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Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis

N. Midzi[a], A.E. Butterworth[b,c], T. Mduluza[d,*], S. Munyati[a], A.M. Deelder[e], G.J. van Dam[e]

[a] *National Institute of Health Research, Harare, Zimbabwe*
[b] *London School of Hygiene and Tropical Medicine, London, UK*
[c] *Biomedical Research and Training Institute, Harare, Zimbabwe*
[d] *University of Zimbabwe, Department of Biochemistry, P.O. Box MP167, Mount Pleasant, Harare, Zimbabwe*
[e] *Department of Parasitology, Leiden University Medical Centre, Leiden, The Netherlands*

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Summary Rapid diagnostic tests are needed for the implementation and monitoring of national schistosomiasis control programmes. The field applicability of the circulating cathodic antigen (CCA) urine reagent strip for the diagnosis of *Schistosoma haematobium* infection was evaluated among 265 pre- and primary schoolchildren aged 2—19 years in a rural area of Zimbabwe. The CCA strip was compared with egg detection before and six weeks after treatment with praziquantel. Pre-treatment prevalence (overall 40.4%) and intensity of infection, as determined by egg counts, increased with age. CCA and parasitological results were significantly correlated ($P < 0.001$), although concordance was slight ($\kappa = 0.21$). Discordant results were mainly attributable to CCA-positive, egg-negative individuals. Correlations and levels of agreement improved significantly with age ($P < 0.001$, $\kappa = 0.40$) and intensity of infection ($P < 0.001$). Praziquantel treatment led to 'cure' in 90.9% and 70.5% of children as measured by the egg detection and CCA methods, respectively. An arbitrary gold standard was constructed that included both CCA and egg detection results. Using this standard, the sensitivities of the CCA test were 88.2% and 95.8%, respectively, for pre- and post-treatment results. The improved version that is field applicable now has an acceptable role in the field diagnosis of *S. haematobium*.

1. Introduction

In recognition of the public health impact of schistosomiasis and soil-transmitted helminths, the WHO has set a minimum target for the control of morbidity due to helminth infection: regular treatment should be given to at least 75%

* Corresponding author. Tel.: +263 4 334052; fax: +263 4 333407.
E-mail address: mduluza@medic.uz.ac.zw (T. Mduluza).

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of all primary schoolchildren at risk of morbidity due to schistosomiasis and soil-transmitted helminths.[1] In order to achieve this goal a strategy has been adopted to involve governments and policy-makers in public health sectors so that they can prioritize schistosomiasis control in their national agendas. This has resulted in many countries in sub-Saharan Africa setting up national plans of action for controlling schistosomiasis, while some have already implemented school-based deworming programmes.[2–4] However, the progress of such large-scale programmes needs regular monitoring and evaluation in order to optimize the use of limited resources, determine treatment efficacy and test for the possible emergence of drug resistance.[5,6]

There is therefore a need for sensitive, field-applicable, cheap and reliable diagnostic tools for regular schistosomiasis screening in endemic communities where mass treatment interventions are being undertaken. Currently, direct parasitological methods are used to detect *Schistosoma* ova in urine, stool or tissue biopsies, with the Kato-Katz[7] and urine filtration methods[5] being widely recommended.[8] However, these techniques have limitations, as single or limited repeat egg counts may miss light infections because of poor sensitivity and day-to-day fluctuation in egg excretion.[9–11] This will lead to a significant underestimation of community prevalence. Several repeated egg counts on successive days are needed to obtain reliable results, but these are time-consuming, labour-intensive and are also met with poor patient compliance.

The use of urine reagent strips in the diagnosis of urinary schistosomiasis is well accepted and documented.[12] However, their sensitivity is affected by age and gender.[13] False-positive reactions may be more common in reproductive women due to contamination of urine with vaginal secretions.[14]

ELISA for the detection of adult worm gut-associated glycoproteins (circulating anodic antigen [CAA] and circulating cathodic antigen [CCA]) have become valuable alternatives to parasitological methods for the diagnosis of human schistosomiasis.[15–17] Both antigens can be detected in the serum and urine of patients with schistosomiasis and their levels are sensitive and specific markers of the presence and intensity of infection.[18] In particular, detection of CCA in urine is a potentially useful and non-invasive method.[19] However, the ELISA techniques used for the determination of CAA and CCA levels in serum and urine require well-equipped laboratories and specialized laboratory staff.

More recently, CCA reagent strips for rapid schistosomiasis diagnosis in urine have been developed.[20,21] Studies carried out so far to determine the field applicability of the rapid urine CCA strip for the detection of schistosomiasis infection have demonstrated that the method is valuable for the detection of *S. mansoni* in endemic communities.[21,22] However, reports evaluating its possible use in the detection of *S. haematobium* infections have been contradictory.[22,23] Subsequently, however, the technique has been improved (G.J. van Dam, unpublished data), and the objective of the present study was to evaluate its field applicability in Zimbabwe for the diagnosis of urinary schistosomiasis and for monitoring treatment efficacy.

2. Materials and methods

2.1. Study site and population

The study was carried out in the Nyama resettlement area in Karoi District, Mashonaland West Province of Zimbabwe. The province has moderate *S. haematobium* and low *S. mansoni* prevalence.[24] A total of 265 pre- and primary schoolchildren at Nyama Primary School, median age 10 years and age range 2—19 years, were randomly enrolled into the study. The 2- and 3-year-old children were brought to school by their parents, who wanted their children to be screened for schistosomiasis. Four adults also attended and requested treatment, but were excluded from the analysis.

2.2. Ethical issues

The study was part of the large-scale National Schistosomiasis and Soil-Transmitted Helminths (STH) control programme in Zimbabwe that is being implemented in two phases: a national survey (planning phase) and a control phase, in which communities will receive treatment following stratification of the districts according to prevalence.[8] The Medical Research Council of Zimbabwe approved the large-scale project, which has also received support from the Ministry of Health and Child Welfare and the Ministry of Education, Sports and Culture. The community in Nyama resettlement area requested the National Schistosomiasis control team to investigate the schistosomiasis situation in their area. The District Health and Education Executives approved the study. Oral informed consent was obtained from the school headmaster, parents and children following health education given by the research team.

2.3. Parasitological investigations

Urine specimens collected between 10:00 and 14:00 h on two consecutive days were processed using the standard urine filtration method.[5] A single stool specimen was collected and processed using the Kato-Katz method at baseline and the six week post-treatment follow-up.[7] The baseline survey took place in December 2006.

2.4. Urine CCA strips

CCA strips were prepared based on the same immunochromatographic principle as the previous version prepared by van Dam et al.,[21] with some improvements made to the original format. Unbacked nitrocellulose (PRIMA 125, Whatman, Dassel, Germany) was fixed on a GL-45569 backing (G&L, San Jose, CA, USA), with a sample pad and an absorbent pad. As the test line, 0.75 mg/ml MAb 54-5C10-A in 5 mM borate + 1% ethanol pH 8.8 was sprayed on the card, and as the control line, goat anti-mouse (Sigma, San Jose, CA, USA) 0.2 mg/ml in 5 mM borate pH 8.8 was used. The carbon conjugate was prepared using MAb 54-4C2-A following the same standard protocol as previously described. After conjugation, 0.75 μl of this solution per 25 μl of a sucrose-containing drying buffer was added to microtitre plate wells and dried

overnight in a 37 °C incubator. In dry surroundings, strips and dried conjugate are stable for more than a year. To perform the test, 75 μl of running buffer and 25 μl of urine sample (before filtration) were added to the wells, mixed well with the colloidal carbon, after which the strip was placed into the wells. Strips were read wet after 30 min, or left to dry, and scored against a series of five standards for each group of 50 samples. The standards consisted of negative urine and four artificial samples containing 10, 100, 1000 and 10 000 ng of semi-purified worm antigen (containing CCA) per ml. The CCA strip was considered negative when the test line was weaker than the standard 100 ng/ml test line and positive when the test line was equal or stronger. Strips were initially read in the field, dried and stored in plastic bags, returned to Harare, and read independently and blindly by a second observer. Agreement between observers for binary (positive or negative) results was good ($\kappa = 0.88$, $P < 0.001$). This was not considered further, and the field data were used for subsequent analysis. The same procedure was repeated at six weeks after treatment.

2.5. Treatment

Participants who had schistosome ova in urine, whose urine was CCA-positive, or who had both ova in urine and were CCA-positive received a single dose of praziquantel (40 mg/kg). A second treatment was given to those individuals who still had detectable schistosome ova six weeks after the first treatment.

2.6. Statistical analysis

Data were entered in Excel (Microsoft Corp., Redmond, WA, USA), checked for accuracy and analyzed with Stata 9 (Stata Corp., College Station, TX, USA). Correlations were tested by the Fisher's exact and Spearman's rank correlation tests, and agreement between binary variables by determining kappa values. A P-value of ≤ 0.05 was considered statistically significant. Specificity, sensitivity and positive and negative predictive values were calculated using two gold standards: egg-positive by the urine filtration test or infection-positive by either egg- or CCA-positivity (assuming 100% specificity of the CCA result). Cure rates were calculated from individuals for whom a complete set of results was obtained before and six weeks after treatment. Cure rates were calculated as the proportion of individuals positive by urine examination and/or urine CCA strip before treatment who became negative six weeks after treatment.

3. Results

3.1. Study group characteristics

The study group contained 125 (47.0%) males, the proportion of which increased significantly with age, ranging from 45.6% among 2—7 year-olds to 60.5% among 12—20 year-olds ($P = 0.011$). A significant increase in the prevalence and intensity of *S. haematobium* infection with age was observed ($P < 0.001$) (Figure 1). No infections with *S. mansoni* were detected.

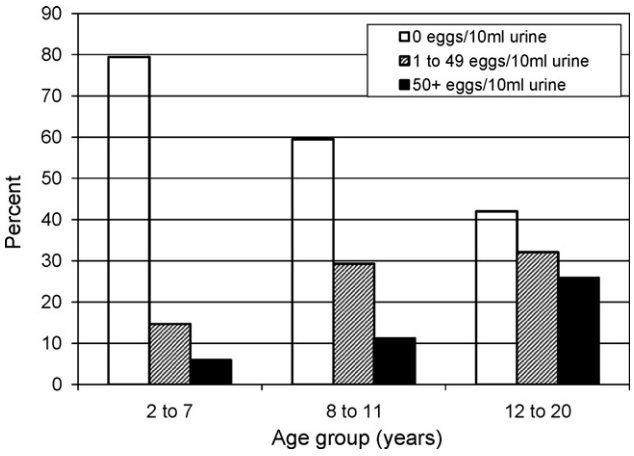

Figure 1 Distribution of *Schistosoma haematobium* egg count by age group.

3.2. Pre-treatment prevalence of infection and correlation between the tests

Overall, the pre-treatment prevalence of schistosomiasis determined by the standard urine filtration technique was 40.4%, while that determined by the CCA strip was 65.0%. The relationship between the urine CCA strip results and *S. haematobium* egg counts before treatment is shown in Table 1. There was a significant correlation between the CCA strip reaction and the urine filtration method, but the degree of agreement was only slight ($P < 0.001$, $\kappa = 0.21$). Discordant results were largely attributable to CCA-positive/egg-negative reactions. When the participants were classified into different age groups the correlation and concordance between the two tests improved with age, being best in the oldest age group ($P < 0.001$, $\kappa = 0.40$). The discordances were worst in the youngest age group (2—7 years). Table 2 shows CCA reactions categorised by intensity of infection. The proportion of CCA-positive reactions increased significantly with increasing egg intensity ($P = 0.001$).

3.3. Diagnostic characteristics of the CCA strip before treatment

The sensitivity of the CCA strip in comparison with the filtration method, the gold standard, is shown in Table 1. Overall sensitivity before treatment was 78.5%. When the study population was stratified by age group, the sensitivity of the CCA strip method increased from the youngest age group (64.3%) to the oldest age group (84.8%). Overall specificity was 44.3%, ranging from 41.5 to 54.3% from the youngest to the oldest age groups.

3.4. Diagnostic characteristics of the tests after treatment

The diagnoses by the urine filtration method and the CCA strip after praziquantel treatment are shown in Tables 3 and 4. With both methods there was a marked reduction in positive reactions after treatment, although the

Table 1 Baseline correlations between the diagnosis of *Schistosoma haematobium* infection by the urine filtration technique and the circulating cathodic antigen (CCA) strip test by age group

Urine filtration technique	CCA reaction		*P*-value	κ	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive *n* (%)	Negative *n* (%)						
Overall (*n* = 265)								
Positive	84 (31.7)	23 (8.7)	<0.001	0.21	78.5	44.3	48.8	75.3
Negative	88 (33.2)	70 (26.4)						
2—7 years (*n* = 67)								
Positive	9 (13.4)	5 (7.5)	0.767	0.03	64.3	41.5	22.5	81.5
Negative	31 (46.3)	22 (32.8)						
8—11 years (*n* = 116)								
Positive	36 (31.0)	11 (9.5)	0.047	0.17	76.6	42.0	47.4	72.5
Negative	40 (34.5)	29 (25.0)						
12—20 years (*n* = 81)								
Positive	39 (48.1)	7 (8.6)	<0.001	0.40	84.8	54.3	70.9	73.1
Negative	16 (19.8)	19 (23.5)						

PPV: positive predictive value; NPV: negative predictive value.

'cure rate' determined by CCA (70.5%) was less than that suggested by urine filtration (90.9%) (Table 3). The correlation between the two methods remained significant after treatment ($P < 0.001$), although the κ value was slight (0.27) (Table 4).

3.5. Diagnostic characteristics of the tests using a combined gold standard

The results obtained using a combined gold standard (infection-positive by either egg- or CCA-positivity, assuming 100% specificity of the CCA result) are shown in Table 5. The correlation of the urine CCA strip with the combined gold standard improved significantly for pre- and post-treatment results (κ = 0.80 and 0.87, $P < 0.001$). The overall sensitivity of the CCA strip method improved to 88.2% and 95.8% for pre- and post-treatment results, respectively.

4. Discussion

We observed 79% sensitivity in pre-treatment CCA strip reactions in urine samples from individuals who had active *S. haematobium* infection as determined by the urine filtration method. Our results contrast with those obtained by Stothard et al.,[22] who failed to detect CCA positivity among study groups living in endemic communities in Zanzibar, Niger and Burkina Faso. They were unable to detect infection among patients who had heavy *S. haematobium* infection, as classified by the standard urine filtration method, using a batch of urine CCA strips that were able to detect *S. mansoni* infection in Uganda. They concluded that CCA strips could not be used for the detection of *S. haematobium*. Our contrasting findings could be explained by technical improvements made to the strips, while using the same anti-CCA monoclonal antibodies (G.J. van Dam, unpublished observations). Alternatively, regional influences might be involved, as Ayele et al.[23] found 52% sensitivity of the original CCA strip in urinary schistosomiasis in Ethiopia. Further studies to evaluate the new CCA strip in other areas with urinary schistosomiasis are required.

The observed significant correlation between the pre-treatment results of the CCA strip and egg detection, together with the increasing proportion of positive results given by the CCA strip with increasing egg count, suggests that the CCA strips are indeed detecting *S. haematobium*

Table 2 Circulating cathodic antigen (CCA) strip reactions by intensity of infection with *Schistosoma haematobium*

S. haematobium eggs/10 ml urine)	CCA reaction		*P*-value
	Positive *n* (%)	Negative *n* (%)	
0	88 (55.7)	70 (44.3)	< 0.001
1—49	51 (73.9)	18 (26.1)	
50+	33 (86.8)	5 (13.2)	

Table 3 Diagnosis of *Schistosoma haematobium* infection by the urine filtration and circulating cathodic antigen (CCA) strip methods, 6 weeks after treatment

Post-treatment	Pre-treatment *n* (%)	
	Positive	Negative
Urine filtration		
Positive	6 (9.1)	2 (4.8)
Negative	60 (90.9)[a]	40 (95.2)
CCA		
Positive	23 (29.5)	0
Negative	55 (70.5)[a]	2 (100)

[a] Percentage cure as determined by each method.

Table 4 Correlations between the results given by the circulating cathodic antigen (CCA) strip and urine filtration, six weeks after treatment among treated children

Urine filtration test	CCA reaction		*P*-value	κ	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive *n* (%)	Negative *n* (%)						
Positive	5 (83.3)	1 (16.7)	<0.001	0.27	83.3	77.0	22.7	98.3
Negative	17 (23.0)	57 (77.0)						

PPV: positive predictive value; NPV: negative predictive value.

infection. Van Etten et al.[25] and de Clercq et al.[16] similarly observed positive correlations between CCA ELISA and egg counts, although Kremsner et al.[26] did not. However, the agreement in our study between CCA strips and egg detection was only slight (κ = 0.21). The fact that the majority of discordant results were CCA-positive but egg-negative suggests that the CCA test is either more sensitive or less specific than egg detection. There is no direct evidence from this study for the first hypothesis, but there are various supportive arguments:

1. Egg detection, the primary gold standard, was based on the examination of only two urine samples collected on two separate days. This is known to be insensitive, due to daily fluctuation in egg excretion.[10,11,16,27,28] Various studies showing CCA-positive, egg-negative cases have suggested false-negative parasitology as the explanation.[18,22,23,25]
2. The level of agreement between CCA strip results and egg counts increased with the intensity of infection, supporting observations made elsewhere.[15,29]
3. Agreement between CCA strips and egg detection increased with age, suggesting poor sensitivity of egg detection in lightly infected younger children.
4. The proportion of samples positive for *S. haematobium* fell markedly after treatment, not only for egg detection, but also for CCA strips. The effect was not as marked for CCA (cure rate 70.5% detected by CCA strips vs. 90.9% for egg detection), but even so, the reduction in the CCA response following treatment suggests that active infection is being measured by the CCA strip. In addition, of 33 cases treated only on the basis of CCA strip positivity (egg-negative), 25 (76%) became CCA-negative after treatment.
5. G.J. van Dam et al. found almost complete specificity (92%) of the CCA strip test in a group of schoolchildren (*n* = 126) from Kenya, after combining the results from 12 stool examinations and urine CCA-ELISA (unpublished data).

The observed CCA-positive, egg-negative discordances in the present study could have been due to the genus cross-specificity of the CCA strip test, which could have detected both forms of schistosome in a single test, a factor that could have contributed to the observed higher prevalence of schistosomiasis obtained using the CCA strip (64.4%) compared to the species-specific urine filtration method (40.4%). It is difficult to ascertain whether the Kato-Katz method detected truly negative individuals, since we did not perform multiple egg counts required to improve the sensitivity of this parasitological method.[16,25,27,30]

Working on the assumption that the CCA strip is 100% specific, we constructed an artificial gold standard based on either egg or CCA positivity. This resulted in a higher sensitivity for the CCA strip than (duplicate) egg counts, both before and after treatment (88.2% vs. 55.4% and 95.8%

Table 5 Diagnostic characteristics of the circulating cathodic antigen (CCA) strip and urine filtration methods using a combined gold standard[a]

	Positive *n* (%)	Negative *n* (%)	*P*-value	κ	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urine filtration								
Pre-treatment combined gold standard								
Positive	108 (55.4)	87 (44.6)	<0.001	0.40	55.4	100	100	44.6
Negative	0	70(100)						
Post-treatment combined gold standard								
Positive	8 (32.0)	17 (68.0)	<0.001	0.20	32.0	100	100	77.0
Negative	0	57(100)						
CCA								
Pre-treatment combined gold standard								
Positive	172 (88.2)	23 (11.8)	<0.001	0.80	88.2	100	100	73.3
Negative	0	70(100)						
Post-treatment combined gold standard								
Positive	23 (95.8)	1 (4.7)	<0.001	0.87	95.8	100	100	98.3
Negative	0	57(100)						

[a] Infection-positive by either egg- or CCA-positivity (assuming 100% specificity of the CCA result).

vs. 32.0%, respectively). The combination of two or more tests to screen parasitic diseases in epidemiological studies is increasingly being used in order to improve the sensitivity of field diagnostic methods to allow more accurate determination of the burden of disease when planning treatment intervention programmes.[30,31]

In contrast to CCA ELISA of serum or urine, the CCA strip test can be performed and read in the field as it does not require sophisticated laboratory equipment. The sample (urine) collection is non-invasive and therefore readily acceptable. The strips are less labour-intensive even than a single urine egg count examination. A single urine sample is sufficient for a conclusive diagnosis using the strips, compared to multiple egg counts on successive days required for the parasitological method.[25,27,30] However, the use of the improved urine CCA strip in national control programmes will depend on cost. Assuming that the cost of the test is reasonable, then the strip is potentially a valuable tool for the initial screening of communities/schools for the allocation of control strategies and in the follow-up of treated communities/schools. However, if both high sensitivity and specificity are needed in individual diagnosis, then more work is required to evaluate the specificity of the urine CCA strip in a tropical area non-endemic for schistosomiasis but endemic for soil-transmitted and other helminths.

Authors' contributions: NM, AEB, SM, TM, AMD and GJD contributed to the concept and design of the study protocol; NM, TM and AEB carried out the clinical assessment and parasitology; AEB, NM, TM and GJD carried out the analysis and interpretation of the data; NM, GJD, TM and AEB drafted the manuscript. All authors read and approved the final manuscript. TM and NM are guarantors of the paper.

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Conflicts of interest: As part of his research activities at the Department of Parasitology of the Leiden University Medical Centre, GJD developed the urine CCA strips that were used in the current report. He is also involved in a new business enterprise focusing on making the strips available at affordable cost for healthcare use in developing countries. All other authors have no conflicts of interest.

Ethical approval: The Medical Research Council of Zimbabwe approved the project.

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