A Color Absorption Strategy for Chromatic Analysis by Using a Portable 3D-printed Tube Holder Coupled with a Notebook Screen and a Smartphone

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Abstract

Background: A colorimetric assay is the simplest detection method to interpret results through discoloration in a solution that is visible to the naked eye. However, visual interpretation has some limitations regarding the variability in individual color perception caused by several factors such as color blindness and experience, which needs trained and experienced personnel to determine between positive and negative results.

Objectives: This study aimed to develop a color readout method for differentiating between positive and negative colors based on color absorption strategy by using a portable 3D-printed tube holder device, a notebook screen, and then readout by a smartphone. After that, our strategy was used to analyze the colorimetric loop-mediated isothermal amplification coupled with malachite green dye (cLAMP-MG) assay for detecting cestodes belonging to the genus *Raillietina* as a colorimetric assay model.

Methods: A portable 3D-printed tube holder was designed using 123D design software and printed by using a 3D printer with a black polylactic acid filament. Color absorption can be easily analyzed using a notebook screen as a chromatic light source and a smartphone camera coupled with the ColorAssist application as a color detector. Moreover, a convex lens (15× magnification) was placed in front of the back camera's own lens of iPhone 13 (f = 1.6 mm) in order to increase the image magnification and focus on the reaction tube in close range. To conduct the experiment, the cLAMP-MG solution in a 0.2 mL tube was inserted into the tube holder hanging on a red notebook screen. The red light from the notebook screen passed through the solution in the tube, and the transmitted light was measured by a ColorAssist on smartphone and were calculated according to the following formula:

$$\text{Absorption}_{\text{sample}-\text{negative control}} = -\log\frac{R_{\text{p}}}{R_{\text{p0}}} + \log\frac{R_{\text{n}}}{R_{\text{n0}}}$$

where R_{p} and R_{n} are the color values of sample and negative control, respectively. R_{p0} and R_{n0} are the red color values of the background (light source) before measuring the sample and negative control, respectively. For a cut-off value setup, the empirical rule was applied to compute a cut-off value from negative controls according to the following: the three-fold standard deviation of the negative controls (3SD) were calculated to confirm with 99.7% confidence that a reaction higher than the cut-off is considered a positive result. This method was used to analyze the results of cLAMP-MG validation, including analytical and clinical evaluations, using a total of 180 reaction tubes for testing.

Results: Negative controls were calculated to define the cut-off value in color absorption difference between positive and negative results, which was found to be 0.0286. All positive results showed absorption values higher than the cut-off with an initial 0.0341 up to 0.2001, which is consistent with the results that were analyzed by gel electrophoresis. The results of colorimetric analysis based on the color absorption did not show false positive or negative results in analytical sensitivity, analytical specificity, and clinical testing. Meaning that our method could assist in differentiating between positive and negative colors of the cLAMP-MG assay

Conclusion: Our color readout method is rapid, simple, and easy to interpret after the ending of the reaction, indicating the possibility to be applied in other colorimetric assays of a wide range of pathogens for supporting farm management and solve animal health problems.

Keywords: Colorimetric assay**,** Chromatic absorption, Colorimetric LAMP, *Raillietina* spp., Malachite green

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