Low-Cost and Rapid Identification of Counterfeit Antimalarial Drugs on Paper

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Introduction

With the emergence of artesunate antimalarial counterfeiting in Southeast Asia and sub-Saharan Africa, we present the production of a rapid, inexpensive and simple colorimetric-based testing kit for the detection of counterfeit artesunate in order to preserve life and prevent the development of multi-drug resistant malaria. The kit works based on paper microfluidics which offer several advantages over conventional microfluidics, and has great potential to generate inexpensive, easy-to-use, rapid and disposable diagnostic devices. Here, we have developed a colorimetric assay that is specific to artesunate and turns yellow upon addition of the analyte. The test can be done within minutes, and allows for a semi-quantitative analysis of the artesunate tablets by comparing the developed yellow color on the paper test to a color-coded key chart that comes with the kit. A more accurate and precise analysis is done by utilizing a color analysis app on an iPhone that measures the color intensity of the developed color on the paper chip. A digital image of the chip was taken and analyzed by measuring the average grey intensity of the color developed on the paper circle. A plot of the artesunate concentration versus the average grey scale intensity was generated.

Materials and Methods

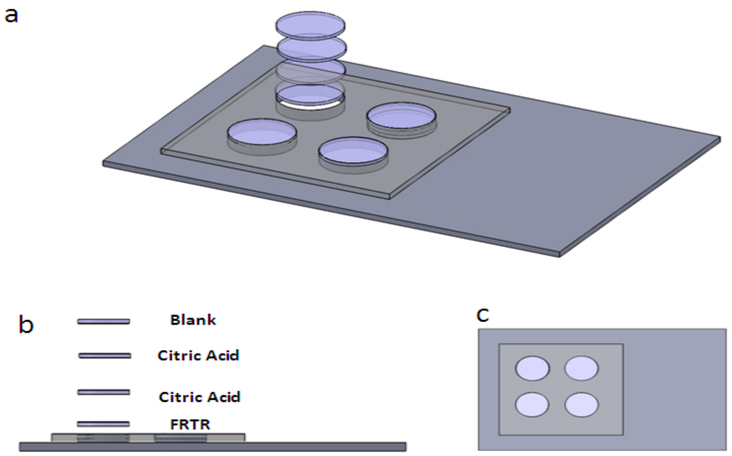


Figure 1. Schematic diagram of the paper test kit. A 3D expanded view of the overall paper test kit (a). A side view illustrating the four layers of the device, top to bottom, a blank absorbent pad, two buffering layers of dried citric acid, and a layer of Fast Red TR salt (b). A top view of the paper test kit showing the four paper testing devices (c).

❖ Paper Microchip Fabrication

- Whatman filter paper (Grade 3 1003-055) or glass microfiber GF/B (Sigma Aldrich, St. Louis, MO, 631778, USA) was cut into 8-mm diameter holes with a hole punch.
- The 8-mm dots were then pretreated with different reagents and allowed to dry prior to stacking (allowing for vertical fluid flow).
- The paper dots were assembled and secured in a plastic holder composed of poly(methyl methacrylate) (PMMA) with a plastic lamination on one side to secure the dots in place
- This assembly was then placed on the Artesunate Colorimetric Field Test Coupon (Figure 1)

❖ Antimalarial Testing

- Artesunate standards (ranging from 1-20 mg/ml) were prepared by diluting a 60 mg/ml stock solution with 1 M NaOH
- Three commercially available drugs were selected containing one antimalarial compound:
  - •Artesunate (Artesunate for Injection, Guilin Pharmaceutical, China)
  - •Artemeter (Artem, Kunming Pharmaceutical Corp, China)
  - •Dihydroartemisinin (Arterakine, Pharbaco Central Pharmacy, Vietnam)
- Drug samples were prepared at 5 mg/ml and tested against artesunate standards

Results

❖ pH dependence of the FRTR colorimetric detection

The specificity of the FRTR colorimetric detection is dependent on the pH of the reaction. The reaction must be maintained at pH 4 in order to be specific to artesunate, and not give positive test results in the presence of other antimalarial drugs.

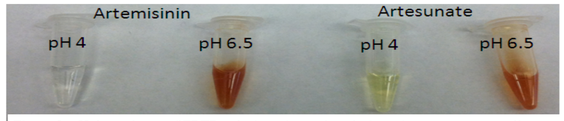


Figure 2. pH dependence of FRTR colorimetric test in solution. Artesunate turns yellow at pH 4 while artemisinin gives no color. However, both anti-malarials turn orange at pH 6.5

❖ Specificity of the Chip Design

Both artesunate and artemisinin were tested on 4-layer and single layer chip designs. The 4-layer design (as shown in Figure 1) successfully maintained the appropriate pH for the specificity of the artesunate test (Figure 3a). The buffering ability of the 4-layer design was modeled by applying 1M NaOH to a two-layered citric acid chip and testing the pH of the resulting solution at the base of the paper chip. It was confirmed that this arrangement gives a pH reading between 3.5 and 4.0 (Figure 3b)

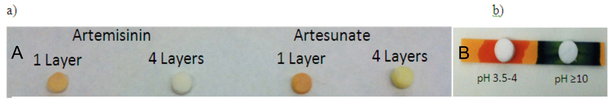


Figure 3. a) pH dependence of FRTR colorimetric test on paper. b) pH test of 1M NaOH solution applied to 2 layers of paper preloaded with citric acid buffer (left) and a blank (right).

❖ 4-Layer Chip Design for Semi-Quantification of Artesunate

Varying artesunate concentrations (0.5 mg/ml to 10 mg/ml) were applied to the 4-layer paper devices (as shown in Figure 1) and allowed to incubate for 5 minutes. The concentration was determined based on a calibrated color scale. The increasing color intensity was consistent with the increasing amount of artesunate in the sample (Figure 4).

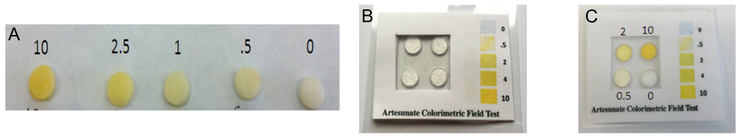


Figure 4. Semi-quantitative data utilizing the designed paper test device. A) From left to right: decreasing concentrations of Artesunate from 10 mg/mL to 0 mg/ml. The artesunate field test kit prior to (B) and after (C) artesunate introduction (concentrations shown in mg/ml).

❖ Paper Test Kit Specificity for Artesunate

- Three Anti-malarial drugs at concentrations of 5 mg/ml were tested on the paper test kit
  - Artesunate (Guilin)
  - Artemeter (Artem)
  - Artemisinin (Artirakine)
- Only artesunate gave a positive result (development of a yellow color)
- Without reliable access to counterfeit drugs this was used as a proof-of-concept

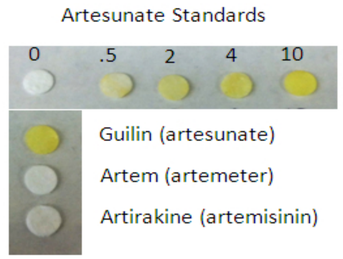


Figure 5. Semi-quantitative data utilizing the paper test device. From left to right is increasing concentrations of Artesunate from 0 mg/mL to 10 mg/ml. Anti malarial drugs prepared at 5 mg/ml compared to the standard.

❖ iPhone based color sensor for Artesunate Quantification

- The iPhone app ColorAssist was used to measure RGB values for each devices (Figure 6)
- RGB values were converted to grey scale and plotted versus artesunate concentration
- The data showed that the intensity of the yellow color was proportional to the amount of artesunate present in the sample. With artesunate concentrations ranging from 0.0 to 20.0 mg/ml, a linear calibration plot was obtained with a detection limit of 0.98 mg/ml (Figure 6).

Results

❖ iPhone based color sensor for Artesunate Quantification

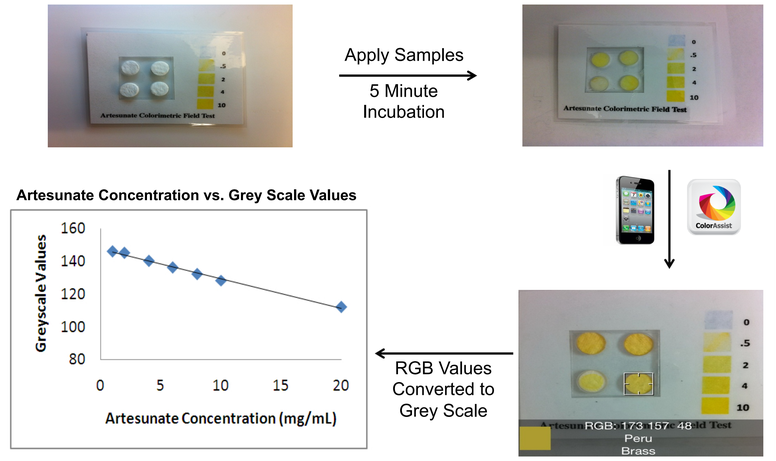
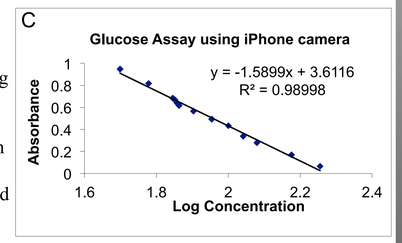
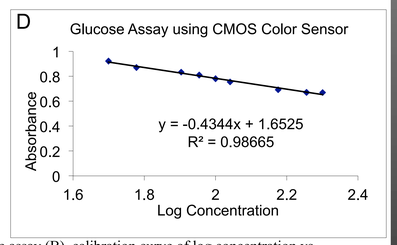


Figure 6. Flow chart for artesunate quantification with an iPhone equipped with ColorAssist. The mean intensity of the color produced was plotted versus the artesunate concentration (0.0 to 20.0 mg/ml). The plot shows very good linearity (R^2 value = 0.9945) with detection limit of of 0.98 mg/ml.

Conclusions & Future Work

We have demonstrated a proof-of-concept for detecting counterfeit drugs using a paper based colorimetric assay. This approach is suitable and can serve as a rapid, simple, and cost effective means for the end user to test the authenticity of the drug in question. Additionally, the simple detection technique could benefit government officials and local pharmacies as a rapid screening method to combat the growing problem of drug counterfeiting today. Our future studies include optimizing the assay with lower limit of detection and improved image analysis by developing a paper device and a CMOS color sensor that would enhance the sensitivity of the measurement, and applying this method of detection to colorimetric glucose assays. (Figure 7).





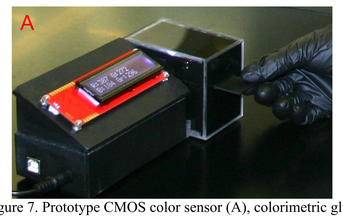


Figure 7. Prototype CMOS color sensor (A), colorimetric glucose assay (B), calibration curve of log concentration vs. absorbance generated using ColorAssist on an iPhone (C), calibration curve of log concentration vs. absorbance using the prototype CMOS color sensor (D).

Acknowledgments

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❖ Notes and References

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