

but not in response to an extending tray. In other words, pivot cam 4610 slips or pivots when cartridge tray 4320 retracts as illustrated in FIGS. 46(c)-(d), but remains rigid to push traveler block 4520 when cartridge tray extends as illustrated in FIGS. 46(e)-(f).

**[0321]** With reference to FIGS. 24 through 26, performance of an assay using a preferred cartridge of the invention will be described. This exemplary method will be described in the context of a two-step multiplexed binding assay using antibodies as binding reagents and ECL as the detection methodology, however, it will be readily apparent to the skilled practitioner that the described fluidic operations can be used in a variety of different assay formats (e.g., binding assays using other classes of binding reagents, enzymatic assays, etc.) and with a variety of different detection technologies. It is also apparent that the sequence of operation discussed below may vary according to differences in the configuration of a particular cartridge as well as differences in the particular assay to be performed.

**[0322]** During operation, the pump vent line valve may be used to enable and disable pressurization of the system for more precise fluid control; when the pump's vent is opened, the system returns to ambient pressure very quickly. Typical fluid draw operations, i.e., routing of fluid within and throughout the fluid network, involve closing the pump vent valve and opening i) one or more (preferably, one) cartridge vent valves, e.g., the sample, air, reagent chamber A and/or reagent chamber B vent valves and ii) one or more (preferably, one) control valves, e.g., waste chamber A or waste chamber B control valves. Therefore, a slug of fluid will move along a path through the fluid network in the cartridge when the fluid channels comprising that path is vented to air at one end and subjected to either pressure or vacuum at the other end.

**[0323]** A user selects the appropriate cartridge for carrying out a desired measurement and introduces sample to the sample introduction port of a cartridge and, preferably, seals a closure on the sample introduction port. The cartridge is inserted into the cartridge reader. Preferably, the cartridge will include features that ensure the cartridge is inserted in the proper orientation; e.g., by incorporating identifying marks to show which direction it should be placed on the tray and/or mechanical features that guide the user to place it in the correct orientation. After the user has successfully prepared and inserted the cartridge, reading/processing of the cartridge is performed by the cartridge reader upon receiving an indication from the user that the read cycle should commence (alternatively, the reader may automatically begin operation upon confirming that a properly prepared cartridge has been properly inserted into the cartridge reader). The subsequent reading of the cartridge is preferably automated; e.g., the cartridge reader's electronic control system (computerized control system or the like) automatically processes and reads the cartridge.

**[0324]** The automated sequence of operations to be performed by the cartridge reader will now be described. Preferably the cartridge includes machine readable indicia, e.g., barcode, that is detected and processed by the cartridge reader. For example, processing of the machine readable indicia, may allow the cartridge reader to verify that a valid, readable barcode has been detected and thereafter determine the operational parameters for the present read cycle; i.e., determine the set of assays/tests to be performed, extract any relevant instrument configuration parameters and verify the

expiration date. In certain preferred embodiments, the cartridge reader may prompt the user for any data that it requires; e.g., operator ID, sample or patient ID, and the like. Additionally, if the cartridge is capable of running a panel of test, the user may be able to select which test(s) within the panel should want be performed.

**[0325]** Preferably, the reader has a cartridge handling sub-system that mechanically engages the cartridges and moves/aligns it into position. Preferably, this process includes positioning the cartridge within a light-tight enclosure. The reader also makes the appropriate fluidic and/or electronic connections to the cartridge and, optionally, breaks or pierces any reagent modules (e.g., reagent ampoules) present in cartridge reagent chambers. As discussed above, in one preferred embodiment, the cartridge handler's motion would be physically coupled to the fluidic and electronic handlers (and, optionally, the reagent module release mechanism) such that upon positioning the cartridge within the light tight enclosure the electrical contacts and the fluidics manifold engage the cartridge at their respective engagement points (and, optionally, the reagent module release mechanisms releases reagent from any reagent modules). Next, where required or preferred, the electronic control system begins operating a heater in order to bring the cartridge to the appropriate predetermined temperature and maintain the cartridge at such target temperature. In certain preferred embodiments temperature regulation may be controlled by a microprocessor employing a proportional derivative control to control a heater that will maintain the target temperature; preferably a suitable algorithm is employed.

**[0326]** Once the cartridge has been maintained at the target temperature for a predetermined amount of time, the fluid handler may begin processing the cartridge for reading; i.e., assemble the assay. Reference to FIG. 26 will be made to illustrate the intermediary states of the cartridge reader and the position of fluid within the fluid network of cartridge 2500 during a 2-step assay format. As presented in FIG. 26, the starting state of the cartridge 2500 (panel 2601) is illustrated and depicts the location of the constituent fluids within the fluidic network. Assay assembly preferably consists of metering specific volumes of sample fluid, reconstituting dried reagents in the sample fluid and incubating the sample fluid in the detection chambers. Predetermined valves are opened in a prescribed sequence in accordance with the desired fluid flow paths to be assumed by the constituent fluids.

**[0327]** According to the present embodiment in which two read chambers are present and will be utilized for testing the sample, two equal lengths of sample fluid (i.e., slugs) will be drawn; the length of the sample slugs is determined by the volume of the read chambers. The sample slugs are delimited from one another by introducing a slug of air between the two sample slugs. Accordingly, sample chamber vent valve 2412 and a waste chamber vent valve 2442A are opened and the pump vent is closed. The pump is subsequently activated to aspirate/draw the sample from sample chamber 2510 (preferably, overcoming a capillary break provided by a Z-transition that is used to prevent leakage of the sample from the sample chamber) into sample conduit branch 2515A. In this and other pumping steps, a pressure sensor (not shown), preferably, detects the pressure created by the operation and provides confirmation that the pump is aspirating/dispensing fluid properly. When fluid is detected at sensor 3 (see FIG. 26, 2602), the pump vent valve is opened and the pump motor is deactivated. The sample chamber vent valve 2412 and waste