

chamber vent valve **2442A** are then closed. Similarly, sample is drawn into sample conduit branch **2515B** by operating the pump with sample chamber vent valve **2412** and waste chamber B vent valve **2442B** open (see FIG. 26, panel **2603**). Defined slugs of sample fluid are drawn into the sample conduit branches by operating the pump with air vent valve **2422** open as well as the waste chamber A and B vent valves **2442A-B** (see FIG. 26, panel **2604**). In this and subsequent steps, two slugs may be moved simultaneously through sample conduit branches **2515A** and B by holding both waste chamber vent valves open or sequentially through the branches by opening one at a time.

[0328] The sample conduit branches, preferably, comprise dry reagent pills (preferably containing one or reagents selected from blocking agents, pH buffers, salts, labeled binding reagents, and the like). One or more of the conduit branches may also comprise spiked analyte for spike recovery controls. In order to reconstitute the dried reagent, the two sample fluid slugs are moved back and forth across the pill zone a predetermined number of times by opening air vent valve **2422** and waste chamber vent valves **2442A** and/or B and operating the pump to alternate between applying positive and negative pressure to the waste chamber vents (FIG. 26, panels **2605-2606**). The two sample fluid slugs may be moved back and forth simultaneously or mixing of the two slugs may be accomplished in series. The number of repetitions that the sample fluid is cycled across the pill zone may be dependent upon a number of factors, including but not limited to, size/volume of reagent dried reagent pill, composition of reagent pill, drying method employed at the time of reagent deposition/pill formation, and the like. In accordance with preferred embodiments, the number of repetitions that need to be carried out by the fluid handler subsystem can be cartridge specific and can be automatically ascertained by the cartridge reader from the information encoded in the machine-readable indicia affixed/incorporated onto the cartridge. The number of repetitions may be predetermined through empirical results but may also be determined in-situ through the use of one or more sensors adapted and configured to measure the degree of mixing of the reagent(s) and sample fluid; e.g., use of optical sensors (transmittance or reflectance), electrical sensors (impedance, conductance, resistance, and the like).

[0329] The sample fluid slugs are now moved into their detection chambers **2550A** and **2550B** by operating the pump with air vent valve **2422** and waste chamber vent valve **2442A** open until the sample slug is detected at sensor **7** and by operating the pump with air vent valve **2422** and waste chamber vent valve **2442B** open until the sample slug is detected at sensor **8** (FIG. 26, panels **2607-2608**). The sample slugs are incubated in the detection chambers to allow constituents of the sample (e.g., labeled binding reagents, analyte, control analyte, etc.) and immobilized binding reagents within the detection chamber to bind to form binding complexes in the detection chamber. Preferably, a mixing operation is employed to enhance the rate of these binding reactions. Preferably, mixing is achieved by moving the fluid slugs back and forth in the detection chamber by a process analogous to that described for reconstituting the reagent pill (optionally, using sensors **1**, **2**, **11** and **12** to provide stopping points in each direction). The aspirate and dispense operations are repeated a predetermined number of times, or until the degree of mixing desired has been achieved/detected. After completion of the incubation step, the air and waste chamber vent

valves are used to draw the slugs out of the detection chambers and into waste chambers **2540A** and B (FIG. 26, panels **2609-2610**).

[0330] Preferably (as shown), the assay process includes a wash step for removing sample and unbound labeled reagents from the detection chamber. The wash uses a wash reagent (preferably, a buffered solution, more preferably comprising a non-ionic surfactant such as Triton X-100 and most preferably comprising an ECL coreactant such as TPA or PIPES) stored in reagent chamber A **2530A**. If the wash reagent is in a reagent module (preferably, ampoule) and the module hasn't been opened, it is opened now. Optionally, the remaining sample fluid is first routed back into the sample chamber to prevent contamination of the wash reagent: first wash reagent is drawn from reagent chamber A **2530A** into one of the sample conduit branches by operating the pump to apply negative pressure with reagent chamber A vent valve **2432A** and the corresponding waste chamber vent valve **2442A** or B open (and, preferably, overcoming a capillary break provided by a Z-transition in the reagent conduit); then excess sample is drawn into the sample chamber by operating the pump to apply positive pressure to the waste chamber vent with the sample chamber vent valve open (FIG. 26, panels **2611-26120**). Wash reagent is then drawn from reagent chamber A **2530A**, through detection chambers **2550A** and **2550B** and into waste chambers **2540A** and **2540B** by operating the pump with reagent chamber A vent valve **2432A** and waste chamber vent valves **2442A** and/or **2442B** (simultaneously or sequentially) open (FIG. 26, panels **2613-2616**). As shown, in particularly preferred embodiments, the wash fluid may be segmented, i.e., broken up by one or more slugs of air. It has been observed that wash fluid alternating with air within the detection chambers increases the effectiveness of the clean cycle. Segmenting the wash fluid can be accomplished by periodically and temporarily opening the air vent valve **2422** and simultaneously closing the reagent chamber A vent valve **2432A** so that air is drawn into the sample conduit. Timing and duration of these operations would dictate the size and frequency of the air slugs introduced into the segmented wash fluid slug.

[0331] In the two step format, one or more labeled detection reagents may be incubated in the detection chambers in an additional incubation step. Preferably, the detection reagent solution is prepared by reconstituting a dry reagent pill comprising the detection reagents with an assay diluent contained within reagent chamber B **2530B**. If the assay diluent is in a reagent module (preferably an ampoule) and it is not already broken, it is broken now. The assay diluent is drawn into elongated reagent conduit **2535** by aspirating at one of the waste chamber vents while opening reagent chamber B vent valve **2432B** until the assay diluent reaches sensor **13** (FIG. 26, panel **2617**). A defined volume of assay diluent is prepared by closing reagent chamber B vent valve **2432B** and opening air vent valve **2422** and continuing to aspirate at the waste chamber vent; reconstitution of the dry reagent in the elongated reagent conduit is promoted by alternating the pump between positive and negative pressure so as to move the slug back and forth over the dry reagent pill (FIG. 26, panel **2618-2619**). In a process analogous to the introduction of sample to the detection chambers, the slug of detection reagent solution is i) distributed between the sample conduit branches **2515A** and B, ii) introduced to the detection chambers (**2550A** and B), incubated in the detection chambers while moving the slugs back and forth in the chambers to