

## Evaluation of PCR and Millipore Filtration Method for the Diagnosis of *Schistosoma haematobium*: A Comparative Study

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### Abstract

**Introduction:** Diagnosis of *Schistosoma haematobium* infection among children poses a significant clinical challenge. Adults with chronic infection as well as lightly-infected children, pass few eggs in the urine that are frequently overlooked when the commonly-used diagnostic methods are utilized. The objective of this study is the evaluation of two diagnostic approaches (Polymerase Chain Reaction (PCR) and millipore filtration method as conventional parasitological method commonly applied in the diagnosis of schistosomiasis).

**Materials and method:** This study was conducted at Al Zaidab district, River Nile State, Northern Sudan (17° 20' N-17° 39' N and 33° 46' E-33° 57' E) on the west bank of the River Nile. The area is known endemic with urinary schistosomiasis since early 1920s. From 93 school children, 50 ml of fresh voided urine specimens were collected. 10 ml were passed through the Millipore filter and examined under the microscope for detection and egg count. 40 ml were passed through Whatman No. 3 filter paper for further DNA extraction. Extracted DNA was amplified by PCR using a set of primers to detect Dra 1 gene fragment at 121 bp.

**Results:** Twenty-three of 93 (24.7%) were positive for eggs, all with hematuria. 44 of 93 (47.8%) showed parasite-specific DNA, 43 with hematuria. The sensitivity and specificity of millipore microscopy of urine were 52.3%, and 100%; and for PCR were 95.4%, and 96%, respectively.

**Conclusion:** It was concluded that Millipore filtration method is suitable for diagnosis of acute symptomatic urinary schistosomiasis in children, and PCR is reserved for clinically susceptible case. Filter paper extraction method was found feasible and should be considered as a transport tool for large scale molecular studies.

**Keywords:** *Schistosoma*; *Haematobium*; millipore; PCR

### Introduction

There are more than 200,000 people die from schistosomiasis each year [1]. This occurs in 74 countries, where there are 700 million people at risk; 240 million of them are affected. Elimination of schistosomiasis as a public health problem and interruption of transmission in endemic foci, are the strategic key factors of the World Health Organization for 2025 [2].

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Human schistosomiasis mainly caused by three species of the genus *Schistosoma*; *S. haematobium*; *S. mansoni*; and *S. japonicum* [3]. Human acquires schistosomiasis by cercarial penetration when contacts infested freshwater. Adult couple worms reside in the blood vessels of the gut or the bladder. Symptoms start with the egg deposition. Acute urinary schistosomiasis is usually accompanied by terminal hematuria. Chronic infection usually associated with life-threatening conditions such like bladder cancer, esophageal varices, periportal fibrosis, and other morbidity [4,5].

The diagnosis of acute urinary schistosomiasis is achieved by microscopic examination of urine, which is the gold standard diagnostic method. The usage of millipore filtration of urine increases the sensitivity of urine examination [6].

The sensitivity of the conventional parasitological methods for the detection of light infections is doubtful [7] and is extensively investigated [8].

Serological tests could be useful especially for chronic infection, though they are less specific. Rapid diagnostic tests (RDTs) are now available for screening purposes [9,10]. Dipsticks continue to serve as useful adjuncts for monitoring community prevalence [11].

Detection and quantification of *Schistosoma species* DNA in shown to be a powerful and specific diagnostic tool for detection of *S. haematobium* infections, with higher sensitivity compared to microscopy [12].

### Materials and Methods

This is a cross-sectional and descriptive study of qualitative and quantitative approaches in which 93 school children (boys and girls, age 6-12 years) were enrolled.

**Study area:** The study was conducted at Al Zaidab district, River Nile State, Northern Sudan (17° 20' N-17° 39' N and 33° 46' E-33° 57' E) at the west bank of the River Nile. The area is known endemic with urinary schistosomiasis since early 1920s.

50 ml of fresh voided urine was collected from each participant, and subjected to standard urine macroscopic examination. 10 ml sample was filtered through millipore filter and examined under microscope for detection and egg count. The rest of the specimen was filtered through Whatman paper No 3 (Whatman International, Maidstone, England) for further DNA extraction.

### DNA Extraction

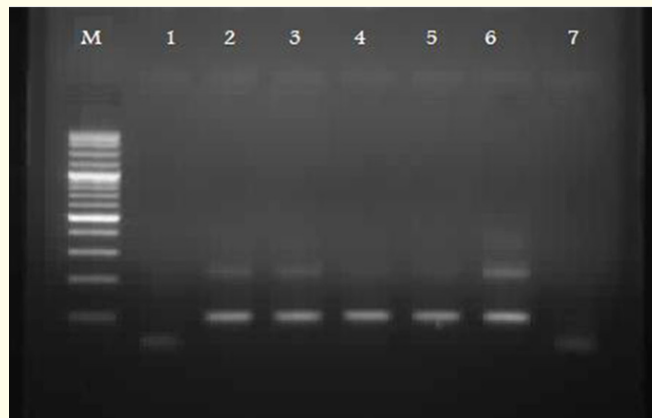
DNA Extraction was achieved from the filter paper using the Qiagen QIAamp® DNA extraction mini-kit (Qiagen Sciences, MD).

The PCR amplification was carried out employing specific primers (forward: 5'- GATCTCACCTATCAGACGAAAC-3' and reverse: 5'- TCACAACGATAGACCAAC-3') for specific amplification of Dra 1 repeats of *S. haematobium*. The PCR product was visualized in 2% agarose gel under UV transilluminator and the result was processed using computerized gel documentation system

### Results

Among the study population, 44 individuals showed hematuria. *S. haematobium* eggs were detected in 23 urine specimens (24.7%), all of them were with both hematuria and proteinuria. *S. haematobium* genomic DNA was detected in 44 urine specimen (47.3%) at the 121 bp size band (Figure 1), only two were without hematuria.

The sensitivity and specificity were calculated based on the presence of hematuria as a marker of a disease. Thus, it was found that the sensitivity and specificity of millipore microscopy of urine were (52.3%, and 100%), and for PCR (95.4%, and 96%) respectively.



**Figure 1:** Detection of 121 bp of *Dra1* repeat sequence of *Schistosoma haematobium* employing *S. h.* primers. M = 100 bp size markers; lane 1 = negative control; lanes 2 = positive control; lanes 3-4 = egg-positive samples; lanes 5 and 6 = egg-negative samples; lane 7 = egg-negative and yielded negative PCR, from Alzaidab, Nile State, Sudan.

## Discussion

In this study, a novel urine filtration method was used to extract DNA from 93 filter papers and subjected to *Dra1* for *S. haematobium* genomic DNA detection. Equal samples were filtered through millipore filters to detect *Schistosoma haematobium* eggs trapped during the filtration process.

The passage of eggs through the urine can be complicated by host and parasite physiological and periodic interaction [13], as well as the individual immune response and nature of pathological damage in the bladder tissue. Diagnostic approaches have to rely on the specific needs of epidemiological or clinical studies. For instance, the use of the urine reagent strips test, in an epidemiological situation when rapid, sensitivity, and low cost are important and specificity can be considered a secondary factor, can be considered as feasible. In these situations the population under study is usually children where the correlation between haematuria and parasitemia is high. However, in clinical practice, this may not be applicable in case of adults, where sensitivity and specificity should be on the maximum level. This was demonstrated by Koukounari and colleagues who evaluated the efficacy of different diagnostic tools including conventional microscopy, antigen detection, medical imaging, and detection of haematuria [7]. Their study showed that it was difficult to define a “gold” standard for diagnosis of this parasite in adults. This study gives evidence to the feasibility of using conventional PCR as the technique of choice, since the equipment is recently available, and the parasite DNA was stable and easy to transport without freezing.

Obeng in Ghana [14], and Lier in China [15], both used real-time PCR to detect molecular evidence of *Schistosoma* infection; they concluded that it was expensive and cost-ineffective for diagnostic and epidemiological surveys. As a means to detect the presence of blood-borne pathogens, the isolation of DNA from urine has been applied to malaria and conceptually this could also be applied to the detection of *Schistosoma* DNA fragments in urine. The repeat nature and volume of *Schistosoma* DNA, as shown by Hamburger and others suggested that the *Dra 1* fragment would be a good target and this appears to be the case described in this study [16]. Despite the fact that it is difficult to distinguish between DNA fragments in urine and those extracted from eggs, it is worth mentioning that *Dra 1* fragments were detected in specimens whether eggs were found or not. The detection of *Dra 1* also was independent of the number of eggs passed in samples. Thus, it is likely that the DNA was present free in the urine. The technique seems to be useful both clinically and in the field and the filtration technique facilitates transport and storage of urine specimens required for this analysis. Additional studies are supposed to be ongoing to expand this work in adult populations in Sudan.

### Conclusion

As a conclusion, the syringe millipore method for the detection of *Schistosoma haematobium* egg in urine samples is a reliable in cases of severe and perhaps moderate infections. The method is convenient for outdoor and field practice. Nevertheless this method fails to detect situations of chronic or light infections.

The PCR technique is a very efficient, accurate and sensitive tool for the detection of the parasite, no matter how mild the infection is. The processing of sample must be carried out inside the laboratory, yet the dried samples of urine collected on filter paper in the field remain durable to adverse conditions and this facilitates storage and transportation from the field to the laboratory without the need for freezing and can stay viable for several months. Despite the fact that it is difficult to distinguish between DNA fragments in urine and those extracted from eggs, it is worth mentioning that *Dra 1* fragments can be detected in specimens where eggs were found and in specimens where no eggs were present.

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