

labeled binding reagents (preferably antibodies) for the assays, stabilizing reagents, and/or other additives such as blocking reagents. For assays employing nitrous acid as an extraction reagent, the dry assay reagent preferably comprises sufficient base (preferably, the base form a pH buffer such as Tris, Hepes, phosphate, PIPES, etc.) to bring the pH of the sample to between 4-10, more preferably between 5-9, more preferably between 6-8. The dissolved reagents may be mixed into the sample by moving the sample back and forth in the fluid line, using sensors to ensure that the liquid remains within a defined region of conduit.

[0345] The sample containing the reconstituted assay reagents is then drawn into detection chamber 3230, where immobilized binding agents (preferably antibodies) are present on individual binding zones that are, more preferably, located on electrodes in an electrode array. The sample is incubated for a specific time period over the binding zones, either in a static mode or under mixing, during which time the analyte and labeled binding reagent can bind to each other and/or to the individual binding zones. Mixing is performed by moving the sample back-and-forth between sensors at the end of the read chamber.

[0346] Sometime before, during, or after sample incubation, a positive control assay is also performed in the other binding chamber: wash buffer is pulled from the wash buffer storage chamber 3240 to sensor #2 by pulling vacuum on vent port 3264 with vent port 3241 open to air. A fluid slug is metered by closing vent port 3241 and opening vent port 3244 to introduce air behind the metered fluid as it is drawn toward control detection chamber 3250. The metered fluid slug is then drawn over and dissolves dry control reagents 3252. These reagents, preferably, include labeled binding reagents (preferably antibodies), defined amounts of the analytes for the assays (to provide positive controls), stabilizing reagents and/or other assay reagents. The positive control sample, comprising the metered wash buffer slug and rehydrated control reagents, is then incubated in the control detection chamber 3250 either in a static fashion or with mixing by moving the sample between sensors located at the end of the control binding zone.

[0347] Following the incubation steps, the positive control sample is drawn into waste chamber 3254 and the extracted swab sample is drawn into the waste chamber 3228. Both detection chambers are washed in a consecutive or simultaneous manner by drawing wash buffer from wash buffer chamber 3240 through the detection chambers and into their corresponding waste chambers (waste chamber 3228 for detection chamber 3230 and waste chamber 3254 for control detection chamber 3250). The wash reagent used during the wash step is preferably segmented by introducing air at vent port 3244. After washing, both the control and sample binding zones are filled with wash buffer to complete the fluid sequence. Advantageously, wash reagent flows through detection chamber 3230 in a direction opposite that in which sample was introduced into chamber 3230. This reverse flow wash ensures the efficient removal of any components in the sample and/or extraction buffer that could interfere with a measurement in the detection chamber.

[0348] Preferably, the binding of analyte and/or labeled binding reagents to binding domains in the detection chambers is measured by an ECL measurement as described above for cartridge 2500. ECL is initiated by applying the desired electrical potentials to electrodes supporting the binding zones. The positive control binding zones in detection cham-

ber 3250 will provide a positive signal for each assay and may be used to provide assurance that the assay reagents onboard the cartridge have not degraded. The ECL signal from any of the sample binding zones in detection chamber 3230 indicates the presence of analyte binds to that capture zone or competes with the binding of a labeled reagent to that capture zone.

[0349] A preferred embodiment of the performance of an assay using cartridge 3700 and reader 4300 is described below, the description focusing on aspects that differ from the operational steps described above for other embodiments of the cartridge and reader. The operational description includes the use of a preferred valve configuration shown in FIG. 37. The basic operations that are used to move fluid in this preferred embodiment (i.e., opening vent ports on one side of the fluid to be moved to air and applying positive or negative pressure to a vent port on the other side of the liquid) will be apparent and are not always described. During operation, the instrument continually monitors to make sure that fluid fronts pass appropriate optical sensors and do not pass by the protection sensors at the vent ports. If inappropriate fluid movement is detected, the instrument may stop the processing of a cartridge or implement corrective actions.

[0350] A sample is collected on a swab with a pre-defined weak point (as described in the text above) is inserted in sample chamber 3720. During insertion into the curved chamber, the swab head breaks off and is retained by retaining features 3721a and 3721b as the swab shaft is removed. The cartridge cap is then sealed. If lot-specific parameters for the lot of cartridges are not stored on the instrument, the user can download them onto the instrument through its network interface or through connection of an external memory device (EEPROM, memory chip, RFID, barcode, etc.). Alternatively, these settings are stored on memory attached to the cartridge. The user may enter patient and operator information into the reader GUI, if desired, and then inserts the cartridge into cartridge tray 4320 until latched in place with latch 4420 (as shown in FIG. 44). Through the GUI, the user instructs the reader to begin processing and the cartridge tray is drawn into the reader and aligned with the reader's cartridge processing sub-components (as described in FIG. 43 and the associated text description) which includes mating the cartridge to the appropriate electrical and fluidic connections.

[0351] Once the cartridge is correctly positioned, the reader uses ampoule breaking assembly 4200 to break the extraction reagent buffer ampoule (which in the case of a flu typing/subtyping panel is, preferably, a low pH buffer as described above). The pump is used to aspirate air from the extraction reagent chamber through the sample chamber and into collection component 3726 until liquid reaches the collection component optical sensor indicating that the correct volume of extracted sample has been collected (described in greater detail in FIG. 38 and the accompanying description). Optionally, the extraction process can use an air-segmented stream of extraction buffer by alternating between aspirating from the extraction buffer chamber vent and the air port vent.

[0352] The sample is metered into the assay flow cell channels by applying pressure at the collection component vent and connecting the left or right waste vents to ambient (to meter into the left or right channels) until the sample fronts reach optical sensors 2a and 2b in the two channels (as numbered in FIG. 37(b)). Air is drawn to the collection chamber vent from the air port vent to finish the metering process. Air