Short Report: Ultra–Low-Cost Urine Filtration for *Schistosoma haematobium* Diagnosis: A Proof-of-Concept Study

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Abstract. Simple, efficient, and cost-effective strategies are needed for urine sample preparation in the field diagnosis of infection with *Schistosoma haematobium*. In this proof-of-concept study, we evaluated inexpensive and widely available paper products (paper towels, school workbook paper, and newspaper) to gravity-filter urine containing 60 eggs/mL of *Schistosoma haematobium*. Eggs were reliably visualized by light microscopy by using single-ply paper towels as urine filters. This filtration method has broad applicability in clinical and public health settings in resource-constrained environments.

Schistosomiasis is a disease caused by infection with a blood-borne fluke acquired through direct contact with contaminated fresh water. Approximately 200 million people are infected worldwide, with the vast majority residing in sub-Saharan Africa.¹ Urogenital schistosomiasis, which is caused by *Schistosoma haematobium*, accounts for approximately two-thirds of all cases^{2,3} and has a broad range of clinical features, ranging from hematuria and genital ulceration to bladder cancer and infertility.^{2–4} Rural, impoverished, and marginalized populations are disproportionately affected.⁵

The World Health Organization is facilitating mass drug administration (MDA) campaigns for several neglected tropical diseases, including the provision of praziquantel in regions with high burdens of schistosomiasis.⁶ Lower prevalences of infection are found in regions that implement MDA campaigns.^{7,8} Effective and inexpensive diagnostic tools will be essential for *S. haematobium* case detection in clinical and public health settings, especially in regions with an infection prevalence below the threshold for MDA campaigns.

There are several low-cost diagnostics strategies for schistosomiasis; urine reagent strips evaluating for hematuria are somewhat sensitive but have a wide range in specificity for *S. haematobium* diagnosis.^{9–11} Similarly, the urine circulating cathodic antigen test is sensitive for *S. mansoni*, but lacks sensitivity for *S. haematobium*.^{12,13} Urine filtration and light microscopy remain an important diagnostic procedure for detection of *S. haematobium* infection. However, techniques used for concentration of eggs from urine involve either centrifugation or filtration by using specialized microporous membranes, which are relatively expensive for use in low-income countries. Simplifying sample preparation in the field before microscopy is vital for efficient and cost-effective diagnostic strategies in resource-constrained settings. The goal of this proof-of-concept study was to evaluate inexpensive, rapid, and effective modes of urine filtration for *S. haematobium* by using widely available and affordable commercial products, and to compare these approaches with a conventional concentration approach.

Ethical approval for this proof-of-concept diagnostic project was granted by the Institutional Review Board of the University of Cape Coast, Cape Coast, Ghana (IRB/UCC) and the Department of Laboratory Technology.

Urine samples from 10 patients with recently microscopically confirmed, high-intensity infection with *S. haematobium* were included in this proof-of-concept study. *Schistosoma haematobium* infection was confirmed in individual patients by centrifugation and pooling of 10 mL of urine collected between 10:00 AM and 2:00 PM and subsequent light microscopic examination for diagnosis and quantification of eggs. Egg quantification in the pooled urine specimen was confirmed by using light microscopy under 10× and 40× objective lenses by a senior expert microscopist. Urine was pooled such that the same infection intensity (60 eggs/mL in our study) would be evaluated by each filtration device.

Three paper products were evaluated as experimental urine filters: paper towels, newspaper, and paper from a student workbook. All papers were procured locally and available for purchase from local shops for less than 2.75 Ghanaian Cedi per product, which is equivalent to approximately \$1.00 US dollar (USD). Multiple filters could be constructed from each individual purchase such that the cost of each filter cost was an estimated \$ 0.01–0.03 USD.

For the proof-of-concept project, each type of filter paper was tested five times. Paper was rolled into a cone, fitted into a funnel, and placed over a glass, as shown in Figure 1A. Ten milliliters of urine containing *S. haematobium* eggs was poured into the bottom of the rolled filter paper cone, which enabled gravity filtration of urine through the paper into a cup below. After filtration, we used scissors to cut off the bottom of the paper cone into which urine was poured. We placed this paper on a glass slide and examined it for ova by using conventional light microscopy under 10× and 40× objective lenses for identification of *S. haematobium* eggs by an expert microscopist. The presence or absence of eggs was recorded. The funnel was washed after each use to avoid potential contamination.

Urine filter paper was easy to procure, and the filtration set-up was constructed without difficulty. Passing 10 mL of urine through each type of filter paper took less than one minute. We were easily able to visualize *S. haematobium* eggs on 5 of 5 (100%) paper towel-filtered samples by using conventional light microscopy (Figure 1B). We were not able to visualize *S. haematobium* eggs with either the workbook

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FIGURE 1. (A) Gravity filtration device with single-ply paper towel as filter for urine. (B) Egg of *Schistosoma haematobium* filtered by singly-ply paper towel and imaged by light microscopy. Scale bar = 100 μm .

paper (0 of 5, 0%) or newspaper (0 of 5, 0%) products because of issues with tearing of the paper when transferring it to the microscope and the way light was refracted off of the paper.

In this qualitative, proof-of-concept study, we demonstrate that widely available and inexpensive paper products may be used for urine filtration in the diagnosis of *S. haematobium* infections. Currently, urine microscopy remains the gold standard for *S. haematobium* diagnosis. Diagnostic approaches for this infection are dependent on resources available; some researchers use urine centrifugation and others use gravity or syringe filtration systems. For field diagnostic procedures, the World Health Organization recommends passing 10 mL of urine through a plastic device with an O-ring rubber seal and Millipore filter paper (Millipore Corporation, Billerica, MA) (pore sizes of 12–20 μm) to capture *S. haematobium* eggs.¹⁴ Millipore filter paper is then removed and evaluated under light microscope for the presence or absence of eggs. Although quite effective, Millipore paper is expensive and may cost \$1–2 USD per filter. In addition, the O-filter system should be cleaned between each use to avoid contamination and false-positive results. The expense of filter paper and time required for cleaning of syringe devices are not advantageous in busy clinics or large public health screening programs.

We demonstrated that regular paper towels were capable of capturing *S. haematobium* eggs and were sturdy enough to be transferred from the filtration device for evaluation under a light microscope. This technique has the benefits of costing pennies, is extremely easy to perform, and the materials are readily available in local shops in most towns and cities. Unfortunately, newspaper and school-book paper were not as successful for *S. haematobium* filtration. Newspaper and school-book paper were very challenging to work with when wet. Both had issues with tearing when being transferred from the funnel to glass slide. In addition, we were not able to visualize eggs with these paper products, possibly because of the way light refracted off their surfaces and distortion of the eggs. Furthermore, given the issues of tearing when transferring these paper products onto glass slides, sample loss could have occurred. Other low-cost filtration devices have been developed and might show promise in the field diagnosis for *S. haematobium*. However, these still require purchasing specialized medical equipment¹⁵ and using conventional microscopy.

There were several limitations to our study. We did not quantify *S. haematobium* eggs on experimental filter paper, and this will be important if this method is to be used in epidemiologic studies. In addition, only high-intensity test samples were used, and only expert microscopists evaluated the filter paper. Future studies should evaluate other low-cost paper products for filtration, diverse infection intensities, and the role of non-expert microscopists in public health and clinical settings. In addition, given the simplicity of this diagnostic platform, we envision portable light microscopic devices,¹⁶ including mobile phone microscopy,¹⁷ to aid in evaluation of filtered urine for *S. haematobium* infection. This procedure may enable poorly equipped peripheral clinics and public health services in rural or remote communities to facilitate diagnosis of this neglected tropical disease.

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REFERENCES

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J, 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 6: 411–425.
2. Van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, Habbema JD, Engels D, 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Trop* 86: 125–139.
3. King CH, Keating CE, Muruka JF, Ouma JH, Houser H, Siongok TK, Mahmoud AA, 1988. Urinary tract morbidity in schistosomiasis haematobia: associations with age and intensity of infection in an endemic area of Coast Province, Kenya. *Am J Trop Med Hyg* 39: 361–368.
4. El-Bolkainy MN, Mokhtar NM, Ghoneim MA, Hussein MH, 1981. The impact of schistosomiasis on the pathology of bladder carcinoma. *Cancer* 48: 2643–2648.

5. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J, 2008. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 118: 1311–1321.
6. World Health Organization, 2006. *Preventive Chemotherapy in Human Helminthiasis: Coordinated Use of Anthelmintic Drugs in Control Interventions: A Manual for Health Professionals and Programme Managers*. Geneva: World Health Organization.
7. Fürst T, Ouattara M, Silué KD, N'Goran DN, Adiossan LG, Bogoch II, N'Guessan Y, Koné S, Utzinger J, N'Goran EK, 2013. Scope and limits of an anamnestic questionnaire in a control-induced low-endemicity helminthiasis setting in south-central Côte d'Ivoire. *PLoS ONE* 8: e64380.
8. Sesay S, Paye J, Bah MS, McCarthy FM, Conteh A, Sonnie M, Hodges MH, Zhang Y, 2014. *Schistosoma mansoni* infection after three years of mass drug administration in Sierra Leone. *Parasit Vectors* 7: 14.
9. Wilkins HA, Goll P, Marshall TF, Moore P, 1979. The significance of proteinuria and haematuria in *Schistosoma haematobium* infection. *Trans R Soc Trop Med Hyg* 73: 74–80.
10. Mott KE, Dixon H, Osei-Tutu E, England EC, 1983. Relation between intensity of *Schistosoma haematobium* infection and clinical haematuria and proteinuria. *Lancet* 1: 1005–1008.
11. Bogoch II, Andrews JR, Dadzie Ephraim RK, Utzinger J, 2012. Simple questionnaire and urine reagent strips compared to microscopy for the diagnosis of *Schistosoma haematobium* in a community in northern Ghana. *Trop Med Int Health* 17: 1217–1221.
12. Ayele B, Erko B, Legesse M, Hailu A, Medhin G, 2008. Evaluation of circulating cathodic antigen (CCA) strip for diagnosis of urinary schistosomiasis in Hassoba school children, Afar, Ethiopia. *Parasite* 15: 69–75.
13. Coulibaly JT, Knopp S, N'Guessan NA, Silué KD, Fürst T, Lohourignon LK, Brou JK, N'Gbesso YK, Vounatsou P, N'Goran EK, Utzinger J, 2011. Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire. *PLoS Negl Trop Dis* 5: e1384.
14. WHO Expert Committee, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organ Tech Rep Ser* 912: i–vi, 1–57.
15. Gyorkos TW, Ramsan M, Foun A, Khamis IS, 2001. Efficacy of new low-cost filtration device for recovering *Schistosoma haematobium* eggs from urine. *J Clin Microbiol* 39: 2681–2682.
16. Stothard JR, Kabatereine NB, Tukahebwa EM, Kazibwe F, Mathieson W, Webster JP, Fenwick A, 2005. Field evaluation of the Meade Readiview handheld microscope for diagnosis of intestinal schistosomiasis in Ugandan school children. *Am J Trop Med Hyg* 73: 949–955.
17. Bogoch II, Andrews JR, Speich B, Utzinger J, Ame SM, Ali SM, Keiser J, 2013. Mobile phone microscopy for the diagnosis of soil-transmitted helminth infections: a proof-of-concept study. *Am J Trop Med Hyg* 88: 626–629.