

0.6-10  $\mu\text{IU/mL}$  at 10 weeks old, 0.4-7.0  $\mu\text{IU/mL}$  at 14 months and gradually dropping during childhood and puberty to adult levels, 0.4-4.0  $\mu\text{IU/mL}$ .

**[0040]** No commercial TSH assay cassette currently on the market is able to provide a visual readout for TSH level below about 0.5  $\mu\text{IU/mL}$ . As such, TSH cassettes cannot currently be used for routine diagnosis of hyperthyroidism. It is believed that by using the devices and methods disclosed herein, that the detection limit can be extended and that TSH cassettes can be used for routine diagnosis of hyperthyroidism and other thyroid related endocrine conditions.

**[0041]** Referring now to FIG. 5, a graph depicting the relationship between the illumination angle on a lateral-flow chromatographic immunoassay cassette and a fluorescent signal obtained from the lateral-flow chromatographic immunoassay cassette at a variety of angles and a variety of loading concentrations is illustrated.

**[0042]** FIG. 6 shows preliminary results for assaying thyroid stimulating hormone dissolved in phosphate buffered saline using the prototype testing device shown in FIG. 8. The limit of detection is 0.005  $\text{mIU/mL}$ .

**[0043]** While the testing in the present application has been conducted with TSH cassettes, it is believed that the same or similar principles can be applied to cassettes adapted for other types of tests.

## II. Methods for Detecting at Least One Analyte of Interest in a Sample

**[0044]** In one embodiment, a method for detecting at least one analyte of interest in a sample is disclosed. The method includes (1) providing a lateral-flow chromatographic immunoassay cassette that includes at least one ligand immobilized thereon, wherein the at least one ligand is capable of capturing an analyte of interest on the lateral-flow chromatographic immunoassay cassette (2) applying a liquid sample to the lateral-flow chromatographic immunoassay cassette, wherein the sample includes at least one analyte of interest, (3) coupling the lateral-flow chromatographic assay cassette to a sample holder configured to angle the lateral-flow chromatographic assay cassette in relation to a detector device, and (4) observing the presence of the at least one analyte of interest by elastic light scattering. In one embodiment, the device includes an illumination source, a miniature spectrophotometer, at least one optical fiber capable of transmitting an illuminating light from the illumination source to the lateral-flow chromatographic assay cassette, a collimating lens capable of transmitting a signal from the lateral-flow chromatographic assay cassette to the miniature spectrophotometer, and an adjustable variable angle stage configured for holding the lateral-flow chromatographic assay cassette at an angle greater than or less than zero degrees in relation to the illuminating light and the miniature spectrophotometer, wherein the illuminating light and the and the miniature spectrophotometer are positioned to illuminate at least a portion of the lateral-flow chromatographic assay cassette and optimize an elastic light scattering signal from the lateral-flow chromatographic assay cassette.

**[0045]** In one embodiment, a single immunoassay device may contain multiple types of different antibodies each conjugated with different dyes (e.g., quantum dots) and multiple capture bands each immobilized with different antibodies. 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.

**[0046]** 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.

**[0047]** In one embodiment, an ultraviolet light source is positioned at a certain angle to the LFA and the detector (e.g., a detection fiber or a miniature spectrophotometer). In one embodiment, a capture band may be queried by taking a reading from the control line of the LFA as a baseline, then a reading from the capture band, and determine the difference in photon intensity. Signal intensity (i.e., the amount of scattered light that is detected) decreases as the concentration of the analyte of interest increases.

**[0048]** 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.

**[0049]** In one embodiment, the timing the observing 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 by observing the at least one control substance. For example, the TSH assay is read 10 minutes after the diluent is applied. By monitoring the position of the wave front or the appearance of the control line, we can 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 added, detecting when liquid has traveled the length of the membrane, and combinations thereof.

**[0050]** The positive control substance can also be used for calibrating the test. This device is intended to provide quantitative results. To do this requires calibrating the test. In one embodiment, the test may be calibrated by adding a quantity (e.g., a known quantity) of a positive control substance (e.g., a solution of fluorescent particles) to the diluent. The light source will generate light at wavelengths to optimize the detection of the test line and to illuminate the positive control substance. The digital camera device can detect the amount of light that is scattered or emitted by the positive control substance and use this information to normalize the scattering or emission from the analyte of interest. In addition to providing calibration data, the positive control substance can be used to time the reaction and to demonstrate that the assay functioned correctly. In addition, the test can be further calibrated or quantified by including a color scale that is printed on the