

## Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis

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### Abstract

An evaluation of a commercially available antigen capture dipstick that detects schistosome circulating cathodic antigen (CCA) in urine was conducted in representative endemic areas for intestinal and urinary schistosomiasis in Uganda and Zanzibar, respectively. Under field-based conditions, the sensitivity (SS) and specificity (SP) of the dipstick was 83 and 81% for detection of *Schistosoma mansoni* infections while positive predictive (PPV) and negative predictive values (NPV) were 84%. Light egg-positive infections were sometimes CCA-negative while CCA-positives included egg-negative children. A positive association between faecal egg output and intensity of CCA test band was observed. Estimating prevalence of intestinal schistosomiasis by school with dipsticks was highly correlated ( $r = 0.95$ ) with Kato-Katz stool examinations, typically within  $\pm 8.5\%$ . In Zanzibar, however, dipsticks totally failed to detect *S. haematobium* despite examining children with egg-patent schistosomiasis. This was also later corroborated by further surveys in Niger and Burkina Faso. Laboratory testing of dipsticks with aqueous adult worm lysates from several reference species showed correct functioning, however, dipsticks failed to detect CCA in urine from *S. haematobium*-infected hamsters. While CCA dipsticks are a good alternative, or complement, to stool microscopy for field diagnosis of intestinal schistosomiasis, they have no proven value for field diagnosis of urinary schistosomiasis. At approximately US \$2.6 per dipstick, they are presently too expensive to be cost-effective for wide scale use in disease mapping surveys unless Lot Quality Assurance Sampling (LQAS) strategies are developed.

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### 1. Introduction

There has been renewed interest and commitment to implement national control programmes directed against schistosomiasis and soil transmitted helminthiasis (Engels et al., 2002; Utzinger et al., 2003). With inexpensive anthelmintic chemotherapy, the central focus is

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upon morbidity reduction (Savioli et al., 2002; Fenwick et al., 2003) and The World Health Organization has issued drug delivery guidelines based upon infection prevalence thresholds (Montresor et al., 2002). If, for example, a school-based survey finds local infection prevalence of schistosomiasis equal to or exceeds 50% then annual mass drug administration to the whole community is advised. Schistosomiasis has a complex focal distribution, and hence the disease landscape is usually aggregated and patchy (King, 2001). In such a complex landscape where the prevalence of infection can vary widely, even between adjacent schools, it may be required to visit a large number of schools to collect prevalence data not only to better estimate the initial disease burden but also to review and revise subsequent drug delivery through time (Kabatereine et al., 2004).

While there are WHO-recommended protocols for mapping the distribution of schistosomiasis the methods differ between urinary and intestinal forms of the disease. For example, with *Schistosoma haematobium* infections questionnaires have been advocated for rapid and inexpensive screening of high-risk schools based upon the frequency of self-reported blood in urine (Lengeler et al., 2002); for *S. mansoni* infections, however, microscopy of faecal smears remains the preferred option (Booth et al., 1998; Lengeler et al., 2002; Montresor et al., 2002). Even though a rapid diagnostic test (RDT) specific for schistosomiasis is now commercially available (see below), RDT methods have yet to find application within large-scale schistosomiasis control programmes (Hamilton et al., 1998; Engels et al., 2002) despite their growing use for other diseases, such as, malaria (Lon et al., 2005) and bubonic plague (Chanteau et al., 2003).

Following from exciting developments within immunodiagnosis, particularly focusing upon schistosome antigen capture and detection in patient's urine (Deelder et al., 1994; De Clercq et al., 1997; Kahama et al., 1998; Polman et al., 2000; van Lieshout et al., 2000), a simple to use diagnostic dipstick specific for schistosome circulating cathodic antigen (CCA) was developed (van Etten et al., 1994; van Dam et al., 2004). With further refinements in content and format, a new lateral flow CCA dipstick, namely the "schistosomiasis one step test", became commercially available in 2003 and was produced for research diagnostic purposes by European Veterinary Laboratory (EVL), Woerden, Holland (see <http://www.evlonline.nl>). In common with other attractive features of antigen capture RDTs, such as storage, portability and durability, the CCA dipstick has some unique advantages. In particular, patient's urine and not

faeces is used which allows convenient testing of those who have failed to produce stool. Moreover, the CCA dipstick is in a very useful format for quick diagnosis in the field, which could not only speed up the specimen collection process but also facilitate in quicker dissemination of prevalence results, and hence local disease burden, to the surrounding community.

Like other RDTs, the CCA dipstick is easy to use, requiring minimal staff training, and interpretation of results is straightforward as an internal control is included. As CCA antigens are genus cross-specific, the test does not discriminate between urinary and/or intestinal schistosomiasis which, from a control perspective, has the advantage of capturing, but not discriminating, both forms of disease in a single test. A limitation for its widespread use, however, is the current cost of each CCA dipstick, presently retailing between US \$2.6 and \$4.6 contingent upon numbers requested/packaging requirements, and is not re-useable. Careful consideration is therefore needed to determine the performance and most cost-effective application of the CCA dipstick and in this paper the CCA dipsticks were evaluated under field-based conditions for diagnosis of schistosomiasis in selected regions within Uganda and Zanzibar, representative of endemic areas for either *S. mansoni* or *S. haematobium*, respectively.

## 2. Subjects and methods

In Uganda, two areas were chosen reflecting the major ecological zones and transmission landscapes of intestinal schistosomiasis where the national control programme was implementing a phased-in control strategy within a sub-county in each of 18 districts (Kabatereine et al., in press). Five sentinel schools have each been chosen within Hoima and Mayuge districts where the health status of a cohort of children is being longitudinally monitored each year. Visiting these schools at intervention baseline during April (Hoima) and July (Mayuge) 2003 accompanying the longitudinal monitoring team before administration of anthelmintics provided a suitable access opportunity and logistic support for this evaluation exercise (Fig. 1).

### 2.1. Diagnostic evaluation within Ugandan sentinel schools

Table 1 details the nine sentinel schools within Hoima and Mayuge that provided a wide range of prevalence and intensity of *S. mansoni* for diagnostic assessment.

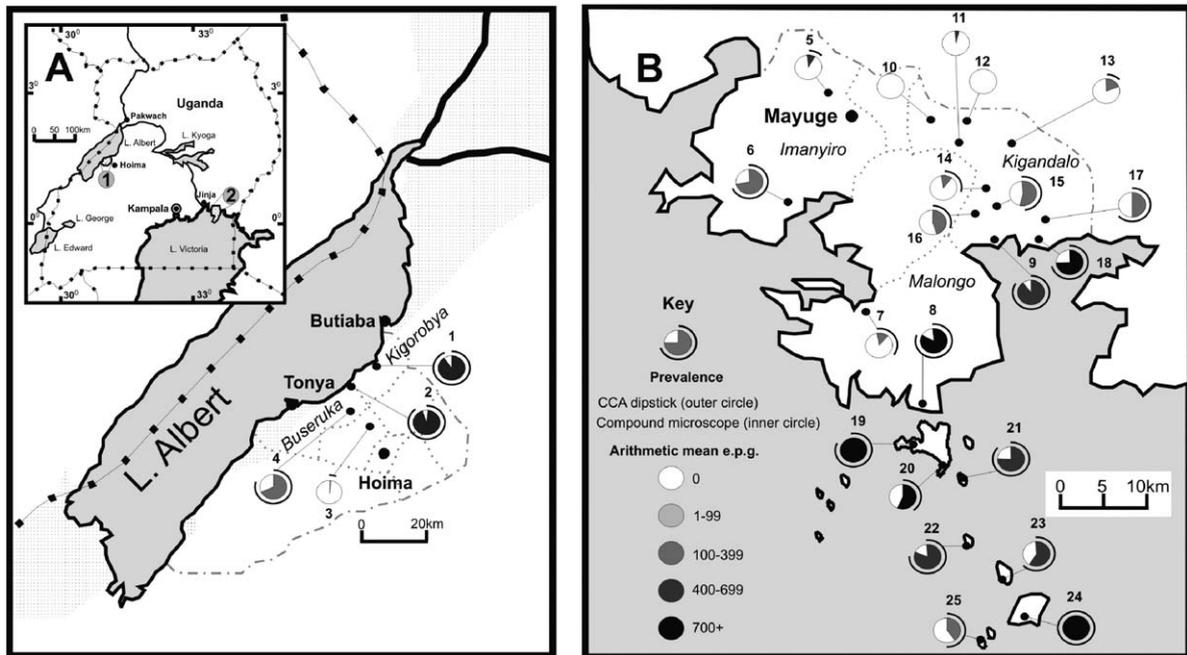


Fig. 1. A schematic map of Uganda with the locations of 25 schools in Hoima and Mayuge districts shown (in the sentinel schools 1–9, 30 children in each school were examined whereas in mapping schools 10–25, 20 children were examined). (A) Inset: schematic map of Uganda with shaded areas 1 and 2 showing the study areas. Map of the 4 chosen schools in Hoima district annotated by the detected prevalence of *S. mansoni* infections employing Kato-Katz technique and CCA dipsticks. (B) Map of the 21 chosen schools in Mayuge district annotated by the detected prevalence of *S. mansoni* infections and CCA dipsticks.

Four schools in Hoima were chosen instead of five, since the first school acted as an in-school training day, the results of which were not included in this study. The geographic location for each school was recorded using a hand-held global positioning system (GPS) unit (*e-trex*, Garmin, Olathe, USA). A total of 30 children, 11-years-old and of matched sexes provided both stool and urine samples for examination. All urine samples were tested using Bayer *Haemastix*<sup>®</sup> to detect micro-haematuria and if any positives were found, the urine sample was analyzed for the presence of *S. haematobium* eggs using standard 10 ml urine filtration method (Montresor et al., 2002). Stool samples were individually screened through a 212  $\mu\text{m}$  metal sieve and two separate Kato-Katz thick faecal smears (41.7 mg) were prepared for each child according to Katz et al. (1972). Smears were read at the school site using a compound microscope with natural light source and all *S. mansoni*, as well as other helminth eggs observed, were recorded separately and egg counts were then transformed to the number of eggs per gram (e.p.g.) of stool. Stool microscopy remains the pragmatic WHO accepted ‘gold standard’ for routine epidemiological surveillance in the field (Montresor et al., 2002), these data were then designated to be

the ‘field gold standard’ for comparisons with CCA dipsticks.

## 2.2. CCA dipsticks—diagnostic evaluation in Ugandan sentinel schools

To gain a practical working knowledge of the CCA tests, a total of four Vector Control Division technicians were trained during a single day workshop in Kampala and a further ‘in-school’ training day in Hoima in the use of the RDTs following manufacturer’s instructions. To help document the CCA test reaction, a digital photograph was used to grade the test band reaction intensity from: negative (–), trace positive (tr), single positive (+), double positive (++) and triple positive (+++). Each technician was assessed to ensure that a shared level of competence had been attained and all tests were carried out in the field under supervision. Duplicate spot checks were performed on CCA tests that were either invalid or could not be easily assigned to the above band reaction intensities. The sensitivity (SS), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) were calculated for each school and then data were then combined or pooled and SS, SP, PPV

Table 1

The identity and geographic locations of schools included in the study with the associated prevalences and intensities of *S. mansoni* infection

Primary school			Intestinal schistosomiasis		
Number <sup>a,b</sup>	District	Name GPS coordinates	Microscopy		CCA
			Prevalence (%)	Geometric mean intensity (e.p.g.)	Prevalence (%)
1 <sup>a</sup>	Hoima	Runga N 01° 43'.989, E 31° 18'.450	90	330	95
2 <sup>a</sup>	Hoima	Kibihiro N 01° 40'.305, E 31° 15'.076	94	647	90
3 <sup>a</sup>	Hoima	Kibanjwa N 01° 29'.633, E 31° 17'.049	3	0	3
4 <sup>a</sup>	Hoima	Kasyeni N 01° 34'.860, E 31° 11'.187	62	210	77
5 <sup>a</sup>	Mayuge	Ikulwe N 00° 26'.617, E 33° 28'.939	10	1	14
6 <sup>a</sup>	Mayuge	Lwanika N 00° 21'.363, E 33° 26'.597	73	21	70
7 <sup>a</sup>	Mayuge	Bukizibu N 00° 15'.698, E 33° 30'.913	14	1	40
8 <sup>a</sup>	Mayuge	Bwondha N 00° 10'.658, E 33° 31'.300	86	155	82
9 <sup>a</sup>	Mayuge	Bugoto Lake View N 00° 19'.419, E 33° 37'.697	90	162	85
10 <sup>b</sup>	Mayuge	Bweza N 00° 25'.317, E 33° 34'.326	0	0	0
11 <sup>b</sup>	Mayuge	Peterson Memorial N 00° 24'.373, E 33° 35'.720	5	0.5	0
12 <sup>b</sup>	Mayuge	Nakazigo N 00° 25'.210, E 33° 36'.158	0	0	0
13 <sup>b</sup>	Mayuge	Bugulu N 00° 24'.097, E 33° 38'.446	15	1	10
14 <sup>b</sup>	Mayuge	Matovu Parent's N 00° 21'.633, E 33° 37'.224	10	0.5	30
15 <sup>b</sup>	Mayuge	Butumbula N 00° 20'.757, E 33° 37'.799	55	10	50
16 <sup>b</sup>	Mayuge	St. Andrews Bugoto N 00° 20'.522, E 33° 36'.551	45	7	30
17 <sup>b</sup>	Mayuge	Musubi Islamic N 00° 20'.042, E 33° 40'.240	50	7	65
18 <sup>b</sup>	Mayuge	Musubi Church of God N 00° 18'.665, E 33° 39'.925	75	48	70
19 <sup>b</sup>	Mayuge	Gori N 00° 08'.240, E 33° 33'.029	100	147	80
20 <sup>b</sup>	Mayuge	Jagusi Island N 00° 07'.418, E 33° 34'.487	60	20	40
21 <sup>b</sup>	Mayuge	Kaaza Island N 00° 06'.675, E 33° 35'.996	75	51	90
22 <sup>b</sup>	Mayuge	Serinyabi Island N 00° 03'.141, E 33° 36'.358	80	70	75
23 <sup>b</sup>	Mayuge	Bumba Island N 00° 01'.321, E 33° 38'.193	65	41	60
24 <sup>b</sup>	Mayuge	Sagitu Island S 00° 00'.633, E 33° 39'.244	100	722	100
25 <sup>b</sup>	Mayuge	Masulya Island Junior S 00°02'.733, E 33° 37'.102	40	3	50

<sup>a</sup> Sentinel school.<sup>b</sup> Mapping school.

and NPV were calculated for all sentinel schools. The invalid test rate (ITR) of the CCA dipstick was calculated as total number of invalid tests where a control reaction band failed to form divided by the total number of tests used.

### 2.3. CCA dipsticks—mapping evaluation for Ugandan school prevalences

The aim of this survey was to examine the practicalities and performance of CCA dipsticks for use within a rapid mapping survey across 16 primary schools. To improve the workload of the technician to an acceptable daily level for conducting both microscopy and CCA tests at each school, the total number of children surveyed at each school was reduced such that 20 children provided stool and urines and a single Kato-Katz thick faecal smear was prepared for each child. The details of the schools visited are presented in Table 1. The geographic location of each school was recorded and

shortest distance to the lakeshore (where it was assumed infection would take place) was calculated with ArcMap 8.3 software (ESRI, Redlands, USA). The SS, SP, PPV and NPV values were calculated for each school and then calculated for all prevalence mapping schools.

### 2.4. CCA dipsticks—Ugandan total evidence

All available data collected during the sentinel and prevalence surveys were pooled to form a total evidence matrix recording the infection status of 590 children. The SS, SP, PPV and NPV values were then calculated. A relationship between *S. mansoni* infection intensity (e.p.g.) and CCA test band intensity was investigated using standard boxplots and a relationship between *S. mansoni* school prevalence estimates, as determined by Kato-Katz thick smears and CCA dipsticks, was investigated using correlation and linear regression with S-PLUS 6.0 (Insightful Corp., Seattle, USA).

### 2.5. CCA dipsticks—diagnostic evaluation of urinary schistosomiasis in Zanzibar

To assess the performance of the CCA dipsticks where only *S. haematobium* was endemic, two schools on Unguja Island (Zanzibar), Tanzania were visited during June 2003 (Stothard et al., 2002). The prevalence of urinary schistosomiasis, as assessed by standard 10 ml urine filtration, was 73% in Ghana Primary School (S 6° 02'.605, E 39° 18'.070) and 64% in Mwera Primary School (S 6° 08'.626, E 39° 16'.334). At each school a total of 25 urines were selected to be representative of both schistosome egg-positive (ranging from light to heavy egg counts) and egg-negative specimens; the selected egg-positive urines were also graded visually for gross haematuria and with reagent strips *Hemastix*<sup>®</sup> for microhaematuria. Duplicate CCA tests were performed on selected urine samples, those shown to be egg-positive/(micro)haematuric ( $n=35$ ) as well as those egg-negative/non-haematuric ( $n=15$ ). In addition, single CCA spot-tests were performed on five heavy egg-positive (>50 eggs 10 ml) with gross haematuria from children reporting for examination at the Pemba Public Health Laboratory, Pemba Island (Zanzibar), Tanzania.

### 2.6. CCA dipsticks—laboratory reference adult schistosomes

To investigate further the performance of CCA dipsticks on detection of African schistosomes, aqueous lysates of several available schistosome species were each prepared from five adult worm-pairs stored in liquid nitrogen at the Natural History Museum (NHM), London, UK. A 5  $\mu$ l solution from a 1:20 dilution of adult worm lysate was applied to the CCA dipstick for the following species: *S. mansoni* NHM Acc. no. 3913 and 3484 from Senegal, *S. haematobium* NHM Acc. no. 3883 from Zanzibar, *S. bovis* NHM Acc. no. 1388 from Kenya, *S. intercalatum* NHM Acc. no. 3627 from present-day Democratic Republic of Congo (formerly Zaire), *S. guineensis* NHM Acc. no. 3827 from Cameroon and *S. rodhaini* NHM Acc. no. 3961 from Burundi. In addition urine from infected animals (mice and hamsters) were collected and applied to the CCA dipstick following manufacturer's instructions.

### 2.7. Anthelmintic treatment and ethical approval

All children examined in the surveys were treated with an appropriate medication of praziquantel (single oral dose at 40 mg/kg) as calculated by children's height according to the amended WHO height pole (Montresor

et al., 2005) and also a single dose of 400 mg albendazole regardless of their infection status (Montresor et al., 2002). Ethical approval for this study (application 03.36) was granted by National Health System Local Research Ethics Committee (NHS-LREC) of St. Mary's Hospital, London as well as approval from the Uganda Ministry of Health and the Ugandan National Council of Science and Technology, Kampala.

## 3. Results

### 3.1. Diagnostic evaluation—CCA sentinel school data

A total of 9 schools were visited, 4 in Hoima district and 5 in Mayuge district comprising a total of 120 and 150 children, respectively. Double Kato-Katz thick smears were examined microscopically for each child. The prevalences and geometric means (e.p.g.) of infections are shown in Table 1. The overall prevalence of *S. mansoni* in the 9 schools was 58% with a total geometric mean (for all children examined) of 11 e.p.g. and arithmetic mean (infected cases only) of 268 e.p.g. The geographic distribution of prevalences and arithmetic means (infected children only) across these 9 schools is shown in Fig. 1. No *S. haematobium* infection was detected. Using the CCA dipsticks for the four schools in Hoima, SS and SP of correctly identifying each child with an egg-positive *S. mansoni* infection was 87 and 90%, respectively while within the five sentinel schools in Mayuge the SS and SP were 89 and 79%, respectively. The ITR of the CCA dipstick was observed to be approximately 8%.

### 3.2. Estimating school prevalence—CCA mapping survey

In light of the ITR, to cater for the 20 children to be tested a total of 22 CCA dipsticks were supplied for use within each school. For the 16 schools, the corresponding prevalence of intestinal schistosomiasis is shown in Fig. 1 and Table 1, with overall prevalence of approximately 48%. Across these data, the mean error between prevalence estimated by the Kato-Katz technique and CCA tests was  $\pm 8.5\%$ .

### 3.3. Detection of *S. mansoni*—overall CCA performance

While there was a positive association of the intensity of the CCA test reaction with the corresponding faecal e.p.g. of the child (Fig. 2), no relationship was found

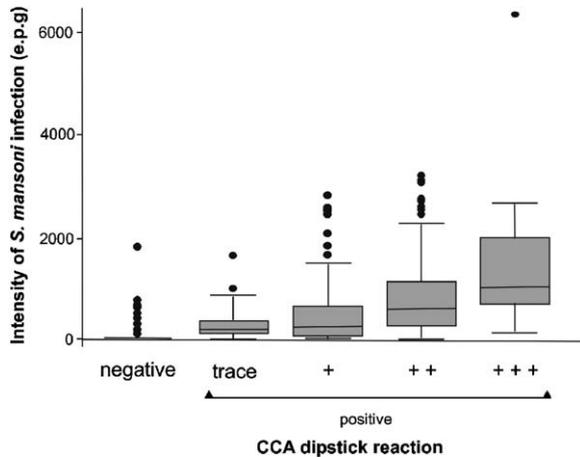


Fig. 2. Boxplot showing the positive association of intensity of *S. mansoni* count with intensity of the CCA reaction dipstick test band. Whilst some infections were egg-negative but CCA positive, a triple positive reaction (+++) is strongly associated with moderate-to-heavy infections.

between faecal e.p.g. and proportion of children incorrectly diagnosed by CCA (Fig. 3). With one exception, for those children exhibiting egg counts higher than 840 e.p.g., there was no discord of infection status between microscopy and CCA dipsticks but it appeared that light egg-positive infections (<100 e.p.g.) were misdiagnosed with the CCA dipstick (Fig. 2). Overall mean infection prevalence of *S. mansoni* was 52%, as determined by the Kato-Katz technique with SS and SP of the CCA dipstick to be 83 and 81%, respectively. Meanwhile, PPV and NPV were both 84%. Plotting the CCA estimates of prevalence against those obtained by Kato-Katz thick smear examination, revealed a positive Pearson correlation coefficient ( $r=0.95$ ). Fitting a linear regression trend line across these data determined a relationship of  $y=0.974x$ , with  $R^2=0.89$ .

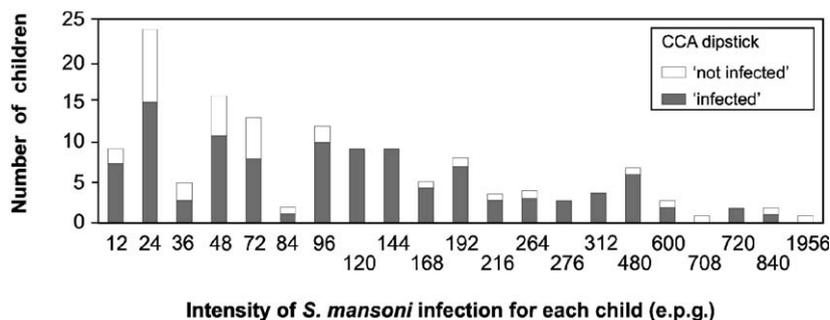


Fig. 3. A histogram of (in)congruence between Kato-Katz thick smears and CCA dipsticks. In sentinel schools where double faecal smears were taken the associated faecal sample was from 83.4 mg of stool. Light infections (<100 e.p.g.) were sometimes incorrectly diagnosed with CCA dipstick as well as several moderate-to-heavy infections. All infections detected in this study with an associated e.p.g. >840, with one exception, were correctly identified with the CCA dipstick.

### 3.4. Detection of *S. haematobium*—overall CCA performance

All urine samples tested with the CCA dipstick on Unguja were deemed negative for schistosome infection. In all cases the positive control reaction zone of the CCA dipstick was clearly visible and tests were also repeated for confirmation. This observation conflicts with the observed schistosome egg status of the 35 urine samples that were patent *S. haematobium* infections (often with >50 eggs/10 ml of urine). In addition, these children came from a highly endemic area for urinary schistosomiasis on Unguja and had various levels of microhaematuria. Eggs were viable as miracidia could be hatched upon exposure to freshwater. Similarly the CCA dipstick incorrectly diagnosed the infection status of the five children reporting to the Pemba Public Health Laboratory.

As a quality control, five CCA dipsticks and associated buffer within the same batch of CCA tests used in Zanzibar were held back and tested in Uganda with patients' urines whom had patent *S. mansoni* infections. All dipsticks used detected infections correctly. Under field conditions, there is no evidence that CCA dipsticks detect *S. haematobium* infections in their present format and formulation.

### 3.5. CCA analysis of laboratory reference schistosomes

All adult worm lysates tested with the CCA dipstick incurred a positive reaction within 20 min, which was judged to be of triple positive intensity. As an additional investigation, while urines from *S. mansoni*-infected mice were shown to be CCA positive, urines from *S. haematobium*-infected hamsters were shown to be CCA negative.

## 4. Discussion

### 4.1. Diagnosis of intestinal schistosomiasis

The evaluation took place over a typical *S. mansoni* transmission landscape found in Uganda (Kabateraine et al., 2004) as high, medium and low prevalence schools were encountered, as illustrated in Table 1 and Fig. 1. While the overall prevalence was 52% across the sample, infections were aggregated to schools such that 15 schools (60%) were high prevalence ( $\geq 50\%$ ), 4 schools (16%) were moderate prevalence ( $\geq 10\%$  and  $< 50\%$ ) and 6 schools (24%) were low prevalence ( $< 10\%$ ). Around Lake Victoria, all high prevalence schools within Mayuge were located within a 5 km zone from the lakeshore, confirming previous observations of Handzel et al. (2003) and Brooker et al. (2005) that distance-to-lake is a strong predictor of infection prevalence by school.

Under these field-based conditions for diagnosis of *S. mansoni*, the SS and SP of the CCA dipstick were 86 and 81%, respectively. As CCA method for detection of infection is based upon a completely different biological marker, i.e. observation of schistosome gut released antigenic proteins (van Dam et al., 1996) rather than visualisation of eggs (Hamilton et al., 1998; Al-Sherbiny et al., 1999), this is perhaps not surprising. While a positive association between faecal e.p.g. and CCA reaction band intensity was observed (Fig. 2) and as previously reported by van Dam et al. (2004), the test often failed to identify children with patent egg infections (Fig. 3). The majority of these cases had light infections ( $< 100$  e.p.g.) although some heavy egg-positive infections were also missed. In those instances where children were CCA-positive but were egg-negative, a simple biological explanation is forthcoming. These children may have harboured adult schistosomes that were either pre-patent in their egg laying capacity or that the gravid egg-producing females only formed a minority of the schistosome population whose total egg production was below the threshold of detection by Kato-Katz examination, which is known to be insensitive (Berhe et al., 2004).

Another possible explanation of the lower SP is that general inflammatory biomarkers excreted in the urine, possessing Lewis-X tri-saccharide epitopes, might cross-react with the CCA dipstick to illicit a non-specific result (van Dam et al., 1996). Indeed the manufacturer of the CCA dipstick has determined this cut-off threshold to minimize such cross-reactivity without compromising SS but instances will occur when the concentration of inflammatory biomarkers in specific cases will be ele-

vated against this general background. A direct consequence can be the designation of 'trace' especially for those users who have slightly better visual acuity than others and an exact determination of a cut-off with any strip test can be difficult (van Dam et al., 2004). In certain schools (for example Bukizubu; school no. 7), this might explain the discrepancy and if 'trace' reactions were downgraded to negative status, agreement would be even better.

### 4.2. Urinary schistosomiasis—CCA diagnosis

As *S. haematobium* was not endemic in the two chosen areas of Uganda, a further evaluation was justified in Zanzibar where only urinary schistosomiasis is endemic (Stothard et al., 2002). The absolute failure of the CCA dipstick to detect *S. haematobium* infections in its present format and formulation gives cause for concern. While previous studies have shown urine-CCA to be useful in diagnosis of urinary schistosomiasis, an ELISA and not lateral flow dipstick format was used (Krijger et al., 1994; De Clercq et al., 1997; Kahama et al., 1998). Clearly while the CCA dipstick is able to detect the presence of *S. mansoni* infections to a comparable level of stool microscopy, for some presently unknown reason it was not able to detect active cases of urinary schistosomiasis on Unguja or on Pemba.

To test if the observed results of the CCA dipsticks on Zanzibar were restricted to East African *S. haematobium*, a further CCA dipstick evaluation took place in a single school in Niger, West Africa. During May 2004, Satoni Primary School (N 19° 28' .348, E 01° 05' .635) was visited and single CCA tests were performed on 20 egg-positive urines. Again all CCA tests failed to detect active urinary schistosomiasis infections. Taking a subset of these tests, which came from a different production batch to those used on Zanzibar, back to Uganda for testing on urine from children with intestinal schistosomiasis showed them to function normally. A similar spot control was conducted in Burkina Faso where CCA dipsticks similarly failed to identify 10 children with egg-patent *S. haematobium* infections. These additional observations further corroborate the findings on Zanzibar and the available evidence clearly shows that the present CCA dipstick has no value in diagnosis of *S. haematobium* infections.

To shed further light on these findings, using adult worm lysates and dilutions thereof, it was shown that in principle the dipstick could capture and detect CCA antigens from several species of adult worm including *S. haematobium* and *S. haematobium*-group species. No doubt lysates contained antigens in con-

centrations in excess to that expected in the urine of active infections, but while urine-CCA from *S. mansoni*-infected mice could be easily detected, that from *S. haematobium*-hamsters could not so the situation in the field was also corroborated in laboratory hosts. The problem may therefore lie within the stoichiometry of the antigen–antibody interaction such that the concentration of urine-CCA typically found in intestinal schistosomiasis must be significantly greater than that found in urinary schistosomiasis or the antibody formulation has a much greater affinity. As another alternative it may be that the urine-CCA of *S. haematobium* infections has become immuno-complexed and is therefore not being captured by the dipstick although we have observed that incubation of urine in dissociation buffer recommended by Krijger et al. (1994) made no subsequent effect.

#### 4.3. Future use of CCA dipsticks—towards LQAS sampling

It is clear that in its present available formulation the CCA dipstick has no value for detection of urinary schistosomiasis. On the other hand, for intestinal schistosomiasis the performance of the CCA dipstick is particularly promising, CCA dipsticks are portable, easy to use and on the whole perform well as an alternative or complement to microscopic examination of faecal smears. The major limitation is their cost, which owing to budgetary constraints means they are presently too prohibitive for routine widespread use within national schistosomiasis control programmes. For example and given the ITR of 8% of the CCA dipstick means the costs alone for examining 30 school children, a sample size recommended by WHO (Montresor et al., 1998), would be approximately US \$86 per school. Moreover this expenditure would increase proportionally as further schools were surveyed. In contrast, while the initial cost of purchase of the table compound microscope can be high (~US \$1500) although much cheaper handheld microscopes (~US \$70) exist (see Stothard et al., 2005), the replenishment costs per school associated with Kato-Katz examinations in comparison are almost negligible (Hamilton et al., 1998).

While it is outside the immediate scope of this paper to develop logistic models for disease surveillance teams using either microscopy and CCA dipsticks, an immediate way to streamline the direct costs of CCA dipsticks would be to use fewer tests per school. This could be achieved by reducing the initial children sample size ( $n < 30$ ) or perhaps by pooling urines in a stepwise manner. Adopting a lot quality assurance sampling (LQAS) strategy, similar to that developed for sampling Kato-

Katz slides as pioneered in Madagascar by Rabarijaona et al. (2003) and more recently in Uganda by Brooker et al. (2005), could be particularly appealing. For example, Brooker et al. (2005) demonstrated that an LQAS strategy of  $n = 15$  (the number of sampled children per school) and  $d_1 = 7$  (the number of infected cases detected) had SS of 100% and SP of 96.4% for correctly identifying high prevalence schools ( $\geq 50\%$ ). In this LQAS strategy ( $n = 15$ ,  $d_1 = 7$ ), sampling stops when either the maximum sample size is met or the number of infected cases is exceeded. In principle if CCA dipsticks were substituted instead of Kato-Katz slides, between 9 and 17 CCA dipsticks need only be utilised, incurring costs of between US \$24 and \$45 per school, thereby almost quartering, or at least halving, direct costs. In addition, the time spent at each school by surveillance personnel could be very short (<1 h), allowing several schools to be visited each day.

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