

step can be used. The amplicons and primers bound in the first capture step are then un-bound using heat or alkaline conditions, then allowed to move to the final detection region, where the capture oligonucleotides capture fully created amplicons.

[0190] In the next step the amplicon arrives in the detection region and the dissolution of the reagent on the wall of the detection chamber **151** occurs. In the preferred embodiment this reagent is streptavidin-labeled alkaline phosphatase which binds to the moiety on the amplicon which is preferably biotin to form a complex of amplicon and the enzyme. This complex can then bind to the capture oligonucleotide on the sensor. Depending on the kinetics the amplicon may also bind first to the capture oligonucleotide and then the labeled enzyme. In the device the time for this step is typically about 5 to 15 minutes.

[0191] In the final step detection reagent is displaced from the detection reagent chamber into the sensing region, thereby displacing any unbound amplicon and labeled enzyme to the waste chamber. Elements **152** and **153** which are constriction that cause turbulence in the region of the sensor may optionally be included to enhance the efficiency of the hybridization step, thus reducing the hybridization time and the amount of wash fluid that is required. In the device the time for this step is typically less than 70 seconds and the amount of wash fluid that is used is about 10 to 50 uL. As stated previously the wash fluid also contains a reagent that enables detection. A trailing portion of the fluid is retained over the sensor, thus enabling the captured alkaline phosphatase to convert the reagent p-aminophenol phosphate to p-aminophenol which is then oxidized at the electrode to give rise to a measurable current. In the device the time for this step is typically less than 1 minute. Positioning of the trailing edge with respect to the sensor may be achieved using a pair of electrodes **155** and **156** forming a conductivity sensor as described above.

[0192] 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.

[0193] In a related embodiment, a second sensor **154** is provided in the detection region to account for any non-specific binding of the streptavidin-labeled alkaline phosphatase to the first sensor. The second sensor is the same as the first but, has a capture oligonucleotide that does not bind to the amplicon. Any signal at the second sensor is subtracted from the signal at the first by the algorithm. The overall time for the assay, from sample entry into the single-use device and insertion into the instrument, takes between about 10 and 20 minutes and generally depends on the specific target and the required number of amplification cycles. When the genetic test is complete and result is displayed by the instrument, the actuation mechanism within the instrument then releases the device and it can be removed and discarded by the user. The instrument is then ready to receive a new single-use device. A significant advantage of the disclosed device and instrument

combination 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. This means the system can be used reliably by those not specifically skilled in analytical laboratory measurement. For example a physician may use the system at the bedside or during a patient's office visit. The system may also be used at remote locations, for example in environmental monitoring and hazard assessment. An added benefit of the design is that it also retains sample residue and amplified material within the device for safer disposal.

[0194] In an alternative embodiment of housing **100**, the extraction chamber **102** contains a filter material **157** and **421**, impregnated with extraction reagents comprising a chelating agent and a chaotropic agent. One wall of the extraction chamber is also composed of heating element with a thermistor for controlling temperature. The filter material is preferably composed of 3 MM Whatman paper and has a carboxylated surface which preferentially binds nucleic acid. When the sample, e.g. blood, enters the extraction chamber, it dissolves the extraction reagent and nucleic acid from the cellular material binds to the filter. This step of the extraction process takes about 0.5 to 2 minutes. A bolus of wash fluid from the wash fluid chamber **122** is then pushed through the extraction chamber and exits into the wash fluid waste chamber **123**, carrying away lysed cellular debris from the sample, while leaving the extracted nucleic acid adsorbed onto the filter. Multiple boluses of wash fluid may be used to ensure a complete wash. A further bolus of wash fluid is then pushed into the chamber and the instrument activates the heating element and controls the temperature of the bolus of fluid to 90° C., by means of the thermistor. This caused the nucleic acid adsorbed onto the filter to desorb from the filter and dissolve in the fluid. The fluid containing the nucleic acid material is then pneumatically transferred to the amplification chamber. In this embodiment the wash fluid is preferably deionized water.

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