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Limited Field Evaluation of a Rapid Monoclonal Antibody-Based Dipstick Assay for Urinary Schistosomiasis

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ABSTRACT

A rapid, visually read monoclonal antibody (MoAb)-based dipstick assay for specific diagnosis of urinary schistosomiasis was field tested with microscopy and the use of hematuria and proteinuria in a schistosomiasis hematobia endemic area in Southern Ghana. The study group consisted of 229 individuals (114 males and 115 females) aged 1 to 86 years; 145/229 (63.3%) of the subjects submitted stool samples from which no *S. mansoni* eggs were detected. However, infections with *Necator americanus* (hookworms) 33.1%, *Ascaris lumbricoides* 2.8%, *Trichuris trichiura* (whipworm) 2.8%, and *Strongyloides stercoralis* 0.7% were detected but did not appear to influence the results of the MoAb-dipstick assay. Urinary schistosomiasis prevalence was estimated as 47.6% by microscopy, 48% by MoAb-dipstick, 39.7% by microhematuria, and 23.6% by proteinuria. The MoAb-dipstick correctly identified 108/109 (99.1%) of microscopically confirmed cases and 118/120 (98.3%) of egg-negative individuals, thereby giving a sensitivity of 99.1% and a specificity of 98.3%. On the other hand, microhematuria and proteinuria were, respectively, 76.1% and 40.4% sensitive, and 94.2% and 92.5% specific when compared to microscopy. Microhematuria and proteinuria had significantly lower sensitivity ($P < 0.001$) than either microscopy or dipstick.

INTRODUCTION

URINARY SCHISTOSOMIASIS caused by *Schistosoma haematobium* affects about 100 million people in Africa and South Western Asia.[1] The pathologic features of infection are the consequence of egg deposition into and around the urinary tract, and routine diagnosis is still achieved by the microscopic detection of *S. haematobium* eggs in urine,[1–3] even though the method is tedious and time-consuming and the sensitivity is limited by great fluctuation of egg output.

Detection of *Schistosoma* genus-specific antigens[1,4] has been shown to be more sensitive, but their routine field use awaits the introduction of rapid dipstick techniques.[1] Genus-specific diagnosis of schistosomiasis is justified by the use of a common drug (praziquantel) in control of schistosomiasis. However, the occurrence of both *S. haematobium* and *S. mansoni* in hyperendemic areas,[5] the reported differences in the efficacy of praziquantel,[1,6–8] and the possibility of the emergence of drug-resistant schistosome species or strains also indicate the epidemiologic importance of specific diagnosis.

This study aims at field testing a new urine-based MoAb-dipstick assay for specific diagnosis of urinary schistosomiasis. The assay uses a *S. haematobium* species-specific IgG1 mouse monoclonal antibody that detects a 29 kDa peptide.[9]

MATERIALS AND METHODS

The MoAb-dipstick Kit

1. *Polyvinylidine difluoride (PVDF) membrane strips.* Four hundred PVDF membrane strips each measuring 0.6 × 7 cm were numbered at one end and wet up to a 3-cm length from the other end by immersion in methanol for 15 seconds. Wet strips were immediately transferred into distilled water in two 50-ml conical tubes (Falcon, Becton Dickinson Labware, Oxnard, California) and transported to the field.

2. *Materials.* The materials used for the MoAb-dipstick included 400 reusable 150-ml capped plastic containers for collecting urine samples, 2 rectangular (20 × 11 × 6 cm) plastic

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bowls for washing, 2 wash bottles, 5 50-ml conical tubes for reagent incubation, 1 10–1000-μl adjustable pipette and pipette tips, and 2 pairs of forceps.

3. *Reagents, Chemicals, and Buffers*. Reagents, chemicals, and buffers, enough for assaying 400 urine samples, were transported to the field. These were 200 ml of 5% skimmed milk in Tris-buffered saline (TBS), pH 7.4; 200 ml of combined reagent consisting of MoAb and goat anti-mouse horseradish peroxidase-conjugated IgG in 0.1% skimmed milk in TBS, and 1 ml of 30% hydrogen peroxide (H_2O_2) both transported on ice; 1 ml of 1M $CoCl_2 \cdot 6H_2O$; 100 mg of 3,3′-diaminobenzidine (DAB) in a 1.5 ml Eppendorf tube; 250 μl of concentrated HCl and 3 liters of TBS, pH 7.4.

4. *Monoclonal Antibody*. The monoclonal antibody (Sh2/15.F) used in this study was produced following immunization of BALB/c mice with precipitated proteins from *S.–haematobium*-infected human urine.[10] Sh2/15.F (IgG_1) was shown to bind a 29 kDa *S. haematobium* species-specific peptide present in the egg stage of both Ghanaian and Egyptian strains of the parasite.[9] Sh2/15.F did not show cross-reactivity with *Necator americanus* (hookworm) egg antigens in the microplate ELISA.[9]

Study area

Field evaluation of the MoAb-dipstick was carried out in a village called Mayera that covers an area of about 5 km^2 and is located 5°41′N and 0°17′W in a *Schistosomiasis haematobia* endemic region of southern Ghana. The vegetation along the banks of the very slow flowing Nsakyi stream on the outskirts of the village consist mainly of grass and a few trees. The weedy stream banks contain large amounts of decaying plant leaves and twigs and are infested with *Bulinus globosus* snails. The Nsakyi constitutes the principal source of water and transmission of urinary schistosomiasis.

Collection and analysis of urine and stool

A total of 20 to 100 ml of urine was collected from each individual and immediately analyzed for microhematuria and proteinuria by reagent strips (Ames Co.; Division of Miles Laboratories, Japan). Urine specimens were also tested in the field for the presence of a *S. haematobium* species-specific antigen by MoAb-dipstick within 4 hours of collection. The specimens were transported to the laboratory on ice at 4° C and examined within 12 hours by microscopy. Each specimen was centrifuged at 400 *g* and the entire sediment was examined microscopically for the presence of *S. haematobium* eggs.

Individuals who gave urine specimens were also given containers to produce stool samples. Stool specimens were transported to the laboratory on ice, stored at 4° C, and examined within 2 weeks for *S. mansoni* and intestinal parasite ova using the Kato-Katz technique.[11]

The MoAb-dipstick Assay Procedure

The wet PVDF membrane strips were distributed one strip per urine sample and left for 30 minutes to trap antigens. The strips were retrieved at the end of the incubation period, rinsed twice, and incubated for 30 minutes with "blocking solution" containing 5% (w/v) skimmed milk in TBS (50 mM Tris and 150 mM NaCl, pH 7.4). The strips were removed from blocking solution and incubated for 1 hour in a combined reagent consisting of the MoAb (Sh2/15.F) and horseradish peroxidase-labeled goat Anti-mouse IgG diluted in 0.1% skimmed milk in TBS. The strips were washed five times in succession, each with excess TBS, pH 7.4. The membrane strips were finally incubated for about 3 minutes in a substrate solution containing 0.05% (w/v) 3,3′-diaminobenzidine (DAB), 0.15% (v/v) hydrogen peroxide H_2O_2 in TBS with 5mM cobalt II chloride hexahydrate ($CoCl_2 \cdot 6H_2O$). The strips were washed with distilled water and the substrate reaction stopped by the addition of a few drops of concentrated HCl. Positive strips appeared bluish-black while negative ones remained colorless.

Determination of the optimum concentrations of MoAb and goat anti-mouse horseradish peroxidase conjugate in the reagent mixture used in the dipstick-ELISA

The MoAb Sh2/15.F (IgG1) in culture supernatant was concentrated hundredfold by filtration through 10,000 MW cut-off pore size membrane (Millipore Corporation, Bedford, MA, USA) and tested in dipstick-ELISA. Briefly, antigen-captured PVDF membrane strips were rinsed in TBS, pH 7.4 for 2 minutes and different strips incubated with a mixture of MoAb and goat anti-mouse-HRPO in a checkerboard titration. Both MoAb and enzyme conjugate were titrated from 1:50 to 1:1200 at twofold dilution in blocking solution. The MoAb and enzyme conjugate dilution that gave the most intense color reactions by visual assessment was noted and used in preparing the reagent mixture for the dipstick-ELISA.

Sensitivity and specificity of the tests

The percentage sensitivity and specificity of the MoAb-dipstick, microhematuria, and proteinuria were calculated as follows:

$$\text{Sensitivity (\%)} = \frac{\text{Number of individuals positive by the test that was also positive by microscopy}}{\text{Total number of individuals testing positive by microscopy}} \times 100$$

$$\text{Specificity (\%)} = \frac{\text{Number of individuals negative by the test that was also negative by microscopy}}{\text{Total number of individuals testing negative by microscopy}} \times 100$$

RESULTS

Results of stool examination

Of the 229 individuals who gave urine specimens, only 145 (63.3%) submitted stool samples. None of the 145 stool samples examined contained *S. mansoni* eggs. However, ova of other intestinal parasites were identified. These included *Necator americanus* (hookworms) 48/145 (33.1%), *Ascaris lumbricoides* 4/145 (2.8%), *Trichuris trichiura* (whipworm) 4/145 (2.8%), and *Strongyloides stercoralis* (1/145 (0.7%). The

TABLE 1. PROBABLE INFLUENCE OF INTESTINAL PARASITISM ON THE MOAB-DIPSTICK ASSAY

Parasites	*Number infected*	*Schistosoma haematobium egg positive*	*MoAb-dipstick positive*
Strongyloides stercoralis	1	0	0
Ascaris lumbricoides	4	1	1[a]
Trichuris trichuria	4	3	3[a]
Necator americanus	48	35	35[a]
Schistosoma mansoni	0	0	0

[a]All individuals positive for urinary antigens were also positive for the *S. haematobium* egg.

probable influence of intestinal parasitism on the MoAb-dipstick results is shown in Table 1. Thirty-five of the 45 *Necator–americanus*-infected individuals who tested positive by the MoAb-dipstick were also positive for *S. haematobium* eggs as determined by microscopy. Likewise, all the persons who were infected with *Ascaris lumbricoides* and *Trichuris trichiura* and were positive for *S. haematobium* urinary antigens also had eggs of the parasite (see Table 1).

Prevalence of urinary schistosomiasis and sensitivity and specificity of the tests

Schistosomiasis hematobia prevalence as determined by microscopy, MoAb-dipstick, microhematuria, and proteinuria are given in Table 2. As shown, the prevalence estimated by microscopy and MoAb-dipstick were similar and significantly higher ($P < 0.05$) than that estimated by proteinuria. Figure 1 shows a comparison of microscopy and MoAb-dipstick in the estimation of prevalence as stratified by age. Both tests showed low prevalence (<10%) in the 1–4 year age group. The prevalence increased sharply and peaked in the 10–14 year age group and remained high in the 15–19 year age group before declining sharply for both tests (see Fig. 1).

The sensitivity and specificity of the four tests (microscopy, MoAb-dipstick, microhematuria, and proteinuria) are shown in Table 3. Out of the 109 *S. haematobium* egg-positive individuals identified by microscopy, one tested negative by MoAb-dipstick, and 25 and 64 were negative for microhematuria and proteinuria, respectively. Taking microscopy as a gold standard, the different tests gave different sensitivities ranging from as high as 99.1% for MoAb-dipstick to 76.1% for microhematuria to 40.4% for proteinura (see Table 3). On the other hand, the occurrence of very few false negatives (see Table 3) coincided with very high specificity (>90%) for each of the tests.

TABLE 2. PREVALENCE OF URINARY SCHISTOSOMIASIS AS DETERMINED BY THE VARIOUS TESTS

Test	*Number tested*	*Number positive*	*Prevalence[a] (%)*
Microscopy	229	109	47.6[b]
MoAb-dipstick	229	110	48[b]
Microhematuria	229	91	39.7[b,c]
Proteinuria	229	54	23.6[b]

[a]Prevalence values with different alphabets are significantly different ($P < 0.05$).

Disparities between microscopy and the MoAb-dipstick

Urine samples from 11 individuals were strongly positive for *S. haematobium* urinary antigens as determined by the MoAb-dipstick but negative for the parasite's eggs following initial microscopy. Repeat examination of fresh urine from these subjects using both tests revealed that mice were actually infected (egg positive) and all 11 individuals continued to test positive by the MoAb-dipstick. The two discordant reactions involved a 10-year-old male and a 20-year-old female. In another disparity, urine from a 30-year-old female was thrice shown to contain *S. haematobium* eggs, but it tested negative for urinary antigens by the MoAb-dipstick on all three occasions.

DISCUSSION

Detection of schistosomal antigens in human specimens is the best of the alternative diagnostic methods introduced so far.[1] This approach has several advantages in that it can be used to diagnose infection, measure the severity of pathologic manifestations in the lower renal tract, monitor the impact of chemotherapy in infected individuals, and investigate ongoing transmission in areas endemic for schistosomiasis. Nevertheless, the routine

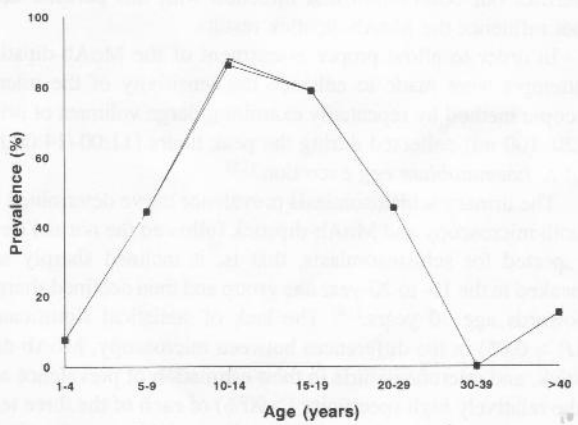

FIG. 1. Urinary schistosomiasis prevalence curves as determined by microscopy (■) and MoAb-dipstick (+).

TABLE 3. SENSITIVITY AND SPECIFICITY OF THE VARIOUS TESTS BASED ON MICROSCOPY

Test	*Number positive*	*Number negative*	*Sensitivity*[c]	*Specificity*[c]
Microscopy	109	120	100	100
MoAb-dipstick	110 (2)[a]	119 (1)[b]	99.1	98.3
Micro-hematuria	91 (8)[a]	138 (25)[b]	76.1	94.2
Proteinuria	54 (10)[a]	175 (64)[b]	40.4	92.5

[a]False positives.
[b]False negatives.
[c]Sensitivity or specificity based on microscopy as a gold standard.

use of antigen detection in diagnosis awaits the development of a rapid dipstick method.[1]

The objective of this study was to evaluate a newly developed MoAb-based dipstick assay for specific diagnosis of urinary schistosomiasis under field conditions. The MoAb (Sh2/15.F) used in the dipstick assay was generated, characterized, and shown to bind a 29 kDa *S. haematobium* species-specific antigen.[9] Sh2/15.F was also shown to detect the species-specific antigen in precipitated proteins from infected human urine[10] and was further utilized in a dipstick-ELISA that was successfully used to detect the antigen in patient urine under laboratory conditions.[12] The rationale behind this evaluation was therefore to compare the MoAb-dipstick assay as performed under field conditions with other schistosomiasis diagnostic methods, namely, microscopy and the use of the microhematuria and proteinuria.

In this study the absence of *S. mansoni* infections in the test population was established. In an earlier study[9] Sh2/15.F did not cross-react with *S. mansoni* crude worm or egg antigen extracts. The observation that all individuals infected with *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Necator americanus* who tested positive by MoAb-dipstick were actually infected with *S. haematobium*, as demonstrated by microscopy, may suggest that those intestinal infections had no influence on the MoAb-dipstick. It should, however, be noted that except for *N. americanus* the number of infections with the other intestinal parasites detected were too few to be used to draw conclusions. In a previous study Amanor et al.[9] showed that Sh2/15.F does not cross-react with *N. americanus* egg antigens in the micro-plate ELISA, which verifies our observation that infection with this parasite does not influence the MoAb-dipstick results.

In order to allow proper assessment of the MoAb-dipstick attempts were made to enhance the sensitivity of the microscopic method by repeatedly examining large volumes of urine (20–100 ml) collected during the peak hours (11:00–14:00 hr) of *S. haematobium* egg excretion.[13]

The urinary schistosomiasis prevalence curve determined by both microscopy and MoAb-dipstick followed the normal trend reported for schistosomiasis, that is, it inclined sharply and peaked in the 10- to 20-year age group and then declined sharply towards age 30 years.[14] The lack of statistical significance ($P > 0.05$) in the differences between microscopy, MoAb-dipstick, and microhematuria in their estimation of prevalence and the relatively high specificity (>90%) of each of the three tests may suggest that they are equally good in performance. However, whereas MoAb-dipstick had a very high sensitivity (99.1%), microhematuria had a lower sensitivity (76.1%, $P > 0.05$).

The ability of the MoAb-dipstick to detect infections that otherwise are detectable only by repeated microscopic examinations may suggest that the antigen detection method is more sensitive. However, the failure of the MoAb-dipstick to detect a parasitologically confirmed case raises interesting questions about its suitability. This failure to detect antigens in confirmed infections, however, appears to be a rare phenomenon and may be the result of other factors.[15] It may be associated with very early infections, which invariably lack detectable antigens. Despite this shortcoming, the noninvasive nature of the MoAb-dipstick together with its ease of application in the field makes it a desirable method in mass control programs. The specific identification of *S. haematobium* infections by this test would also permit differential diagnosis of urinary and intestinal schistosomiasis in hyperendemic areas of Africa[5] and provide more accurate epidemiologic data. Nevertheless, this new assay should be evaluated for its ability to diagnose *S. haematobium* infections in different geographic areas; determine the severity of pathologic manifestations; and monitor the impact of chemotherapy.

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