

and no detection result would be expected after amplification. The quality control steps allowed by the positive and negative amplification and detection chambers are an essential step in gaining FDA CLIA waiver status, for embodiments where this might be required.

[0110] The make up of the reagents used in the master mix and positive control for the target nucleic acid sequence using the LAMP method is defined by the Eiken Chemical Co. Ltd of Japan. Such master mixes include primer mixes for a variety of infectious diseases, including H5 avian influenza for example.

[0111] FIG. 12 shows how the amplification and detection is performed inside the test sample amplification and detection chamber 20. It should firstly be noted that the detection chamber 20 is transparent as the microfluidic device 7 is fabricated from an optically-transparent material. Instrument 8 includes a number of light emitting diodes (LEDs) 30 mounted onto a printed circuit board (PCB) 29. The LEDs 30 are adjacent to one side of the chamber 20, and shine collimated light through the chamber 20 in a direction which is orthogonal to the planar surface of the microfluidic device 7. The LEDs 30 are provided with a particular wavelength to suit subsequent turbidimetric detection.

[0112] The micro magnetic stirrer bar element 22 which is captured within chamber 20 is also constructed from an optically-transparent material. The outer edges of the stirrer bar element are printed with an iron-oxide material 32. This in turn allows a remote magnetic stirrer head 35 (to which is fitted outer magnets 36) to turn the stirrer bar element 22 inside the chamber thereby mixing the fluid contents contained within the chamber without disrupting the light path through the chamber provided by LEDs 30. Magnetic stirrer head 35 is driven by motor 38 via shaft 37, and this motor/stirrer head assembly is part of instrument 8.

[0113] On the reverse side of the test chamber a transparent Indium Tin Oxide (ITO) heating element 39 is printed onto the microfluidic device 7. This ITO heating element 39 makes an electrical contact with the instrument 8 in order to provide isothermal incubation to 62.5° C. as recommended for isothermal amplification for the LAMP method. Because the ITO heating element 39 is transparent, it does not disrupt the light path provided by LEDs 30.

[0114] Adjacent to the ITO heating element 39 is an array of photodiodes 33 mounted on a PCB 34 and which are part of instrument 8. The photodiodes 33 receive light emitted by the LEDs 30, and are able to detect the proportion of light that has been transmitted through chamber 30.

[0115] As the LAMP reaction proceeds, in the event of a positive test the amount of turbidity in the test sample amplification and detection chamber increases over time. After a known period of time, the turbidity level inside chamber 20 will increase to a level where photodiodes 33 are receiving a significantly lower proportion of light from LEDs 30 than they were at the start of the test. Conversely, in the event of a negative test, there will be no turbidity in the test chamber 20, and photodiodes 33 will receive the same proportion of light from LEDs 30 as at the start of the test. Thus, using a simple low cost turbidimetric detection approach, the system is able to diagnose and quantify the presence of the target nucleic acid sequence. Subsequent computer processing by instrument 8 is able to translate and display the results of the turbidimetric detection into clinically useful information which may be easily recorded or interpreted by a non-specialist operator.

[0116] The same process described above is also used in the positive control amplification and detection chamber 17 to verify that the assay has run correctly. Such a positive control step is a mandatory part of quality control in most molecular biology assays.

[0117] FIG. 13 shows an alternative detection embodiment inside the test sample amplification and detection chamber 20. In this case the Light-Emitting Diodes (LEDs) 30 are chosen to have an emission wavelength which corresponds to the absorbance wavelength of a fluorophore included in the master mix. These LEDs may shine through a thin film interference filter 40 (TFIF) which has a narrow bandpass and which allows light of only a short wavelength band to be transmitted through chamber 20.

[0118] The stirring and heating approach using this detection method is the same as was described for FIG. 12.

[0119] Light at the particular wavelength for the fluorophore of interest then causes the fluorophore to emit light at a different wavelength (the excitation wavelength) in the event that the target nucleic acid sequence is present and is undergoing amplification. This phenomenon where light is received by a fluorophore at one particular wavelength, and which causes the fluorophore to emit light at a second particular wavelength is known as a "Stoke's Shift". The excitation light output may then also be passed through a second bandpass filter 41 prior to being received by photodiodes 33.

[0120] As the LAMP reaction proceeds, in the event of any light being received by photodiodes 33, a positive test result will be returned. Conversely, in the event of no light being received by photodiodes 33, a negative test result will be confirmed. The fluorometric detection approach may provide improved sensitivity over the turbidimetric detection method.

[0121] FIG. 14 shows an alternative heating approach for the polymerase reaction in which a secondary chamber 42 is provided within the microfluidic device 7. This chamber 42 is filled with either water or paraffin oil, which is heated in a separate zone to 62.5° C. by instrument 8 via a conventional heating element and recirculated within chamber 42. This heating approach may provide faster heating and more accurate temperature control than ITO heating element 39. Heating chamber 42, and the heating fluid (water or paraffin oil) are transparent so as not to block the light transmitted through the chamber. Alternatively they may be positioned so as not to obstruct a light path from LEDs 30 to photodiodes 33. Heating chamber 42 is also positioned at the minimum distance X from the chamber 20 in order to maximize heat transfer efficiency.

[0122] FIG. 15 shows a system block diagram of all the major elements of the diagnostic system, including the instrument 8, the reagent pack, and the microfluidic cartridge assembly 300 using the example of a reagent pack for the detection of H5 avian influenza. It should be noted from this diagram that instrument 8 may require on-board cooling system (such as thermoelectric cooling elements) to keep the reagents in the reagent pack at a low storage temperature. Optionally, heat labile reagents such as polymerase and primer sets may be dehydrated and stored directly on the microfluidic cartridge 7, and then rehydrated in the elution buffer during the assay.

[0123] On completion of the test, the test result is displayed on the liquid crystalline display 9 of the instrument 8, thereby indicating whether the test result is positive or negative, and (in the event of a positive test) quantifying the amount of the virus or pathogen present. This is shown in FIG. 16. Such a