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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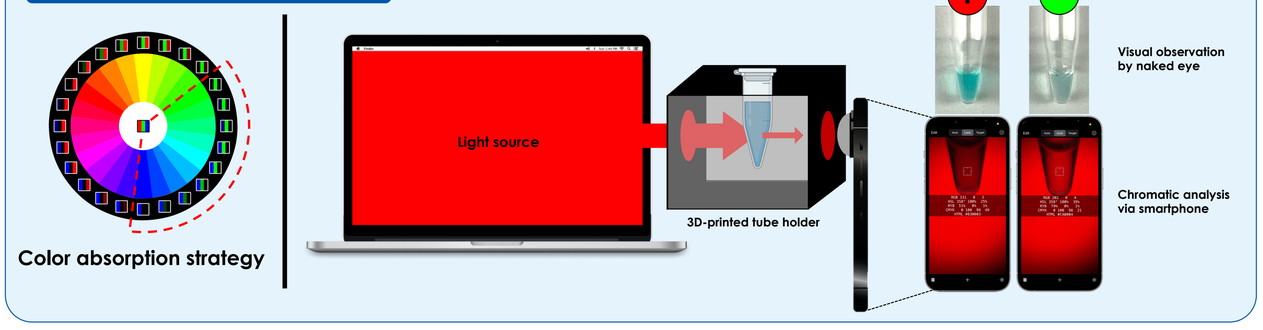
GRAPHICAL ABSTRACT



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 interpret the color

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

1 A portable 3D-printed tube holder design and fabrication

- A device was designed by a 123D design software and printed by a 3D printer with a black polylactic acid filament to prevent light reflection in the device.
- MacBook Air M2 screen was used as a chromatic light source. An iPhone 13's camera and a convex lens coupled with a ColorAssist application on smartphone were applied a color detector.
- cLAMP-MG solution in a 0.2 mL tube was inserted into the tube holder hanging on a notebook screen. The red light from the screen passed through the solution in the tube, and the transmitted light was measured by smartphone

2 Chromatic analysis

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

Where R_{p} and R_{n} are the color values of sample tube and negative control, respectively. R_{p0} and R_{n0} are the red color values of the background (light source) before measuring the sample tube and negative control, respectively.

3 Chromatic threshold setting

Empirical rule was applied to compute a cut-off value from negative controls in analytical sensitivity test (n= 9) according to the following: three-fold standard deviation of the negative controls (3SD), any reaction higher than cut-off is considered a positive result.

4 Utilizations of the color absorption for chromatic interpretation

Our strategy was used to analyze the chromatic results of cLAMP-MG validation, including

- **Analytical sensitivity**: Ten-fold serial dilutions of each *Raillietina* species (*R. echinobothrida*, *R. tetragona*, and *R. cesticillus*), ranging from 1 to 1 × 10^{-9} ng/µL by performing three replicates per species, were used as a template to evaluate shading variability caused by a variety of initial DNA concentrations.
- **Analytical specificity**: The color results after cLAMP-MG reaction of *R. echinobothida*, *R. tetragona*, *R. cesticillus*, and seven related species (*Cotugnia* sp., *Hymenolepis nana*, *Choanotaenia* sp., *Echinostoma miyagawai*, *Heterakis gallinarum*, *Ascaridia galli*, *Gallus gallus domesticus*) were tested.
- **Fecal detection**: Sixty chicken fecal samples were individually collected from a part of the cloaca (approximately 0.25 – 0.54 g) and then extracted using a GF-1 Tissue DNA Extraction Kit (Vivantis) before testing with cLAMP-MG assay. The clinical sensitivity and specificity of color absorption strategy were estimated according to the formula by Lalkhen and McCluskey (2008) by comparing the gel electrophoresis technique.

5 Statistical analysis

- Analytical sensitivity and specificity results were statistically analyzed by one-way ANOVA. Statistical differences were significant at $p < 0.05$.
- Degrees of agreement in decal detection were assessed using Cohen's kappa, ranging from 0 to 1 were interpreted as different degrees of agreement based on the guidelines provided by McHugh (2012). McNemar's test was used to compare between our strategy and gel electrophoresis, with significant differences at $p < 0.05$.

ACKNOWLEDGEMENTS

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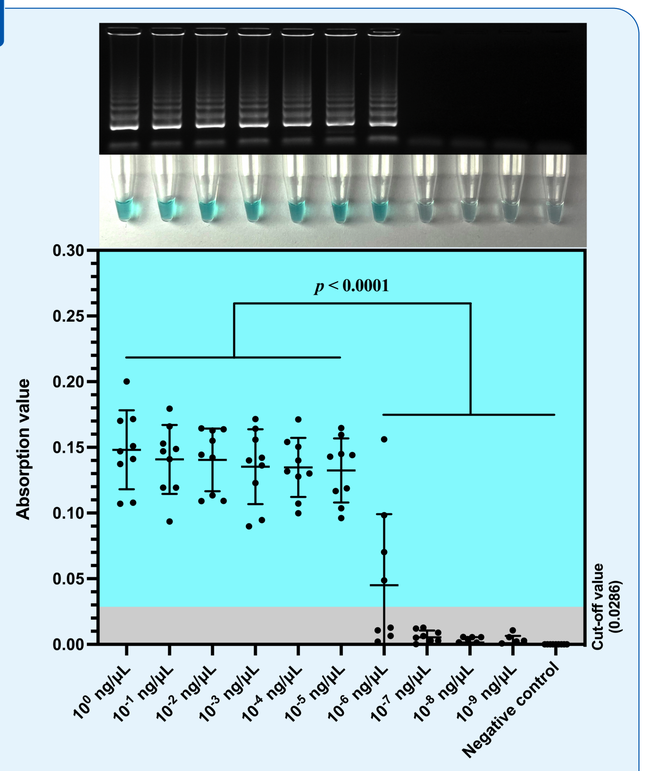
RESULTS AND DISCUSSIONS

Fig. 1 shows DNA samples ranging from 1 to 10^{-9} ng/µL of *R. echinobothrida*, *R. tetragona*, and *R. cesticillus* were tested individually by performing three replicates per species. The analytical sensitivity results analyzed by gel electrophoresis reached as low as 10^{-5} ng/µL for the three species, which was consistent with the color absorption values, ranging from 0.0900 to 0.2001. While the 10^{-6} ng/µL shows an instability of positive results, resulting in the standard deviation below a cut-off value. Therefore, the analytical sensitivity concluded to be 10^{-5} ng/µL or 10 fg/µL. Statistically significant difference was shown between 1 - 10^{-5} ng/µL and 10^{-6} - 10^{-9} ng/µL at $p < 0.0001$. For the threshold setting, nine negative controls were calculated to determine the cut-off value in color absorption difference between positive and negative reactions, which was found to be 0.0286, meaning that a value of absorption above 0.0286 was interpreted a positive result. Mean ± SD is shown on graphs (n = 9).



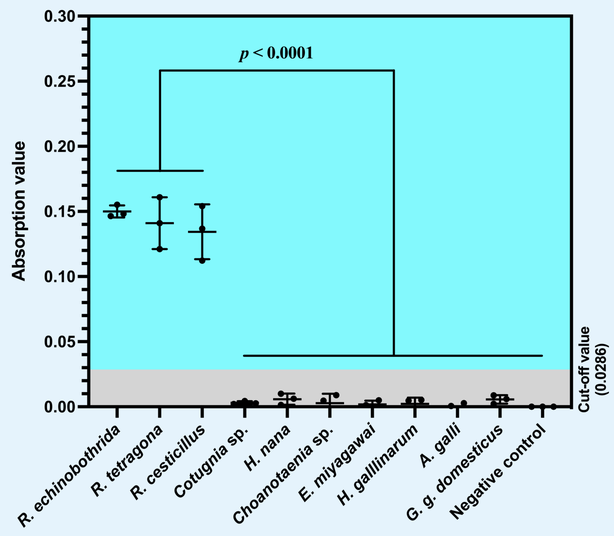


Fig. 2 shows the analytical specificity of the cLAMP-MG assay was evaluated for seven different species, including six parasites and host. The results show that *Raillietina* DNA was amplified without cross-reaction with other species by using discoloration analyzed by color absorption were higher than the cut-off value with initial 0.1123 up to 0.1609, which was also consistent with the statistically significant difference between *Raillietina* spp. and other species ($p < 0.0001$). Mean ± SD is shown on graphs (n = 3).

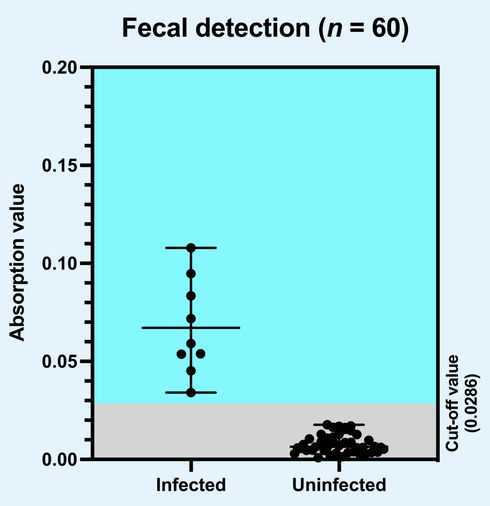


Fig. 3 shows the performance of the color absorption strategy comparing gel electrophoresis for analyzing the cLAMP-MG results (n = 60) that were amplified in the fecal sample. The gel electrophoresis results show that 9 out of 60 samples detected *Raillietina* infection, which was consistent with the color absorption values, ranging from initial 0.0341 up to 0.1079. Thus, the clinical sensitivity and specificity of cLAMP-MG results analyzed by color absorption were 100% and 100%, respectively. Regarding the evaluation of kappa value, the cLAMP-MG results detected by color absorption when compared to gel electrophoresis showed almost perfect of agreement as 1. Furthermore, McNemar's test indicates that our color absorption strategy was not not significantly different from gel electrophoresis.

One limitation of the color absorption strategy in this work is its endpoint analysis, indicating the infect or non-infect results, but it can be unspecified their parasitic loads. Nevertheless, a numerical threshold value can be interpreted the results with high confidence and to solve the problem related to the variability in the color perception of individuals.

CONCLUSION

Our strategy 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.

PUBLICATION

Panich, W., Puttharugsa, C., Tejangkura, T., & Chontananarth, T. (2024). A simple color absorption analysis of colorimetric loop-mediated isothermal amplification for detection of *Raillietina* spp. in clinical samples using a 3D-printed tube holder coupled with a smartphone camera and notebook screen. Microchim. Acta, 191(10), 603.

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