

Accuracy of circulating cathodic antigen tests for rapid mapping of *Schistosoma mansoni* and *S. haematobium* infections in Southern Sudan

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Summary

OBJECTIVE To evaluate the diagnostic accuracy of a circulating cathodic antigen (CCA) urine dipstick test for detecting *Schistosoma mansoni* and *S. haematobium* alongside an integrated rapid mapping survey in Southern Sudan.

METHODS AND RESULTS A total of 373 children aged 5–16 years were included in the study. Of these 26.0% were infected with *S. haematobium* and 24.5% were infected with *S. mansoni*, as identified by urine filtration or single Kato–Katz thick smear, respectively. The CCA performed moderately in detecting *S. mansoni*, with sensitivity of 89.1% and specificity of 74.2%, and poorly in detecting *S. haematobium* infections, with a sensitivity of 36.8% and specificity of 78.9%. This may be a slight underestimate of true CCA accuracy, since only single stool and urine samples were examined by microscopy. The true ‘gold standard’ for comparison would have been the collection of multiple stool samples over consecutive days.

CONCLUSION The poor CCA accuracy for diagnosis of urinary schistosomiasis means that this test is currently not suitable for rapid mapping of schistosomiasis in areas where both *S. mansoni* and *S. haematobium* may be endemic.

keywords schistosomiasis, *Schistosoma haematobium*, *Schistosoma mansoni*, diagnosis, circulating cathodic antigen test, neglected tropical diseases, mapping, Southern Sudan

Introduction

Programmes aiming to control or eliminate neglected tropical diseases (NTDs) are being scaled up in many parts of the world (Ottesen *et al.* 2008; Utzinger *et al.* 2009). As part of this ongoing effort, many countries still need to complete mapping of their NTD endemic areas. To do so reliably and efficiently, there is considerable need to investigate mapping methodologies, including new or improved diagnostic tools (Brooker *et al.* 2010). In

Southern Sudan, NTDs are a major public health problem and efforts are underway to provide the first national assessment of NTDs (Sturrock *et al.* 2009).

This study explores the accuracy of circulating cathodic antigen (CCA) urine dipstick tests for the rapid diagnosis of human schistosome infection in the context of rapid mapping surveys in Southern Sudan. To date, currently available CCA tests have been shown to have moderate to good reliability in detecting *Schistosoma mansoni* (van Dam *et al.* 2004; Stothard *et al.* 2006; Legesse & Erko 2007, 2008; Standley *et al.* 2010a; b), but poor performance for detecting *S. haematobium* (Stothard *et al.* 2006, 2009; Ayele *et al.* 2008; Obeng *et al.* 2008; Midzi *et al.* 2009). Further evaluations of the accuracy of the urine CCA as a point of contact diagnostic tool are underway as part of the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE; <http://score.uga.edu>) initiative in

Disclaimer

This study was made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of Malaria Consortium and do not necessarily reflect the views of USAID or the United States Government.

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several African countries (Utzinger *et al.* 2011). The present study aimed to evaluate the accuracy of CCAs during rapid mapping of human schistosome infection in Unity and Central Equatoria states in Southern Sudan, where both *S. mansoni* and *S. haematobium* are endemic.

Materials and methods

Data collection took place from June to July 2010 in five villages in Unity state and eight villages in Central Equatoria state, Southern Sudan, in coordination with technical support provided by the United States Agency for International Development (USAID) NTD control programme. The work was conducted alongside ongoing integrated rapid mapping for soil-transmitted helminthiasis (STH), schistosomiasis and lymphatic filariasis (Sturrock *et al.* 2009).

Single urine and stool samples were collected between approximately 10 am and 2 pm from enrolled children. CCA tests (Rapid Medical Diagnostics; Pretoria, South Africa) were conducted according to the manufacturer's instructions on single urine samples collected from children aged 5–16 years; a maximum of 70 children were enrolled per village. CCA results were graded according to test band strength, where weak positive was defined by the control band being darker than test band, while a strong positive was defined by a test band darker or the same colour as the control band. Two Kato–Katz thick smears (A and B) were prepared from each stool sample, and 10 ml of urine were filtered through a hydrophilic 12 µm polycarbonate Millipore® membrane and mounted on a microscope slide. All urine samples were also tested for microhaematuria using Hemastix® reagent strips (Bayer Diagnostics; Basingstoke, UK).

Use of CCA tests was restricted to suspected schistosomiasis endemic villages. Summary results were tracked throughout the survey to ensure enrolment of: (i) approximately equal proportions of *Schistosoma*-positive and -negative individuals, according to microscopy results and (ii) approximately equal proportions of males and females, as well as similar ages, for individuals in positive and negative categories. Kato–Katz and urine filtration slides were examined on site for *Schistosoma* eggs by light microscopy: *S. haematobium* and *S. mansoni* infection were recorded as eggs per 10 ml urine or eggs per gram of faeces (EPG), respectively. Slides A and B were read by different technicians in the field, and mean egg count calculated for use in analysis. Ten per cent of samples were randomly selected for a third reading by a supervising technician each day and result corrected if necessary. After the survey, a further 10% of slides were re-read by experienced technicians at the Vector Control Division,

Ministry of Health, Uganda, and corrected according to this reading. Children with *Schistosoma* infection according to microscopy were treated with praziquantel, using a dose pole to determine number of tablets required, in addition to a 400 mg single dose of albendazole for treatment of STH infection.

Data were double entered into Microsoft Excel 2007 (Microsoft Corporation, Seattle, WA, USA) and analysed in STATA 9.0 (Stata Corporation, College Station, TX, USA). Infection prevalence by sex and age were assessed by chi-square test. Categories of *S. mansoni* and *S. haematobium* infection intensity were applied according to World Health Organization guidelines (WHO 2002). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using standard 2 × 2 tables where microscopy results were considered as 'gold standard'. Urine CCA test accuracy was evaluated for any *Schistosoma* infection, where either *S. mansoni* or *S. haematobium* infection was treated as a positive result. CCAs were also evaluated for *S. haematobium* and *S. mansoni* individually: microscopy negative samples needed to be negative for both *Schistosoma* species, and microscopy positive samples were not permitted to have co-infection with the other species. The chi-square test was used to compare CCA test accuracy between *Schistosoma* species. Accuracy of Hemastix® and CCA for diagnosis of *S. haematobium* was compared using McNemar's test, which accounts for the paired nature of the data (Lachenbruch & Lynch 1998).

The integrated rapid mapping protocol received ethical approval from the Directorate of Research, Planning and Health System Development, Ministry of Health, Government of Southern Sudan (GoSS), and from the Ethics Committee of the London School of Hygiene and Tropical Medicine, UK (application no. 5500). CCAs were performed on the same urine samples that were collected as part of the rapid mapping surveys. Clearance to conduct the CCA evaluation alongside the routine surveys was obtained from the Ministry of Health, GoSS.

Results

Full data records were obtained from 373 individuals sampled during the integrated rapid mapping survey at 13 sites in Unity and Central Equatoria. The mean number of children by site was 29 (range 9–57). Fifty-six per cent were male, and mean age was 9.3 years (range 5–15 years).

CCAs were positive for 41.3% of samples: 53.9% of these showed only a weak test band. Microhaematuria was identified by positive Hemastix® result in 29.0% of enrolled individuals. Diagnosis using microscopy identified a total of 45.3% of individuals infected with schistosomes.

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Prevalences of *S. haematobium* and *S. mansoni* were 26.0% and 24.5%, respectively, while 5.1% of individuals had concurrent infection with both species. The majority of *S. haematobium* (76.5%) and *S. mansoni* (52.1%) infections were categorised as light intensity. There was no association between infection and sex, but *Schistosoma* prevalence was higher in children aged 10–15 years than in those aged 5–9 years (52.6% vs. 39.1%, $\chi^2 = 6.8$, $P = 0.009$).

Sensitivity of the CCA in detecting any *Schistosoma* infection was 65.9%, with specificity of 79.0%, if both strong and weak test bands were considered as positive (Table 1). If weak test bands were classified as negative, CCA sensitivity was far lower (37.7%) but specificity improved to 96.1% ($P < 0.001$ for both). When CCA performance was assessed for the two *Schistosoma* species individually, it showed sensitivity and specificity of 89.1% and 74.2% for *S. mansoni*, and of 36.8% and 78.9% for *S. haematobium* (Table 1). CCAs were found to have higher sensitivity and specificity for detection of *S. mansoni* than *S. haematobium* ($P < 0.001$). Furthermore, Hemastix[®] were found to have superior sensitivity (69.6%, 95% confidence interval (CI): 59.7–78.3%) and specificity (84.4%, 95% CI: 79.8–88.4%) for detection of *S. haematobium* than the CCAs ($P < 0.001$).

Overall, 43 individuals had false-positive CCA tests. More false-negative CCA results were seen for *S. haematobium* infections ($n = 48$) than for *S. mansoni* ($n = 9$). However most false-negative CCA results were for light-intensity infections: 88.9% *S. mansoni* false-negatives and 81.3% *S. haematobium* false-negatives.

Discussion

The present study set out to determine the accuracy of a reformulated urine CCA test produced by Rapid Medical Diagnostics for diagnosis of *S. mansoni* and *S. haemato-*

bium infections, with a view to using this test in rapid NTD mapping in Southern Sudan. Our findings are consistent with other evaluations of this test, in that performance was found to be moderate for detection of *S. mansoni* infection, and low for detection of *S. haematobium* (Stothard *et al.* 2009; Standley *et al.* 2010b). Accuracy of the CCA for detection of *S. mansoni* is largely comparable to that of earlier test formulations (van Dam *et al.* 2004; Stothard *et al.* 2006; Legesse & Erko 2007, 2008), while previously identified difficulties surrounding detection of *S. haematobium* infection (Stothard *et al.* 2006; Obeng *et al.* 2008; Midzi *et al.* 2009) have not been resolved.

Daily fluctuations in egg excretion and heterogeneous distribution of eggs within the stool have both been demonstrated to contribute to a reduced sensitivity of Kato–Katz thick smears compared to ‘true’ infection prevalence (Engels *et al.* 1996; Kongs *et al.* 2001; Utzinger *et al.* 2001; Booth *et al.* 2003; Berhe *et al.* 2004). While increasing sampling effort, particularly by collecting stool samples over consecutive days, can greatly improve sensitivity of Kato–Katz in detecting low-intensity *S. mansoni* infections, this has considerable cost implications. It is likely that the prevalence of *S. mansoni* was underestimated in the current study by examination of duplicate Kato–Katz thick smears from a single stool per child. It is therefore possible that apparent false positive CCA results were actually due to light *Schistosoma* infections.

Hemastix[®] are currently used in the rapid mapping methodology as a screening tool before urine filtration. In the current study, Hemastix[®] were found to have superior sensitivity and specificity than CCA tests for *S. haematobium* diagnosis, when compared to urine filtration and microscopy, but Hemastix[®] sensitivity was lower than previously reported from Southern Sudan (Robinson *et al.* 2009). It is possible that the variance is due to differences in transmission intensity, and it will be worthwhile to filter and examine a proportion of Hemastix[®]-negative urine

Table 1 Summary performance of CCA tests, with 95% binomial exact confidence intervals (95% CI): sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

	N	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CCA weak & strong positive:					
Any <i>Schistosoma</i> spp.	373	65.9 (58.2–73.0)	79.0 (72.8–84.4)	72.3 (64.5–79.1)	73.6 (67.3–79.3)
<i>S. mansoni</i> *	276	89.1 (80.9–94.7)	74.2 (68.7–79.2)	52.9 (44.7–61.0)	95.5 (91.8–97.8)
<i>S. haematobium</i> †	280	36.8 (26.1–48.7)	78.9 (72.7–84.3)	39.4 (28.0–51.8)	77.0 (70.7–82.6)
CCA strong positive only:					
Any <i>Schistosoma</i> spp.	373	37.7 (30.3–45.4)	96.1 (92.5–98.3)	88.9 (79.2–95.1)	65.0 (59.4–70.4)
<i>S. mansoni</i> *	276	65.2 (54.6–74.9)	95.8 (92.7–97.8)	83.3 (72.7–91.1)	89.4 (85.4–92.7)
<i>S. haematobium</i> †	280	4.0 (0.8–11.1)	96.1 (92.4–98.3)	27.3 (6.0–61.0)	72.9 (67.1–78.1)

*Individuals with microscopy results positive for *S. haematobium* and negative for *S. mansoni* were excluded.

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samples for quality control purposes during future rapid mapping.

An easy-to-use rapid diagnostic test for detection of schistosome infection has great potential value in resource-constrained settings such as Southern Sudan, where there is a severe shortage of trained laboratory technicians. Furthermore, potential programmatic cost savings have been demonstrated, whereby baseline screening with a CCA could reduce costs when compared to blanket treatment (Stothard 2009). From an operational perspective, ambient conditions in Southern Sudan are likely to exceed the recommended storage conditions for the CCA of 4–28 °C, indicating a need for temperature-controlled storage. Storage of CCAs beyond the recommended temperature may result in reduced sensitivity of the test.

While the current findings indicate that CCA accuracy is less than optimal, this is likely an underestimate, since only single stool and urine samples were examined by microscopy. The current urine CCA may therefore be appropriate for use as a point of care diagnostic test for *S. mansoni*. However, for rapid mapping to determine intervention needs in areas with unknown parasite endemicity, the CCA is not appropriate due to its poor accuracy in detecting *S. haematobium*.

Acknowledgements

We would like to thank the staff of the State Ministries of Health in Unity and Central Equatoria State for their support in implementing the surveys, and all technicians for their dedication during the weeks of strenuous field work. CCAs were provided free of charge to Malaria Consortium for the purpose of this evaluation. Rapid Medical Diagnostics South Africa had no role in study design or decision to publish these results. RAA received additional support from the John-Henry Credland Memorial Fund. SB is supported by a Research Career Development Fellowship (081673) from the Wellcome Trust. USAID funding for integrated rapid mapping was provided through Research Triangle Institute (RTI) to Malaria Consortium.

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