# A parametric study of lateral flow biosensors for measurement of salivary cortisol concentration

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*Abstract*—**A parametric study was performed to investigate the effects of lateral flow assay preparation and construction on measurement of salivary cortisol concentrations. It is shown that a higher concentration of anti-cortisol antibodies is more suitable for measurement of higher concentrations of cortisol. Moreover, it is shown that thinner control and test lines provide more consistent measurement.**

*Keywords*— **Lateral flow assay, salivary cortisol, biosensor.**

## I. Introduction

Typical levels of cortisol within the body vary: cortisol follows a daily cycle, where it peaks 30-40 minutes following awakening [1], [2]. Stress is commonly known to contribute to abnormal levels of cortisol [2]–[6] and sustained abnormal level of cortisol can lead to health problems [2], [7]. Therefore, it is important to monitor the cortisol levels within the body.

Salivary cortisol concentrations have been found to correspond to serum cortisol concentrations, but at smaller concentrations [3], [5]. Current methods of measuring salivary cortisol such as enzyme linked immunosorbent assays (ELISA) are expensive and time consuming. Additionally, the current methods require training and specialized equipment that are not suitable for point of care analysis.

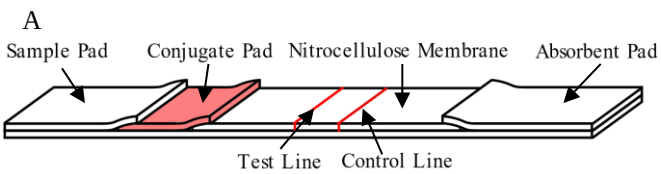
Lateral flow assays (LFA) have been utilized in the detection of a variety of analytes, and have been applied to point-of-care diagnostics [8]–[10]. These assays commonly use a colorimetric approach to measure salivary cortisol [6], [11]. By implementing image processing techniques, it is possible to obtain a salivary cortisol concentration from the LFA [5], [6], [11]. Smartphones have also been implemented in a variety of biosensing applications [5], [6], [12]–[14]. Although the use of smartphones and their cameras have been shown for measuring the salivary cortisol using LFA, the characterization of the effects of different parameters on the measurement accuracy and range has not been shown before.

In this study, smartphone processor, phone cameras and LFAs are used to develop and test a portable point of care device for the measurement of salivary cortisol. A parametric study is performed to investigate the effects of factors such as line thickness and antibody concentration on the measurement accuracy and range.

## II. Methodology

### A. Lateral Flow Assays

Sample pads (Standard 14, GE Healthcare), conjugate pads (Standard 14, GE Healthcare), nitrocellulose membranes (FF 80HP, GE Healthcare) and absorption pads (CF4, GE Healthcare) are used to construct the LFA, as seen in Fig. 1A. Capillary action allows the sample to flow from the sample pad to the conjugate pad, allowing cortisol to attach to the labeled molecule in the conjugate pad. As the sample with the labeled molecule flows through the LFA, the labeled molecules become immobilized at the test and control lines, and this results in red lines forming [8]–[10]. Gold nanoparticles (GNP) (G-30-100, Cytodiagnostics, Canada) were implemented as the labeled molecule, which were conjugated to anti-cortisol antibodies (ab1952, Abcam) to allow the binding of cortisol to the labeled molecule as well as the binding of the label to the test and control lines [5], [6], [11]. The test line was made of a Cortisol-BSA (80-IC20, Fitzgerald, USA) conjugate solution and the control line was an anti-mouse antibody (M8642-1MG, Millipore Sigma) solution, a line of both were applied to the nitrocellulose membrane [6], [11] using a fountain pen [11]. Depending on the concentration of cortisol in the sample, the colour intensity of the test line varies. At low concentration, the test line produce is prominent, while at higher concentration it is faint (Fig. 1B). Known concentration of cortisol solution were prepared and 100 µL was applied to the LFA for assessment.



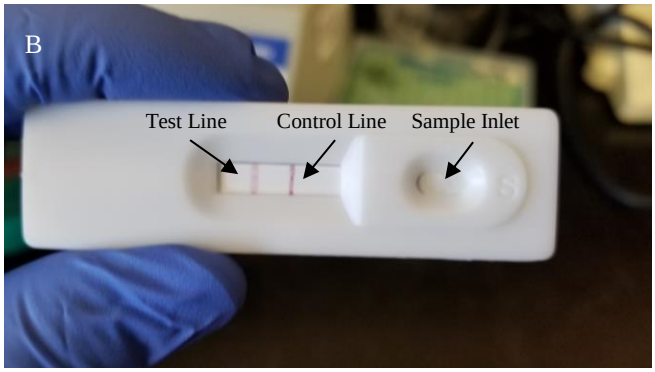



Fig. 1 Lateral Flow Assay. (A) Illustration of the LFA components. (B) LFA reaction to 100 µL of 1 ng/mL cortisol solution.

*B. Image Processing*

A MATLAB image processing algorithm is developed to measure the intensity of both the test and control lines. By applying a sample with known concentrations of cortisol to the LFA, a calibration curve is constructed.

## III. Results and Discussion

Fig. 2 shows the ratio of intensity between the test and control lines for different known concentrations of cortisol. Concentrations used were at 0, 1, 10, and 50 ng/ml solution of cortisol. The gold nanoparticle solution was adjusted to different pH to determine whether more anti-cortisol antibodies attached to the nanoparticles. The pH was adjusted to 7.55, 8.14, and 8.8, and conjugated with 1 µL, 1.5 µL and 2 µL of 1 mg/mL anti-cortisol antibodies per 1 mL of GNP solution, respectively.

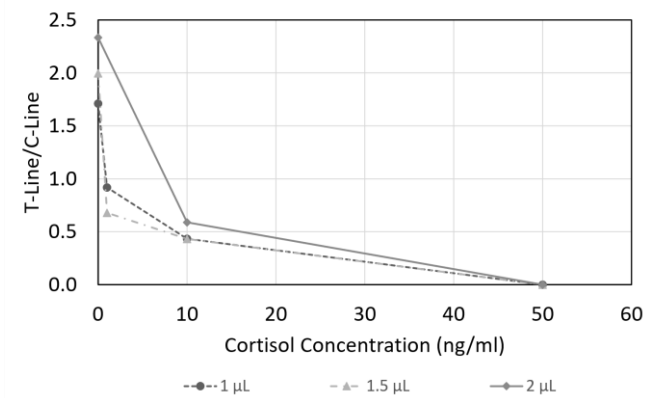



Fig. 2 Effects of increase in anti-cortisol antibodies on the cortisol measurement.

As can be seen the ratio decreases as the concentration of cortisol increases. This is due to the decrease in the intensity of the test line as the concentration of cortisol applied increases. By increasing the antibodies and pH the ratio increases at the lower concentration, which indicates a high intensity of the test line. At a cortisol concentration of 50 ng/ml the ratio is 0 for all, this shows that there is an oversaturation of cortisol, and the test line does not appear. Therefore, a higher concentration of anti-cortisol antibodies is more suitable for the measurement of higher concentrations of cortisol.

To investigate the effect of the test and control line thickness, LFAs were prepared with identical parameters except for the width of the lines. To increase the thickness of the lines, double and triple lines were used. A 1 ng/ml solution was applied to all LFAs, and the ratio of the test and control line intensity where obtained, as seen in Fig. 3.

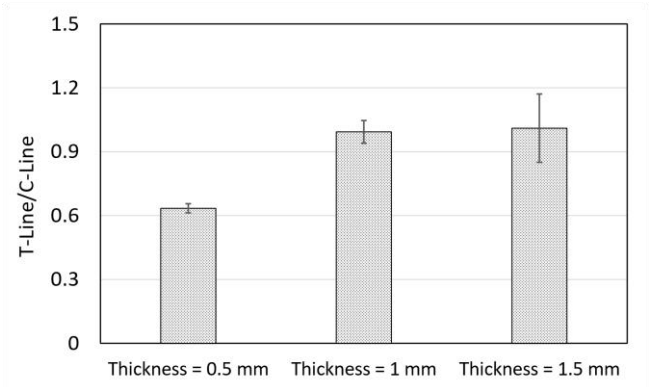



Fig. 3 Effects of control and test line thicknesses on the cortisol measurement. Error bars show the calculated standard deviation (n=3).

As seen in Fig 3, the ratio of the test and control line increases as the thickness of the lines increases. This is due to the increase in the intensity of the test line as more binding sites are applied to the nitrocellulose membrane. The variation from strip to strip also increases as the thickness increases which is indicated by the error bars. This can be attributed to human error when applying the thicker lines. Therefore, thinner lines will provide more consistent results.

## IV. Conclusion

It has been shown that LFAs can be implemented in the measurement of the concentration of cortisol. Further development is required to increase the sensitivity of the LFA and to obtain a test line that appears at the higher concentration of cortisol. Development and implementation of the automated smartphone application will also be conducted.

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