Original Research Article

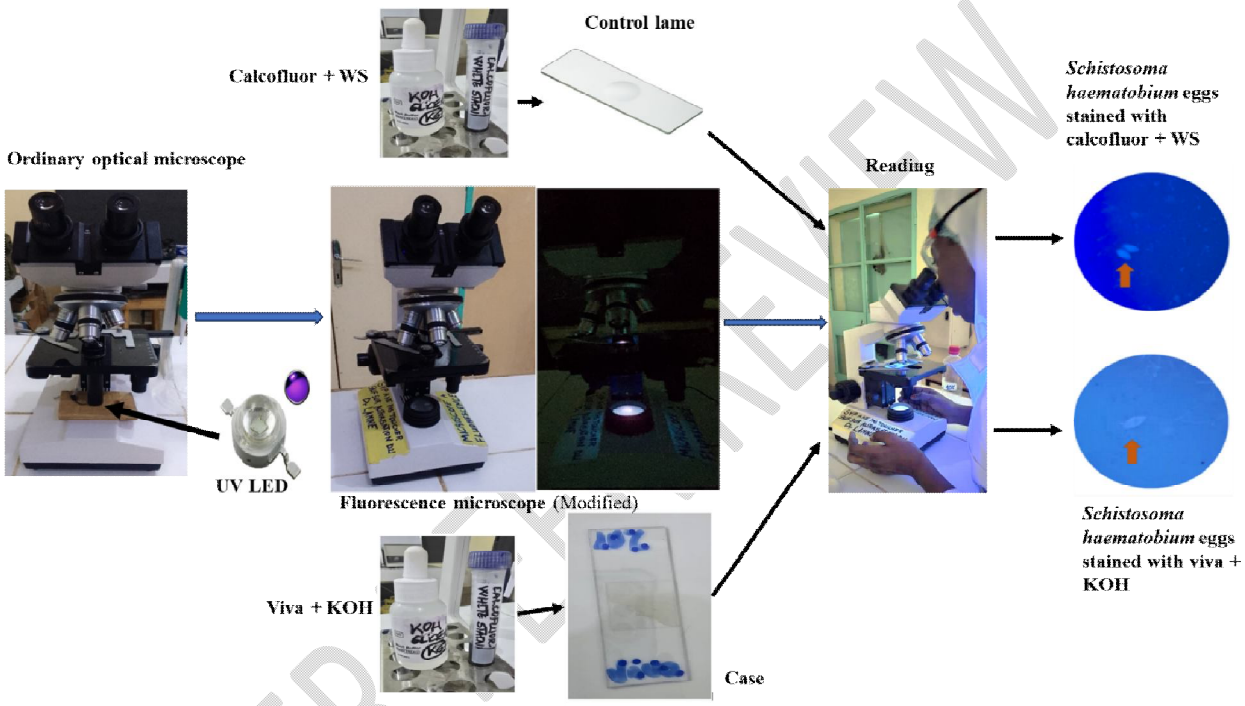
Fluorescence-Based Detection of *Schistosoma haematobium* Eggs Using Optical Brighteners from Laundry Detergents

Abstract

Schistosomiasis, caused by parasites of the *genusSchistosoma*, is a neglected tropical disease that mainly affects populations in sub-Saharan Africa. In this study, we adapted an optical microscope into a fluorescence microscope by replacing its light source with a UV lamp (300-400 nm). This simple modification allowed us to test the effectiveness of Optical Brighteners(OBs), fluorescent compounds present in detergents, for the staining and detection of *Schistosomahaematobium* eggs in urine samples. The results show that OBs allow rapid and reliable detection through visible fluorescenceto observe *Schistosoma haematobium* Eggs, offering a cost-effective alternative to traditional methods. This promising method could improve the diagnosis and surveillance of schistosomiasis in low-resource settings.

Keywords: Schistosomiasis, Optical Brighteners, Diagnostic, Fluorescence microscopy

Graphical abstract



Introduction

Schistosomes are parasites responsible for schistosomiasis, a neglected tropical disease affecting millions of people worldwide, mainly in sub-Saharan Africa. Among schistosome species, *Schistosoma haematobium* is of particular concern due to its association with urogenital schistosomiasis, a disease that can lead to serious complications including haematuria, chronic urinary tract infections and, in some cases, bladder cancer. Surveillance and early diagnosis are essential to control this disease and reduce its prevalence (World Health Organization 2013; Colley et al. 2014).

Traditional methods for diagnosing schistosomiasis rely mainly on direct microscopic observation of eggs in urine or stool samples. However, these methods have important limitations, including reduced sensitivity in cases of low parasite load and a high reliance on operator expertise. To overcome these obstacles, more sensitive and specific techniques, such as fluorescence-based approaches, can be explored (Harrington and Hageage 2003; Vieira et al. 2016).

Optical Brighteners (OBs) are synthetic fluorescent compounds that absorb ultraviolet light (360-365nm) and emit blue fluorescence (415-445nm) and are added to most modern detergents to compensate for unwanted yellowing of clothing (Cao et al., 2009).

Among OBs, two are commonly used: DSBP (4,4′-bis (2-sulfostyry) biphenyl) and DAS1 (4,4′-diamino-2,2′-stilbene-disulfonic acid), both very soluble in the water. It should also be noted that OBs of the diaminostilbene type are non-toxic compounds, stable in a strongly alkaline solution. (Jasperse & Steiger, 1992)

OBs are widely used in industrial applications: like paper and textile whitening also in washing powders (detergents) for their ability to absorb UV rays and re-emit visible fluorescence. This fluorochromes were initially introduced in mycology but are of increasing interest as a potential tool in parasitology (Hageage and Harrington 1984; Harrington and Hageage 2003; Hamer et al. 2006). These chemical compounds could offer an economical and efficient alternative for the staining and detection of parasite eggs such as *S. haematobium*. The observation and testing of the different detergents containing OB must be done with fluorescence microscope.

In this study, we designed an innovative method by adapting a conventional optical microscope into a fluorescence microscope with simple means. This adaptation consisted in replacing the original halogen light source with a UV lamp (300-400 nm), thus allowing the excitation of this fluorescent compounds. We then tested the efficiency of OBs present in some detergents,(Data not shown) to stain *S. haematobium* eggs in urine samples. The aim was to determine whether these OBs could serve as inexpensive and easily accessible fluorescent markers for the rapid and reliable detection of schistosome eggs.

Materials and Methods

1. Materials

The materials used in this study included several essential tools and products. Urine samples tested positive for *Schistosoma haematobium* were used as the basis for the evaluation of Optical Brighteners. Distilled water was used to prepare the solution of the selected detergent *Viva* (Aspira Nigeria, LTD). A modified brightfield microscope

was used to observe the preparations. Other equipment included slides and coverslips, a centrifuge, conical tubes, micropipettes and protective glasses.

2. Methods

2.1 Selection of detergent and preparation of solutions

Viva detergent containing Optical Brighteners was selected for this study due to its local availability and suitable chemical composition. We used Calcofluor White(Tinopal UNPA-GX) as positive control colorant(The CW aqueous solutions were prepared at 0.1%).Thedetergent solutions were prepared at a concentration of 10%.

Each detergent was precisely measured at 5 g, to which 50 ml of distilled water was added. After stirring to ensure complete dissolution, the solutions were stored away from light to avoid any alteration of the fluorescent properties at room temperature.

2.2 Microscope Modification

An ordinary optical microscope has been transformed into a fluorescence microscope. The normal bulb has been replaced by the UV lamp bulb described. High quality 365nm UV LED as light source was used. It has 3 watts output and can be used as a professional UV flash light. This technique was feasible and above all low cost. The use of this fluorescence microscope allowed us to test the different detergents containing OB.

This modification allowed a cost-effective and easily reproducible upgrade, making the instrument suitable for detecting fluorescence emitted by detergentsOBs.

2.3 Parasitological examination of urine

The parasitological analysis began with the selection of samples strongly positive for *S. haematobium* using a conventional microscope. The urine was then centrifuged for 5 minutes, and the supernatant was decanted. A drop of the homogenized sample was placed between the slide and the coverslip, followed by the addition of a drop of 10% detergent solution + Working solution (WS) (10% KOH w/v and 10% Glycerol v/v). The glycerine prevents preparations drying out.

The prepared slides were stored protected from light before being examined under a fluorescence microscope. The observations were documented using a Samsung S24 Ultra phone. The slides were analyzed after 10 minutes under 10x magnification

Results and Discussion

S. haematobium eggs treated with detergent +WSemitted intense and visible fluorescence under UV light. This fluorescence allowed rapid and reliable identification of eggs in urine samples (Fig.1).Additional advantages include the simplicity of the method, low toxicological impact in workers and environment, low fading molecules, and the use of readily available materials, making this approach particularly suitable for low-resource settings.

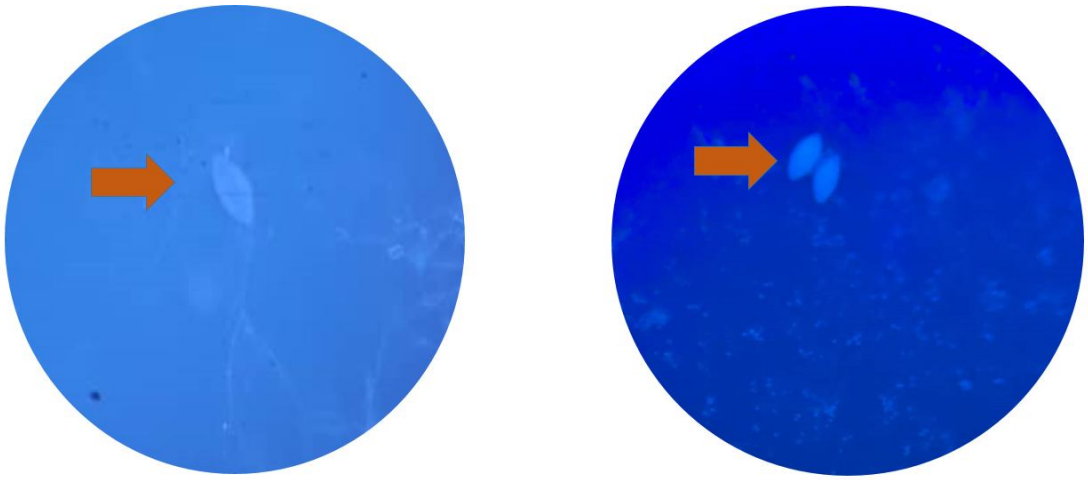


Figure 1.Image showing the fluorescence emitted by *Schistosoma haematobium* eggs after exposure to Optical Brighteners + WS after 10 min. (Left image. Stained with viva + KOH. Right image stained with calcofluor + WS (Magnification of the images 10x)

This study focused on laundry detergents containing optical brighteners and their ability to stain the eggs of the trematode *S. haematobium*. Calcofluor and laundry detergents OBs are fluorochromes that can stain schistosomes eggs and emit blue fluorescence under excitation of light with a wavelength of 365 nm. Laundry detergents containing OBs can effectively stain *S. haematobium* eggs. This study shows that Viva detergents and Calcofluor stain *S. haematobium* eggs after 10 minutes with KOH.

This improvement in coloration could be explained by the presence of a chemical substance such as speckles and polycarboxylate contained in viva detergents (Jasperse & Steiger, 1992). KOH has also been reported to improve the fluorescent ability of OBs (Hageage& Harrington, 1984).

Conclusion

The use of OBs for fluorescent staining of *S. haematobium* eggs represents a significant advance in the diagnosis of schistosomiasis.This method offers a cost-effective and accessible alternative to traditional techniques. The microscope modification is simple and does not require expensive equipment, making it a viable solution for laboratories in endemic areas.

However, further studies are neededabout details on UV light calibration and fluorescence intensity standardization. This allows to evaluate the durability of this method in field conditions and to standardize the procedures to ensure optimal reproducibility. The potential impact of this technique on epidemiological surveillance and control of schistosomiasis could be significant, especially in regions where access to advanced technologies is limited.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: This study does not require approval from an ethics committee, as it does not involve human participants or animal experiments.The sample that was used is the positive sample which serves as a positive control in our laboratory.

Data Availability Statement: The data used to support the results of this study are available upon request from the corresponding author**.**

Disclaimer (Artificial intelligence)

Author(s) hereby, declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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