons, followed by a wash step to remove any unbound unincorporated oligonucleotides which would be involved in the final capture