

the signal from the test and calibration lines. Moreover, instead of assuming a zero value, observing the background signal in the blank region allows the calculation of a true two-point calibration curve, which is more accurate.

**[0079]** In one embodiment, the method may further include providing means for dispensing a known amount of liquid from a sample pad of the assay cassette. Such means may include, without limitation, rollers, presses, rollers or presses that include a stop that determines how much liquid can be squeezed from the samples pad, spring loaded devices that automatically press down on the sample pad to dispense a predetermined amount of liquid, and the like. In one embodiment, the testing device may include means for dispensing a known amount of liquid from the sample pad. For example, the testing device may include a port for inserting the lateral-flow chromatographic assay cassette into the testing device. Such a port may, for example, include a roller or a similar means that rolls over the sample pad and dispenses a selected amount of liquid therefrom when the cassette is inserted into the testing device.

**[0080]** In one embodiment, a single immunoassay device may contain multiple types of different capture moieties (e.g., antibodies) each conjugated with different dyes (e.g., quantum dots) and/or multiple capture bands each immobilized with different capture moieties. A single light source (e.g., an ultraviolet light) illuminates all dyes (e.g., quantum dots) simultaneously, and the detector device (e.g., a digital camera) captures the emitted signals from multiple bands simultaneously.

**[0081]** In one embodiment, analytes of interest assayed on the lateral flow immunoassay cassettes described herein may be detected and quantified by elastic light scattering. The amount of light scattered from a selected region of a lateral flow immunoassay cassette (e.g., a capture band) is highly sensitive to the amount of material in a region illuminated by an incident light. In general, elastic light scattering, coupled with angle optimization, may be as much as 100 times more sensitive than comparable reflectance or fluorescence analysis. Other excitation/detection methods may include surface plasmon detection; Rayleigh scattering, reflectance, diffuse scattering, electrochemical detection, conductivity, fluorescence, magnetic, enzymatic, transmission, absorption, acoustic detection, any other method which is based upon Beer's law, kinetic analysis (e.g., change in signal strength over time), and the like.

**[0082]** In one embodiment, a light source may be positioned at a certain angle to the lateral flow assay cassette and the detector (e.g., a detection fiber or a cell phone camera) or fiber (eventually the cellphone camera CCD). In one embodiment, the reporter(s) may be queried by taking a reading from each reporter and calculating the intensity of the scattered light. Signal intensity (i.e., the amount of scattered light that is detected) decreases as the concentration of the analyte of interest increases.

**[0083]** In an embodiment that includes a cell phone camera or the like, the camera's CCD will take an image. In one embodiment, the image may be taken with a red distance filter. In the image, the calibration standard lines and the test lines will be present. The digital image will then undergo digital image processing with a selected digital processing algorithm to produce a representative image of the color bands for the calibration standard lines and test simultaneously. For example, a digital processing algorithm may (1) identify the areas of interest (e.g., the test line and the at least

two calibration standard lines) in the image taken of the lateral flow immunoassay cassette, (2) calculate an RGB value for each pixel in the image, (3) convert RGB format to xyz format, (4) convert xyz format to Lab color format, (5) assign a numerical value to each of the areas of interest (e.g., the test line and the at least two calibration standard lines), (6) calculate a calibration curve based on the numerical values obtained from the first and second calibration standard lines values, and (7) convert the numerical value for the test line into a concentration value for the analyte of interest in the sample.

**[0084]** In addition, internal controls, such as but not limited to, a control line (e.g., a fluorescent marker) to potentially eliminate or reduce variations in the final signal from manufacturing tolerances of the lateral flow assay cassette may be used to increase the robustness and reliability of the analysis. Additionally, analysis of the white portion of the lateral flow assay cassette may be used as an additional negative control to further improve reproducibility.

**[0085]** The digital processing algorithm is able to convert the numerical value for the test line into a concentration value because the at least two calibration standard lines are selected to provide numerical values that are proportional to non-zero concentration amounts for the analyte of interest. This relationship is clarified by reference to FIG. 9, which shows a graph 700 with Lab value on the Y-axis and concentration on the X-axis. The first and second calibration standards have a known response that relates to known and, preferably, non-zero concentration values for the analyte of interest. Lab values for each of the first and second calibration standards 730 and 740 can be related to a concentration for each 750 and 760 by a simple relationship. By relating observed Lab color values to concentration values 750 and 760, a calibration curve 770 can be generated that can be used to calculate the concentration 790 of the analyte of interest in the sample based on the observed Lab color 780. One will of course appreciate that the calibration curve 770 can also be described by a mathematical formula and that the analysis algorithm may not actually generate a calibration curve, per se.

**[0086]** In one embodiment, the method may further include mixing the liquid sample with a dye conjugate prior to applying the sample to the lateral-flow chromatographic immunoassay cassette. In one embodiment, the dye conjugate is configured to interact with at least one of the analyte of interest or the ligand to provide a visual readout related to the presence or concentration of the analyte of interest in the sample. In one embodiment, the sample includes at least one control substance and at least one analyte of interest.

**[0087]** In one embodiment, the observation of the interaction of the at least one analyte of interest with the at least one ligand immobilized on the lateral-flow chromatographic immunoassay cassette may be timed by observing the appearance of at least one control substance. For example, a thyroid stimulating hormone ("TSH") assay may be read ~10 minutes after a diluent is applied. By monitoring the position of the wave front or the appearance of the control line, it may be possible to eliminate the need to manually time the test. Likewise, by observing the timing of the appearance of a control, the most favorable time for reading the assay can be identified. These could include monitoring the movement of the mobile phase, monitoring the movement of the control substance, timing the movement of the mobile phase, taking sequential images of the test result, detecting when buffer is