Biosensors and Bioelectronics 103 (2018) 138–142

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Mobile phone based ELISA (MELISA)

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ARTICLE INFO

Keywords:
Enzyme-linked immunosorbent assay (ELISA)
Optical sensor
Progesterone
Mobile ELISA (MELISA)

ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) is one of the most important technologies for biochemical analysis critical for diagnosis and monitoring of many diseases. Traditional systems for ELISA incubation and reading are expensive and bulky, thus cannot be used at point-of-care or in the field. Here, we propose and demonstrate a new miniature mobile phone based system for ELISA (MELISA). This system can be used to complete all steps of the assay, including incubation and reading. It weighs just 1 pound, can be fabricated at low cost, portable, and can transfer test results via mobile phone. We successfully demonstrated how MELISA can be calibrated for accurate measurements of progesterone and demonstrated successful measurements with the calibrated system.

1. Introduction

Recently, mobile phones have become a popular platform for developing point-of-care testing systems. The term mHeatlh has been adopted by the World Health Organization to cover medical services and practices that utilize mobile phones or other portable electronics (World Health Organization, 2011). For example, pregnancy complication monitoring can be done on a mobile phone (Archibong et al., 2017; Konnaiyan et al., 2016). Blood pressure monitoring with a mobile phone application for stroke patients is being researched (Jenkins et al., 2016), while other applications are also being developed for diabetic patients (Katz et al., 2012), weight control (Turner-Mcgrievy et al., 2013), patients with sickle cell disease (Cheng et al., 2013), etc. Additionally, mobile platforms can be used for HIV prevention, care, and treatment (Catalani et al., 2013). Mobile phones are already widely available across the globe, including low and middle-income countries. Contemporary cellphones have a number of built-in sensors including: cameras, light-sensors, microphones, etc. Availability of the aforementioned sensors integrated into a convenient mobile platform has enabled a number of mobile phone based diagnostic systems.

Currently, the standard of care platform for conducting a wide variety of tests is ELISA, which is an expensive and complex procedure. It would be immensely beneficial for patients if ELISA was translated to a mobile platform. However, up to date, most of the research was focused on using a phone camera as a read-out, while there is no complete system that allows conducting all of the steps of the ELISA protocol (McGeoug and O'Driscoll, 2013; Vashist et al., 2015). Here, we demonstrate a complete low cost mobile phone based system allowing execution of all of the ELISA steps without any additional equipment and demonstrate its successful application to hormone measurements because there are not many systems focusing on important topics such as measurement of hormone levels. Studies related to point-of-care hormone measurement are very limited and mostly focus on cortisol detection (Zangheri et al., 2015). Here, we describe a new mobile phone based device that simplifies and reduces the cost of current ELISA procedures, and demonstrate measurement of progesterone in whole blood samples.

2. System design

The proposed design of the whole MELISA system is shown in Fig. 1. The device housing is comprised of two separate sections. The inner section of the housing is the site where ELISA procedure steps take place, including incubation and image capture. A backlight LCD screen is located in the bottom of this section. The outer section of the housing is used to store the control circuitry necessary for device operation. The control circuitry includes: an Arduino Mega 2560 microcontroller, two DC solid state relays, and an input/output (I/O) circuit for the microcontroller. A door is designed to block out any light from outside sources and connects to the box by sliding into grooves. The plastic components were designed in Autodesk Inventor and the housing was 3D-printed in polylactic acid (PLA) plastic. The device has two inserts – one for sample heating and the other for image capturing. The heater insert (the water bath) was also designed in Autodesk Inventor but 3D-printed using acrylonitrile butadiene styrene (ABS) plastic rather than PLA because of better resistance of this material to higher temperatures. The heating tray has holders for the ELISA microwells arranged in two lines of five, equidistant from two copper tubes with inserted heating

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https://doi.org/10.1016/j.bios.2017.12.033
Received 27 July 2017; Received in revised form 1 December 2017; Accepted 20 December 2017
Available online 21 December 2017
0956-5663/

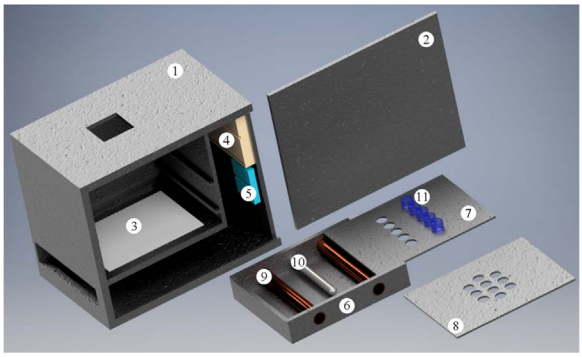

Fig. 1. 3D model of the complete system (artistic rendering). Labeled components: 1) prototype housing, 2) sliding door, 3) LCD backlight screen and Arduino Mega 2560, 4) circuit board, 5) DC solid state relays, 6) water bath, 7) heating tray, 8) image capturing tray, 9) copper tubes with heaters, 10) temperature probe, 11) ELISA microwells.

elements. The water bath slides into the 3D-printed housing with the heating elements and temperature sensor connecting to the Arduino microcontroller, as well as a power supply. The image capturing tray has ELISA microwell holders arranged in a hexagonal pattern for optimal light distribution. The prototype is modular, enabling custom modifications of the whole system. All the parts needed for construction of the prototype (besides a mobile phone) can be purchased for less than $35 and be used off the shelf.

2.1. Incubator design

The first step of ELISA is antibody conjugation with the target protein performed at 37 °C, therefore a system for portable ELISA should include an incubator that can accurately maintain the needed temperature. For uniform heating of the ELISA microwells we use a water bath with two copper tubes integrated with heating elements, and a waterproof temperature probe for continuous temperature monitoring (Fig. 2). The top view of the whole water bath heater in operation is shown in Fig. 2a,b. The thermal image (Fig. 2a), taken with an FLIR camera, demonstrates that the temperature is the highest around the heaters, but is rather uniform between the heaters. A cross-sectional COMSOL simulation of the heating system is shown in Fig. 2c, demonstrating the uniform heat propagation across the water layer. The ELISA microwells are placed in two rows alongside the heaters to ensure identical thermal treatment of all the samples. Since most ELISA reactions take place at 37 °C, accurate control of the heaters is necessary to maintain this temperature. The heaters are controlled through solid state relays by an Arduino Mega 2560 microcontroller. When the temperature probe reads less than 37 °C, the relays are enabled, which powers the heating elements. When the temperature is greater than 37 °C, the relay closes, switching the heaters off. The ELISA microwells are placed in holders, with their outside surfaces contacting the warm water in order to provide the temperature necessary for the conjugation reactions to occur. The water bath and the slower heat dissipation through the copper tubes help to prevent larger temperature fluctuations in the ELISA microwells. As shown in Fig. 2d, the water bath requires 5 min to reach the setpoint of 37 °C from ambient conditions. After reaching the setpoint, the temperature fluctuates within ± 0.8 °C for the duration of incubation (60 min). This precision is sufficient for reproducible ELISA measurements (17OH Progesterone ELISA for Routine Analysis, 2012).

2.2. Optical readout design

The next ELISA step after completing the incubation, is adding a secondary colorimetric antibody followed by quantifying the sample absorption with a plate reader. As a substitute for a plater reader, MELISA uses a smartphone camera and this data is then compared with data obtained using a commercial plate reader. To remove interference from the ambient light while taking an image with a mobile phone

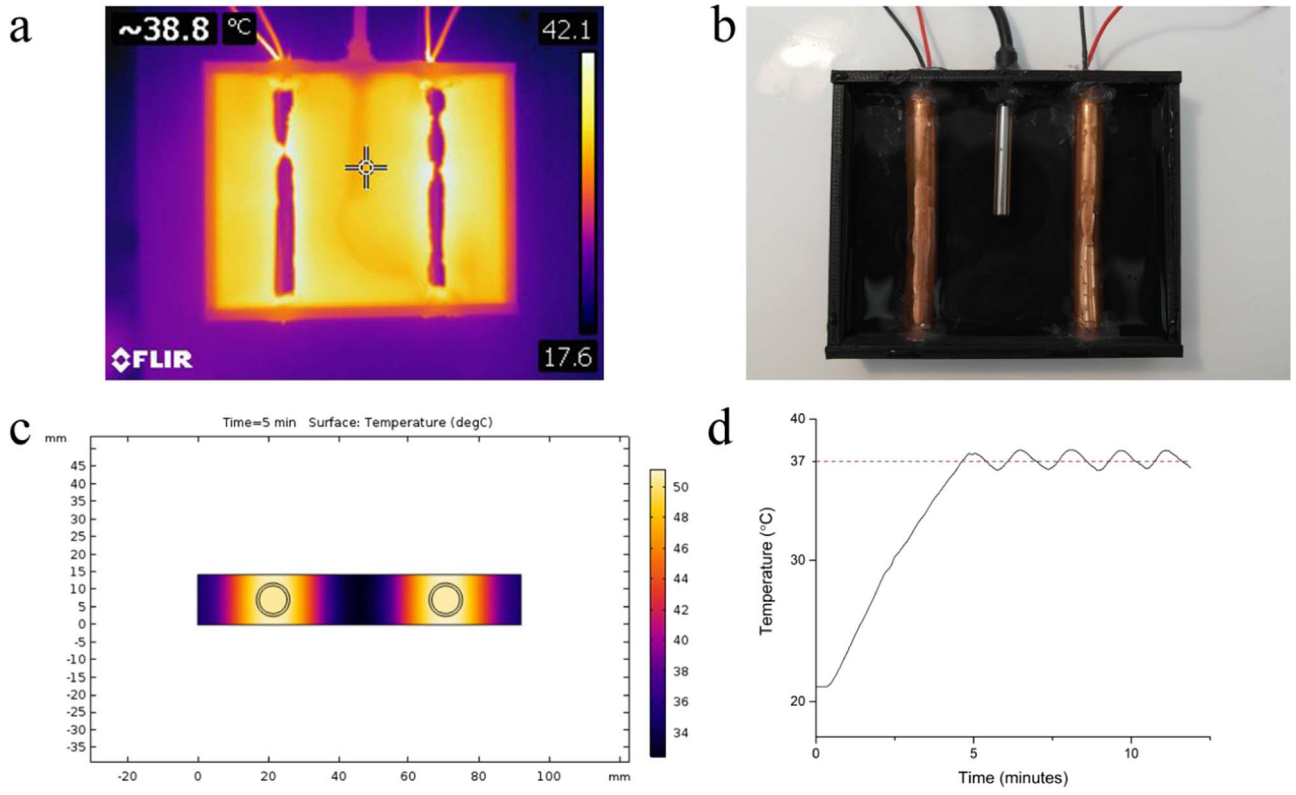

Fig. 2. Thermal characteristics of the water bath. a) Infrared thermal image (top view), b) Real image (top view), c) COMSOL simulation of heat distribution (side view), d) Water bath thermodynamic tuning curve.

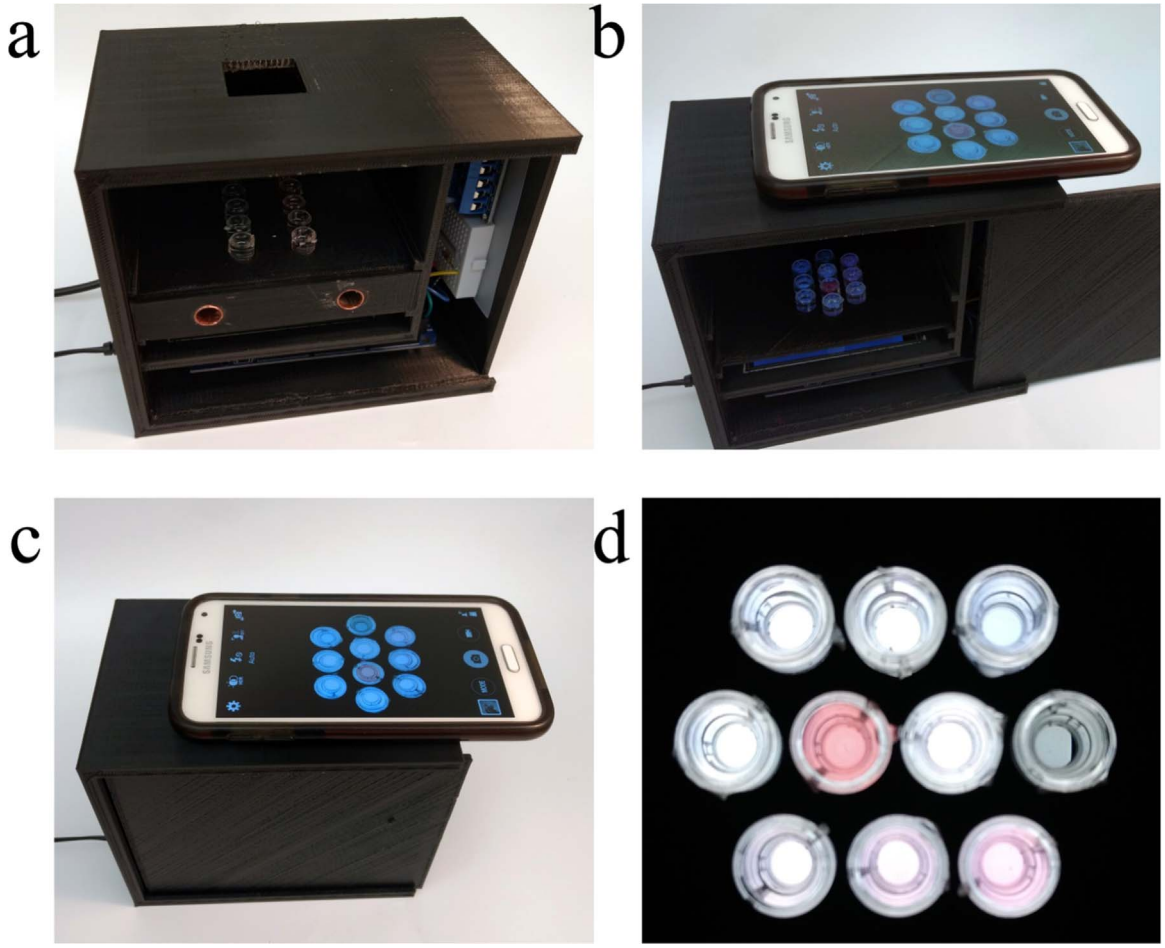

Fig. 3. Demonstration of the prototype. a) Sample incubation mode, b) Loading image capturing tray, c) Image capturing mode, d) Captured sample image (colored dyes for demonstration).

camera, we placed the samples inside of a black box similar to Chromadock (Konnaiyan and Pyayt, 2015). As a light source we used a white LCD screen placed under the samples, and it produced uniform illumination. The camera from the mobile phone was used for the colorimetric measurements, and during the measurements camera parameters such as white balance, ISO, and exposure rate were fixed (Hirsch, 2013).

2.3. Operation

The first step in the ELISA procedure is shown in Fig. 3a. Progesterone samples are added to the microwells and inserted into the sample holder under controlled temperature environment in the water bath. After heating the samples to 37 °C and maintaining this temperature for 60 min, the microwells are removed from the water bath and placed into the viewing tray, which is then placed on the same rails used for the bath, as shown in Fig. 3b. Once the microwell-loaded viewing tray has been placed inside the MELISA device, the door is closed and the LCD screen located below the viewing tray emits light through the bottom of the microwells. In order to capture the images of the samples, a smartphone is placed on top of the MELISA housing, and the camera aperture is aligned with the square-inch hole, with this step shown in Fig. 3c. With all ambient light blocked by both the door and the enclosed design of the box, color contrast is more discernable, focus is improved, and the overall image quality is enhanced. After loading the viewing tray into the housing and closing the door, the color concentrations of the ELISA samples can be recorded with a smartphone. An example of the resulting image captured by a smartphone is shown in Fig. 3d.

3. Evaluation of MELISA

The next step was evaluation of MELISA using actual ELISA measurements. One of the hormones vital to female reproduction health is progesterone, which regulates a number of critical physiological processes, affecting blood pressure and the cardiovascular system in general (Barbagallo et al., 2001). In addition, progesterone significantly influences the ability to conceive and has a major impact on pregnancy overall (Xu et al., 2012; Csapo et al., 1974). Progesterone has a big role in maintaining a healthy immune system and determines the development of neoplastic diseases (Stopińska-Głuszak et al., 2006). Healthy ranges of progesterone concentrations for women in the follicular phase is 0.2 ng/ml–1.3 ng/ml, 1.0 ng/ml–4.5 ng/ml in the luteinic phase, and 0.2 ng/ml–0.9 ng/ml in menopause (17OH Progesterone ELISA for Routine Analysis, 2012). Concentration levels below the clinical range may increase the risk of endometrial and breast cancer (Kim et al., 2013). In addition to ELISA, progesterone levels can also be measured by protein-binding techniques (Yoshimi and Lipsett, 1968), radioimmunoassays (Abraham et al., 1971), and liquid chromatography–tandem mass spectrometry (Inder et al., 2012). These other methods are not designed for point-of-care or at-home use due to the need for specialized equipment and high cost.

To test the MELISA design, a 17OH progesterone ELISA kit (17OH Progesterone ELISA for Routine Analysis, 2012; Elder et al., 1987) was used and results from our measurements were compared with the measurements conducted using current gold standard instrument, a

Fisher Scientific AccuScan plate reader. The ELISA kit contains antibody-coated microplates, calibration samples, and a substrate required for quantitative determination of progesterone concentration. Calibration samples have progesterone concentrations of 0, 0.2, 0.4, 1.6, 6.4, and 19.2 ng/ml, respectively. After incubation and solid-phase washing, horseradish peroxidase (HRP), conjugated with 17OH antigens, binds to anti-17OH progesterone antibodies. The HRP-antibody binding, along with the addition of H_2O_2-tetramethylbenzidine (H_2O_2-TMB) and a sulfuric acid stop solution, results in a color change. The color intensity of the ELISA samples is inversely proportional to the 17OH progesterone hormone concentration. The final step is to measure sample absorption using the current gold standard to validate results.

3.1. Procedure

Blood samples were purchased from Innovative Research and a 17OH ELISA calibration kit was used to measure progesterone concentration with the MELISA device. The blood samples were allowed to sediment, with 50 µL of plasma collected from the top layer of each sample, and dispensed into each sample microwell. After six calibration samples and three unknown plasma samples were loaded in the prototype heating tray, samples were incubated for 60 min at 37 °C. After incubation, the contents of the microwells were washed out with deionized (DI) water and moved to an image capturing tray. Next, 100 µL of H_2O_2-TMB (0.26 g/L concentration) is added to each sample, binding to the 17OH antigens that are conjugated with the HRP chromogenic agent. The image capturing tray is then placed back in the enclosure at ambient temperature with the door closed. After 15 min in the dark, sulfuric acid (0.15 mol/L concentration) stop solution is added to the samples which prepare them for optical reading (17OH Progesterone ELISA for Routine Analysis, 2012).

An image of the samples was captured inside the prototype with the backlight LCD as shown in the Fig. 4. The white balance setting of the camera was used to adjust the color of the captured image based on the light source. The main advantage of using an automatic white balance setting is the color reproducibility for images captured under different light conditions (Hsu et al., 2008). Since a stable light source was used as a background in the experimental setup, the white balance was set to a 'daylight' mode, which normalizes the color values based on the standard daylight illuminant source D65. The autofocus option in a camera can cause blur in images that can add error to the measurements (Brown, 2000). Fixed focus mode was programmed into the mobile application as the distance between the object and the camera is constant, which is determined by the height of the box.

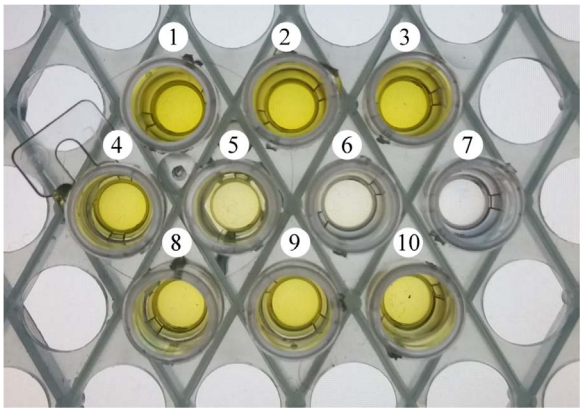

Fig. 4. Progesterone ELISA samples image, 1) 0 ng/ml, 2) 0.2 ng/ml, 3) 0.4 ng/ml, 4) 1.6 ng/ml, 5) 6.4 ng/ml, 6) 19.2 ng/ml, 7) blank, 8–10) unknown concentration.

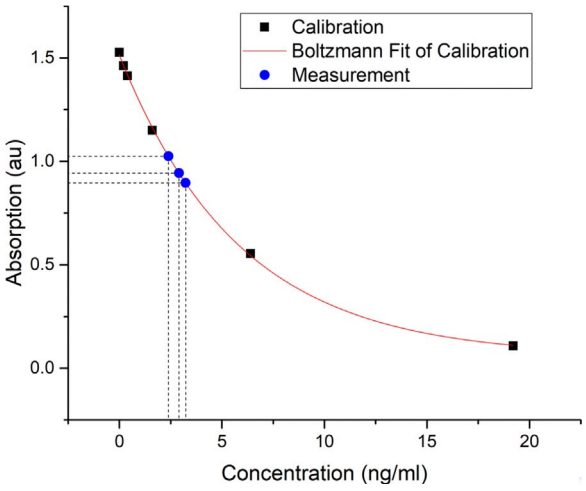

Fig. 5. Plate reader progesterone concentration calibration curve.

3.2. Data analysis

The conventional measurement of progesterone concentrations of the ELISA samples was performed using a commercially available Fisher Scientific AccuScan microplate reader. Monochromatic light with 450 nm wavelength was used by the reader to measure the absorbance of the calibration samples, and they were plotted against their respective concentrations (Fig. 5). An Origin software package was used to fit the data points with a four-parameter Boltzmann Sigmoid function (Cheemalapati et al., 2016) (1):

$$y = A_2 + \left(\frac{A_1 - A_2}{1 + e^{\frac{x - x_0}{dx}}} \right) \quad (1)$$

where x_0 is the center of the Sigmoid; dx is the span of the curve; A_1 and A_2 are the minimum and the maximum values of the curve. The calibration curve obtained using the calibration samples can be used to measure the concentration of unknown samples based on the absorbance value.

In the captured image, samples 1–6 are known progesterone values used for calibration, while samples 8–10 are unknown. RGB values of the calibration samples were measured and plotted against respective concentrations (Fig. 6). The red (R) and green (G) values are insignificant due to the low range of measured values. The blue (B) component has the range required to construct the calibration curve using the four-parameter Boltzmann Sigmoid function (1). Applying the calibration

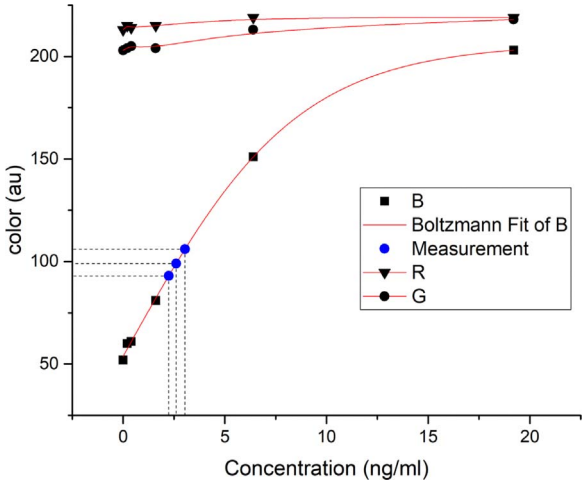

Fig. 6. MELISA (prototype) progesterone concentration calibration curve.

Table 1
Unknown samples calculated values.

Sample number	Plate reader measured concentration (ng/ml)	Prototype measured concentration (ng/ml)
8	2.9	2.6
9	2.39	2.24
10	3.23	3.04

function to the B values of the unknown samples was used to determine the concentration.

The results of measuring the progesterone concentrations of the unknown samples with the commercially available plate reader and with MELISA are shown in Table 1. Comparing the values from the two measurements shows the accuracy of the MELISA prototype is within 10% of the gold standard. This is within value variability of the ELISA kit.

4. Conclusion

Here we introduced a new mobile ELISA (MELISA) system for point of care measurements of progesterone concentration. Multiple samples with known concentrations of progesterone were analyzed using the MELISA system and accuracy was verified using a gold standard plate reader (Fisher Scientific AccuScan). The device consists of a dual-purpose enclosure used for incubation and image capturing of ELISA samples. A water bath heater was used to incubate samples at a target temperature, and then images were captured using a mobile phone and analyzed to measure progesterone concentrations. Adobe Photoshop was used to determine the RGB color components of each sample. The blue color component was used for further analysis due to its sensitivity to the changes in progesterone concentration. It was shown that we can incubate and measure progesterone ELISA samples, extract color values, analyze data and build a calibration curve based on the blue component of RGB data. A close correlation between mobile system measurements and the data from the plate reader was demonstrated. Finally, we showed that the results acquired with our prototype match the gold standard plate reader within 10%.

Acknowledgements

A.P. is supported by NSF Grant # 1701081. A.Z. is supported by USF Presidential Fellowship. Authors want to thank USF AVC staff for their help.

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