**Smartphone-Based Quantitative Immunochromatographic TSH Measurement**

TSH, a glycoprotein hormone made in the pituitary gland, regulates the synthesis and secretion of T3 and T4 by the thyroid gland. TSH is the second most commonly performed clinical laboratory test in the United States; approximately 3.25 million TSH assays are done annually. This document summarizes the method by which i-calQ’s biosensor measures TSH. This method can be used to quantify any lateral flow immunochromatographic assay which generates a fluorescent, chemiluminescent, or visible spectrum signal.

i-calQ’s biosensor consists of two components: an immunochromatographic assay and a smartphone-based reader. The TSH immunochromatography cassette (Figure 1) has two control lines, C1 and C2, which are used for assay calibration and a T (test) line. TSH is measured by adding 30 microliters of capillary blood and two drops of buffer to the sample well (S in Figure 1), waiting 15 minutes, then imaging the cassette’s results lines (Figure 2) with the i-calQ reader attached to a smartphone (Figure 3) and TSH app (Figure 4).

The assay contains two calibration lines used by the app’s image analysis algorithm to generate a standard curve for each cassette at the time of testing. The test line signal falls on the standard curve. Data analysis consists of determining a linear fit between the calibration lines and then fitting the test line to this calibration.

The image acquired consists of vertical lines on the test strip. The color channels of the image are separated and the red channel is used for measurement. A region of interest is determined and all the columns in this region are summed to give an average reading. A plot of the summed columns shows an overall background slope due to uneven lighting and several dips corresponding to the C1, T and C2 lines on the assay (Figure 5).

The overall background is fit with a linear curve and this is subtracted from the data line (Figure 5). A second linear fit is performed using known concentration C1 and it’s resulting pixel value and C2 and its pixel value. This line represents the test’s standard curve (Figure 6). The unknown value for the T line is then determined by extrapolation from calibration curve.

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**Figure 1**
Quantitative TSH Cassette

**Figure 2**
Test Results Used for Image Analysis
Figure 3
i-calQ Reader with Cassette

Figure 4
Analyte-Specific App

Figure 5
Image Analysis Algorithm

Figure 6
Standard Curve