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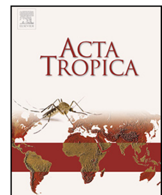

Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for *Schistosoma mansoni* in different transmission settings within Bugiri District, Uganda

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A B S T R A C T

Diagnosis of schistosomiasis at the point-of-care (POC) is a growing topic in neglected tropical disease research. There is a need for diagnostic tests which are affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered to those who need it, and POC is an important tool for disease mapping and guiding mass deworming. The aim of present study was to evaluate the relative diagnostic performance of two urine-circulating cathodic antigen (CCA) cassette assays, one commercially available and the other in experimental production, against results obtained using the standard Kato-Katz faecal smear method (six thick smears from three consecutive days), as a 'gold-standard', for *Schistosoma mansoni* infection in different transmission settings in Uganda. Our study was conducted among 500 school children randomly selected across 5 schools within Bugiri district, adjacent to Lake Victoria in Uganda. Considering results from the 469 pupils who provided three stool samples for the six Kato-Katz smears, 293 (76%) children had no infection, 109 (23%) were in the light intensity category, while 42 (9%) and 25 (5%) were in the moderate and heavy intensity categories respectively. Following performance analysis of CCA tests in terms of sensitivity, specificity, negative and positive predictive values, overall performance of the commercially available CCA test was more informative than single Kato-Katz faecal smear microscopy, the current operational field standard for disease mapping. The current CCA assay is therefore a satisfactory method for surveillance of *S. mansoni* in an area where disease endemicity is declining due to control interventions. With the recent resolution on schistosomiasis elimination by the 65th World Health Assembly, the urine POC CCA test is an attractive tool to augment and perhaps replace the Kato-Katz sampling within ongoing control programmes.

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1. Introduction

Intestinal schistosomiasis, also called Bilharzia, is one of the leading causes of morbidity and disability in many fishing communities lying along large water bodies in Uganda, such as Lakes Albert, Victoria, Kyoga and along the Albert Nile (Nelson, 1958), as well as in rice paddy fields in Eastern Uganda (Bukenya et al., 1994). It is estimated that over 60% of the people in these communities have the disease (Kabatereine et al., 2004); with *Schistosoma mansoni* infections recorded in 64 out of 112 districts of Uganda. By contrast, urinary schistosomiasis, caused by *S. haematobium*, exists in only a few districts near Lake Kyoga and is much rarer (Schwetz, 1951; Rosanelli, 1960). Nation-wide, it is estimated that up to 4 million people are affected and 16.7 million are at risk of schistosomiasis (Kabatereine et al., 2006a). The disease affects both children and adults, with the peak infection and intensity levels being in the age group 10–20 years (Kabatereine et al., 2006b).

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Nation-wide schistosomiasis control in Uganda was initiated in 2003 under the auspices of the Schistosomiasis Control Initiative (SCI) with funding from the Bill & Melinda Gates Foundation (Kabatereine et al., 2006a,b). The objective was to control morbidity through regular chemotherapy of at-risk communities as identified based on the WHO mapping protocol which uses the single Kato-Katz thick smear method for diagnosis (WHO, 2006). Although this test has several advantages such as low cost, high specificity, simplicity to perform and concurrent detection of other helminth species, the counting of excreted parasite eggs in just 41.7 mg of stool is unreliable and inaccurate (Booth et al., 2004; Gryseels, 1996). Consequently, the method has low sensitivity especially in low transmission settings which can become more common after many years of control, at which time excreted egg-outputs fall within infected children (Colley et al., 2013). Although the sensitivity of the Kato-Katz method can be improved through increasing the number of examined smears (de Vlas et al., 1993), such an approach is time consuming and limited by operational challenges and costs. The single Kato-Katz method is also prone to error in estimating the true prevalence of infection as it is affected by day to day variability in egg excretion (de Vlas et al., 1993; Teesdale et al., 1985) especially in 'light' infection intensities when egg-output is sporadic, hence the method cannot adequately guide interventions to meet the current WHO target of disease elimination (WHO, 2012; Standley et al., 2010b).

Consequently, a more sensitive tool such as the "Point-of-Care Circulating Cathodic Antigens" test (POC-CCA, Rapid Medical Diagnostics, Pretoria, South Africa) which is based on direct detection of parasite CCA in host urine and gives results within few minutes has been developed to augment and perhaps replace the Kato-Katz method (Stothard et al., 2009; Standley et al., 2009, 2010a,b). This rapid diagnostic test (RDT) became commercially available in 2003 (Stothard et al., 2006; Standley et al., 2010a) and it is the only rapid assay for diagnosis of *S. mansoni* currently commercially available retailing for approximately 1.75 USD per test (Colley et al., 2013). The POC-CCA cassettes are portable, easy to use; require minimal staff training, data interpretation is simple and has characteristics observed in other Rapid Diagnostic Tests (RDTs) such as those applied in Malaria diagnosis (Colley et al., 2013; Stothard et al., 2006; Coulibaly et al., 2011; Shane et al., 2011; Tchuem Tchuente et al., 2012). This implies that CCA tests can be easily incorporated in the existing health systems for rapid diagnosis and treatment of cases in health facilities (Colley et al., 2013). The POC-CCA test uses a drop of urine on a lateral flow strip to detect the presence of adult worm infection, and has been successfully tested for disease mapping around Lake Victoria in school age children (Standley et al., 2010b) and in Ugandan preschool age children (Sousa-Figueiredo et al., 2013). However, a more comprehensive evaluation of the test was arranged recently by Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) and conducted under a multi-country study involving Cote d'Ivoire (Coulibaly et al., 2011) Kenya (Shane et al., 2011) and Cameroon (Tchuem Tchuente et al., 2012). The combined results of the multi-country study were recently published (Colley et al., 2013) and our current study is part of that multi-country investigations whose objective was to evaluate the CCA diagnostic accuracy for rapid *S. mansoni* mapping in an area in Uganda where the original high prevalence and intensity of infection was significantly reduced through many rounds of MDA with praziquantel (Kabatereine et al., 2007).

Earlier reports had observed high sensitivity results of the POC-CCA assay when compared to the WHO recommended Kato-Katz method prompting a suggestion that the test might be producing many false positives (Stothard et al., 2006; Standley et al., 2010a). As a consequence, the manufacturer produced another CCA version here designated as CCA2 believed to be less sensitive than the original CCA1 assay (Colley et al., 2013). Under the current study, the performance of CCA1 and its alternative version CCA2 were both evaluated against the Kato-Katz thick smear method (Katz et al., 1972) as a reference test.

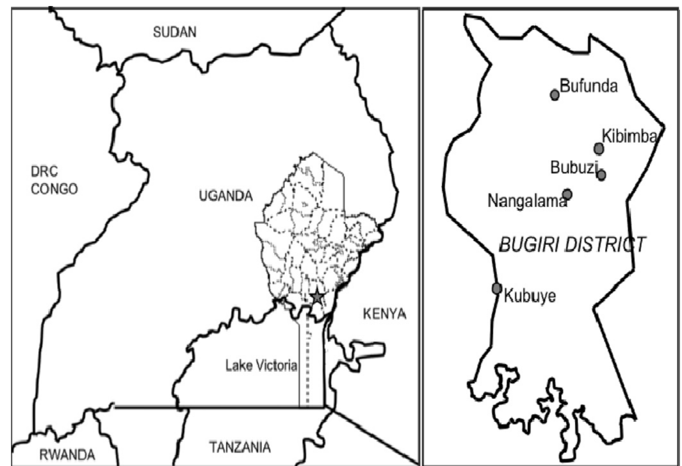

Fig. 1. Map of Uganda showing SCORE Study schools in Bugiri District. Please note that the Star shows location of Bugiri District.

2. Materials and methods

2.1. Study area

The study was conducted in five primary schools in Bugiri district which lie along the shoreline of Lake Victoria in south-eastern Uganda (longitude 33°10′ E, 34 E and latitude 6°1′ N, 12′ E, Fig. 1). Annual mass treatment with praziquantel had been administered in the area for five years before the study begun and the campaign had reduced the endemicity of the disease from high to medium levels.

2.2. Selection of study population and data collection

Following the pre-screening SCORE guidelines using the Lot Quality Assurance Sampling (LQAS, where a maximum of 15 pupils and a single smear is read for each child) (Brooker et al., 2005). Five schools were selected and categorised into three settings based on the WHO helminth threshold. Setting A consisted of only one school in a low *S. mansoni* endemic area. Settings B and C were situated in moderate and high endemic areas, respectively, and each contained 2 schools. In each school, 100 children aged 7–13 years were randomly selected, making a total of 500 subjects. The selected children were asked to provide both stool and urine samples on the first day and another stool sample on each of the next two consecutive days.

2.3. Laboratory methods

Duplicate Kato-Katz thick smears (Katz et al., 1972) were prepared in the field upon receipt of the faecal samples and were read under a microscope within 60 min of slide preparation to determine hookworm status. The slides were again read 24 h later for *Ascaris lumbricoides*, *Trichuris trichiura* and *S. mansoni* and this was repeated for all subsequent stool samples. The intensity of *S. mansoni* infection was expressed as egg count per gram (EPG) of stool for each child (WHO, 2002). The urine samples were tested immediately upon receipt using the POC-CCA assay for the Detection of Schistosoma (Stothard et al., 2006; Standley et al., 2010a). The CCA tests were scored as negative, trace, single positive, double positive and triple positive based on formation and intensity of the

control band on CCA cassettes, as a qualitative measure of intensity of infection (van Dam et al., 2004; Kabatereine et al., 2006b; Midzi et al., 2009). Cases with trace CCA results were considered positive infections.

2.4. Treatment and ethical approval

All children found positive for intestinal schistosomiasis (egg-patency) or positive CCA tests were treated with praziquantel (Distocide®, Shin Poong Pharmaceuticals, Seoul Republic of Korea) at 40 mg/kg body weight. Regardless of infection status, a tablet of albendazole (400 mg) was given to each child for soil-transmitted helminthiasis. This study was approved by the Ugandan National Council of Science and Technology and forms part of the monitoring and surveillance activities of the Ugandan National Bilharzia & Worm Control Programme. Permission from school authorities was sought and children consented before participation in the study.

2.5. Data analysis

The parasitological data was entered in a computer in Microsoft Excel™. Prevalence values with 95% confidence interval of CCA1, CCA2, double, quadruple and sextuple Kato-Katz thick smears in different settings were estimated. Diagnostic sensitivity (proportion of true-positives detected by the test), specificity (proportion of true-negatives detected by the test), positive predictive value and negative predictive value of CCA1 and CCA2 when trace is considered either positive or negative were estimated using Kato-Katz thick smear results of double, quadruple and sextuple as reference tests. However, the lack of a true gold standard test was a limitation; as a result, it was difficult to determine the true sensitivity and specificity of any test. Arithmetic mean of *S. mansoni* faecal egg counts of double; quadruple and sextuple Kato-Katz thick smears were calculated and multiplied by a factor of 24. The intensity of *S. mansoni* infection was categorised according to the WHO threshold into light (1–99 EPG), medium (100–399 EPG) and heavy (400+ EPG) (WHO, 2006).

The Chi-square proportional test was applied to determine the impact of Soil Transmitted Helminths (STH) infections on CCA diagnostic performance by comparing *S. mansoni* prevalence among STH positives vs. STH negatives. To determine impact of increasing intensity of infection on the CCA diagnostic performance, logistic regression analysis model was applied to estimate Odds Ratios for different intensity groups. In this model, the CCA test results were used as outcome and expressed in binary form. To assess the correlation between CCA1 and CCA2 colour reaction categories and *S. mansoni* intensity of infections, ordinal logistic regression analysis for ordinal categorical outcomes was employed. The geometric mean EPG of two Kato-Katz thick smears per stool sample per day served as continuous explanatory variable, whereas the colour reaction of the CCA test was considered as categorical outcome. Day to day variation of Kato-Katz results was assessed using McNemar test (McNemar, 1947) Statistical significance were measured using non overlapping 95% CI and P-values <0.05.

The strength of agreement between different CCA assays when trace results are either positive or negative and Kato-Katz thick smear of double, quadruple and sextuple slides were assessed by a kappa statistic (k) in different endemic settings, as follows: $k<0$ indicating no agreement, $k=0<0.2$ indicating poor agreement, $k=0.2<0.4$ indicating fair agreement, $k=0.4<0.6$ indicating moderate agreement, $k=0.6<0.8$ indicating substantial agreement, and $k=0.8–1.0$ indicating very good agreement (Landis, 1977; Cohen, 1960). In addition, receiver operating characteristics (ROC) curves were applied to compare sensitivity and specificity for the CCA assays against double, quadruple and sextuple Kato-Katz smears as reference. The area under curve (AUC) indicated the probability to identify accurately a true positive case when the result is simultaneously positive and negative for CCA tests. In this test, AUC > 0.7 indicated a high discriminating power.

3. Results

3.1. Study adherence

Overall, a total of 500 pupils with mean age of 10.4 years were included in the study. There were slightly more females included than males (259 vs. 241). The number of children surveyed in epidemiological setting A was 100 and 200 children were included for each of settings B and C. Altogether, 500 children provided both stool and urine samples on the first day while just 496 and 469 children submitted their stool samples for day two and day three, respectively. Of the 469 pupils who provided all the three stool samples, 96 (21%) were from epidemiological setting A while 187 (40%) and 186 (40%) children were from settings B, and C in that order.

3.1.1. Parasitological examinations

Although the pre-screening survey results using the LQAS (Brooker et al., 2005) had identified 3 different *S. mansoni* endemic settings A, B and C, respectively, when 2 x KK method was applied to determine the prevalence of infection basing on "WHO" guidelines (WHO, 2006), the classification was confirmed only in settings A and B as low and moderate but category C which had been classified as "high endemicity", shifted to upper limits of the moderate endemicity (Table 1). Overall, the prevalence of *S. mansoni* increased with increasing number of examined smears (Table 1). When this parameter was assessed under different epidemiological thresholds, the respective prevalence for double, quadruple and sextuple smear readings were; 8%, 11% and 13% in setting A; 23%, 31% and 33% in setting B, and 36%, 47% and 55% in setting C respectively. Overall, the mean intensity of *S. mansoni* infection among positives was 115, 235 and 372 epg in settings A, B and C respectively (Table 1). Considering results from the six Kato-Katz smears, 293 (76%) children had no infection, 109; (23%) were in the light intensity category, while 42 (9%) and 25 (5%) were in the moderate and heavy intensity categories respectively. The overall prevalence of infection with any STH was 31% (154/500). Most of the STH infections were due to hookworms (30%) while the prevalence of *A. lumbricoides* and *T. trichiura* was just 1% and 2%, respectively.

3.2. Prevalence of S. mansoni infection by CCA testing

When trace results were scored as positive, the prevalence of *S. mansoni* based on CCAI assay in settings A, B and C, was 48%, 61% and 72% respectively while it was 9%, 27% and 39% for the CCA2 (Table 1). Similarly, when the trace results were considered negative, the same parameter in settings A, B and C was 20%, 32% and 43% respectively for CCA1 and 5%, 15% and 16% for CCA2, implying that the prevalence of *S. mansoni* increased moving from low to high endemic areas. Overall, the prevalence of *S. mansoni* when data from all settings was combined was 63% and 34% for CCA1, and 28% and 13% for CCA2 when trace result were considered either positive or negative in that order. Hereafter, in all our further analysis, all trace CCA results were considered positive in agreement with (van Dam et al., 2004).

3.3. Comparison of CCA tests against Kato-Katz

Table 1 shows *S. mansoni* prevalence results determined with either CCA assays or by the Kato Katz method. The prevalence of *S. mansoni* by CCA1 was significantly higher than that obtained using the 6 Kato Katz smears (Table 1). In contrast, the difference was

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Table 1
Prevalence of *S. mansoni* and STH infections according to each diagnostic test by epidemiological setting category.

Diagnostic test	Setting A		Setting B		Setting C		Overall	
	No: exam	% Positive (95% CI)	No: exam	% Positive (95% CI)	No: exam	% Positive (95% CI)	No: exam	% Positive (95% CI)
S. mansoni diagnosis								
CCA1 trace (+ve)	100	48 (38–58)	200	61 (54–67)	200	72 (66–78)	500	63 (58–67)
CCA1 trace (−ve)	100	20 (12–28)	200	32 (26–39)	200	43 (36–50)	500	34 (30–38)
CCA2 trace (+ve)	100	09 (3–15)	200	27 (21–33)	200	39 (32–45)	500	28 (24–32)
CCA2 trace (−ve)	100	05 (1–9)	200	15 (10–19)	200	16 (10–21)	500	1 (10–16)
Kato-Katz thick smear readings								
Double	100	08 (3–13)	200	23 (17–28)	200	36 (29–43)	500	25 (21–29)
Quadruple	100	11 (5–17)	199	31 (24–37)	197	47 (40–54)	496	33 (9–37)
Six (sextuple)	96	13 (6–19)	187	33 (26–40)	186	55 (48–62)	469	38 (33–42)
STH infections								
Hookworm	100	17 (10–25)	200	15 (10–20)	200	51 (44–58)	500	30 (26–34)
Ascaris lumbricoides	100	01 (1–3)	200	0	200	02 (0–04)	500	01 (0.1–02)
Trichuris trichiura	100	01 (1–3)	200	1 (0.4–2)	200	03 (1–5)	500	02 (0.6–03)
Any STH	100	18 (10–26)	200	16 (11–21)	200	52 (45–59)	500	31 (27–35)

Please note that there was a dropout of 4 children on day two and 31 children on day three who did not provide their stool samples.

insignificant in settings A and B when CCA2 results were compared to six smears (Table 1). However in setting C, the CCA2 results were significantly lower at 39% compared to the 6 smear results of 55%.

3.4. Sensitivity, specificity and predictive values of CCA tests

Table 2 shows sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different CCA results when compared to Kato Katz double, quadruple and sextuple smears as reference. The CCA1 sensitivity increased with increasing number of Kato-Katz smears and with increasing endemicity. For example, using six smears as reference, the sensitivity of CCA1 was 75%, 84 ad 92% in settings A, B and C respectively. However, CCA2 results did not follow the same trend in that it increased between A and B but decreased between B and C (Table 2). Overall, considering 6 Kato-Katz smears as reference, the sensitivity of CCA2 was insignificant from that of 4 Kato-Katz smears (60% vs. 68%).

Considering six Kato-Katz smears as reference; the specificity of CCA1 was quite low being 55%, 52% and 50% in settings A, B, and C in that order. However, for CCA2, the specificity was very high at 95%, 92% and 85% respectively (Table 2). With both versions of CCA, sensitivity increased with increasing endemicity of infection.

Again Table 2 shows the PPV and NPV of different CCA assays in reference to different numbers of Kato-Katz readings. Considering six Kato-Katz smears as reference tests, the PPV of CCA1 increased significantly with increasing endemicity (Table 2). The NPV value of CCA1 were 94%, 87% and 84% in settings A, B and C in that order compared to 92%, 83% and 63% in the same order for CCA2.

3.5. Agreement between Kato-Katz and CCA assays

Table 3 shows the agreement between the different CCA assays and reference tests of double, quadruple and sextuple Kato-Katz thick smear readings. Using six Kato-Katz smears as a reference test, the agreement of CCA1 were $k = 0.13$, $k = 0.30$ and $k = 0.44$ in settings A, B and C in that order while the agreement of CCA2 was $k = 0.41$, $k = 0.57$ and $k = 0.43$ respectively in the same order. Overall, this agreement was fair ($k = 0.35$) for CCA1 and moderate ($k = 0.53$) for CCA2. Similar results were observed using double Kato-Katz test as reference.

3.6. Effects of *S. mansoni* infection intensities on CCA performance

Our ordinal logistic regression analysis showed that for an increase of *S. mansoni* infection intensity by 1 EPG, the likelihood of a stronger colouration of the CCA1 odds ratio (OR) = 2.52) and the CCA2 (OR = 2.34) is significant both at p, 0.001). Fig. 2 shows the correlation between prevalence o f *S. mansoni* using CCA assays and intensity of infection categories. Generally the positivity of CCA assays increased with increasing intensity of infection in all study settings. For example in setting A, the positivity of CCA1 increased

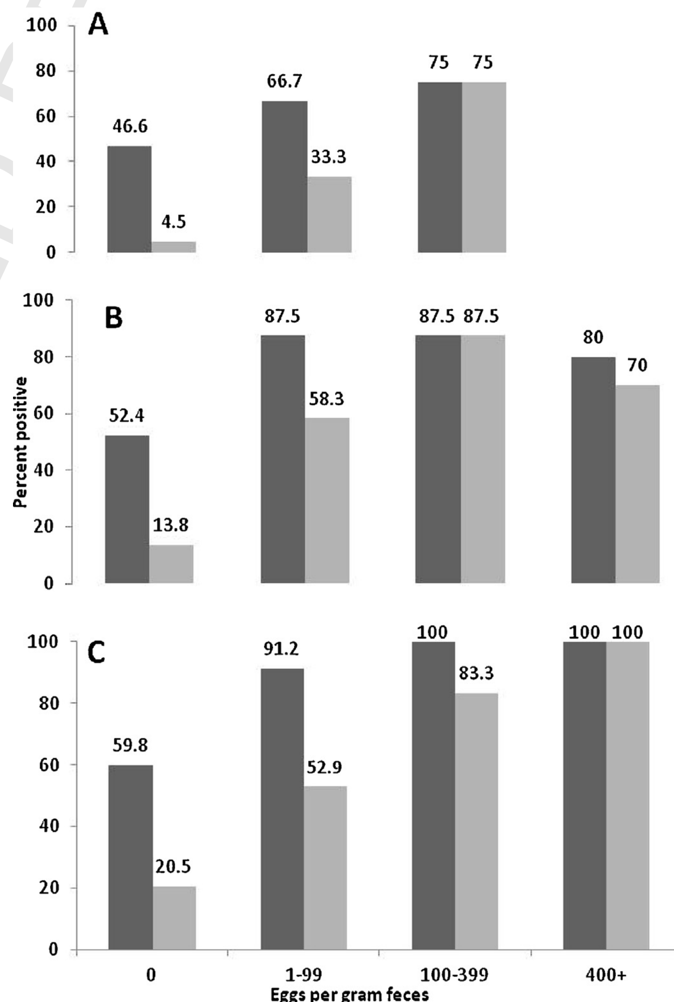

Fig. 2. Correlation between Kato Katz and CCA assays for *S. mansoni* diagnosis. Figure showing correlation between the prevalence of *S. mansoni* using CCAs trace positive (dark and light bars represents CCA1 and CCA2 respectively) and intensity of infection is determined by six Kato-Katz stratified by setting. According to six Kato-Katz thick smear examinations, the prevalence of *S. mansoni* in setting A, B and C was 12.5%, 33.2% and 54.8% respectively.

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Table 2
Q3 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) between different techniques for the diagnosis of *S. mansoni* infections in different settings.

	Setting A (*n* = 100)				Setting B (*n* = 200)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Double KK as 'reference' diagnostic test								
CCA1 trace (+ve)	75 (35–97)	54 (44–65)	13 (4.–25)	96 (87–100)	87 (73–95)	47 (39–55)	32 (24–41)	92 (84–97)
CCA2 trace (+ve)	63 (25–92)	96 (89–99)	56 (21–86)	97 (90–99)	69 (53–82)	85 (79–90)	57 (43–71)	90 (84–95)
Quadruple KK as 'reference' diagnostic test								
Double KK	73 (39–94)	100 (96–100)	100 (63–100)	97 (91–99)	72 (59–83)	100 (97–100)	100 (92–100)	89 (83–94)
CCA1 trace (+ve)	73 (39–94.	55 (44–66)	17 (7–30)	94 (84–99)	85 (74–93)	51 (42–59)	43 (34–53)	89 (80–95)
CCA2 trace (+ve)	46 (17–77)	96 (89–99)	56 (21–86)	93 (86–98)	62 (49–74)	89 (82–94)	72 (58–83)	84 (77–90)
Six KK as 'reference' diagnostic test								
Double KK	67 (35–90)	100 (96–100)	100 (63–100)	96 (89–99)	68 (55–79)	100 (97–100)	100 (92–100)	86 (80–91)
Quadruple KK	92 (62–100)	100 (96–100)	100 (71–100)	99 (94–100)	95 (87–99)	100 (97–100)	100 (94–100)	98 (93–100)
CCA1 trace (+ve)	75 (43–95)	55 (44–67)	19 (9–33)	94 (83–99)	84 (72–92)	52 (43–61)	46 (37–56)	87 (77–93)
CCA2 trace (+ve)	42 (15–72)	95 (88–99)	56 (21–86)	92 (84–97)	61 (48–73)	92 (86–96)	79 (65–90)	83 (75–89)

	Setting C (*n* = 200)				All setting A,B,C (*n* = 500)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Double KK as 'reference' diagnostic test								
CCA1 trace (+ve)	96 (88–99)	41 (33–50)	48 (40–56)	95 (85–99)	91 (85–96)	47 (42–52)	36 (31–42)	94.1 (90–97)
CCA2 trace (+ve)	72 (60–82)	81 (73–87)	68 (56–78)	84 (76–90)	70 (62–78)	86 (82–90)	63 (54–71)	90 (87–93)
Quadruple KK as 'reference' diagnostic test								
Double KK	77 (67–85)	100 (97–100)	100 (95–100)	83 (76–89)	75 (68–81)	100 (99–100)	100 (97–100)	90 (85–92)
CCA1 trace (+ve)	94 (86–98)	45.7 (36–56)	60 (52–68)	89 (77–98)	89 (83–93)	50 (45–56)	47 (41–53)	90 (85–94)
CCA2 trace (+ve)	66 (56–76)	86 (78–92)	80 (70–89)	74 (66–82)	63 (55–71)	10 (86–93)	75 (67–82)	83 (79–87)
Six KK as 'reference' diagnostic test								
Double KK	68 (58–77)	100 (96–100)	100 (95–100)	72 (63–80)	68 (60–75)	100 (99–100)	100 (97–100)	84 (79–87)
Quadruple KK	87 (79–93)	100 (96–100)	100 (96–100)	87 (78–93)	90 (85–94)	100 (99–100)	100 (98–100)	95 (91–97)
CCA1 trace (+ve)	92 (85–97)	50.0 (39–61)	69 (61–77)	84 (71–93)	88 (82–93)	52 (46–58)	53 (47–58)	88 (82–92)
CCA2 trace (+ve)	60 (50–69.)	85 (75–92)	82 (72–90)	63 (54–72)	59 (51–66)	91 (87–94)	79 (71–86)	79 (74–83)

from 47% when no ova was observed to 67% in light infection and finally to 75% in moderate infections. Similar trends were observed in settings B and C.

3.7. Effects of STH infections on CCA assays

To assess the effect of STH infection, we compared prevalence of *S. mansoni* results as assessed by either CCA1 or CCA2 between STH positives vs. STH negatives. Only hookworms, *A. lumbricoides* and *T. trichiura* were considered in this study and the results are summarised in Table 4. In all *S. mansoni* endemic settings, no significant difference in the results by either version of CCA assay was observed based on STH status implying that STH had no impact on the diagnostic accuracy of any of the CCA assays (Table 4).

3.8. Discriminating power of CCA tests: ROC curves and AUC

The discriminating power of CCA test given by ROC and AUC of different CCA assays using two, four and six Kato Katz thick smears are presented in Fig. 3. This was determined after merging together data in all three settings. Considering CCA1, the discriminating power using double, quadruple and sextuple Kato-Katz smears was high (AUC = 0.7). Similarly, when CCA2 were considered, the

Table 3
Agreement between different diagnostic techniques in diagnosis of *S. mansoni* in different setting.

	Setting A		Setting B		Setting C		Overall	
	% agreement	k[a]	% agreement	k[a]	% agreement	k[a]	% agreement	k[a]
Double Kato-Katz as 'reference' diagnostic test								
CCA1 trace (+ve)	56	0.1	56	0.2	61	0.3	58	0.3
CCA1 trace (−ve)	82	0.3	78	0.5	77	0.5	79	0.5
CCA2 trace (+ve)	93	0.6	82	0.5	78	0.5	82	0.5
CCA2 trace (−ve)	95	0.6	84	0.5	77	0.4	83	0.5
Quadruple Kato-Katz as 'reference' diagnostic test								
Double KK	97	0.8	92	0.8	89	0.8	92	0.8
CCA1 trace (+ve)	57	0.1	61	0.3	68	0.4	63	0.3
CCA1 trace (−ve)	79	0.2	83	0.6	79	0.6	81	0.6
CCA2 trace (+ve)	90	0.5	81	0.5	77	0.5	81	0.6
CCA2 trace (−ve)	92	0.5	79	0.4	68	0.3	77	0.4
Six Kato-Katz as 'reference' diagnostic test								
Double KK	96	0.8	89	0.7	82	0.7	88	0.7
Quadruple KK	99	1	98	1	93	0.9	96	0.9
CCA1 trace (+ve)	57	0.1	63	0.3	73	0.4	66	0.4
CCA1 trace (−ve)	77	0.2	84	0.6	75	0.5	79	0.5
CCA2 trace (+ve)	89	0.4	82	0.6	71	0.4	79	0.5
CCA2 trace (−ve)	91	0.4	79	0.5	61	0.3	74	0.4

[a] $k < 0$ indicating no agreement, $k = 0 < 0.2$ indicating poor agreement, $k = 0.2 < 0.4$ indicating fair agreement, $k = 0.4 < 0.6$ indicating moderate agreement, $k = 0.6 < 0.8$ indicating substantial agreement, and $k = 0.8\text{-}1$indicating almost perfect agreement.

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Table 4
Correlation between the prevalence of *S. mansoni* using CCA tests and STH infections by setting.

S. mansoni diagnosis	STH infections			
	No		Yes	
	Number examined	% Positive of *S. mansoni* 95% CI	Number examined	% Positive of *S. mansoni* 95% CI
Considering CCA1 trace as positive				
Setting A	82	51 (40–62)	18	33 (9–58)
Setting B	168	61 (53–68)	32	59 (41–77)
Setting C	96	67 (57–76)	104	77 (69–85)
All	346	60 (55–65)	154	68 (61–76)
Considering CCA2 trace as positive				
Setting A	82	22 (13–31)	18	11 (01–22)
Setting B	168	34 (27–41)	32	21.9 (07–37)
Setting C	96	34 (25–44)	104	51 (41–61)
All	346	31 (26–36)	154	40 (32–48)

Number of children provided one stool sample ($n = 500$ in all setting) in each of study setting stratified by STH infection status (No and Yes). Observed prevalence of *S. mansoni* (95% CI) as detected by different CCA assays when traces are considered positive in each of the setting.

discriminating power was still high compared to different reference tests, with values of AUC (0.75–0.78) > 0.70.

3.9. Comparisons of day-to-day Kato-Katz results and CCA tests

The results showed that 21/176 (12%) pupils were positive by Kato-Katz thick smear method but not by either of the CCA assays. Of the 21 children, 18 (86%) were positive for only a single stool and a single slide and had light infections, the overall EPG being 19. Surprisingly however, 3/21 children shed eggs in all their 3 consecutive stool samples, all their 6 stool smears were positive and had heavy egg count, the overall EPG being 901.3.

4. Discussion

Earlier studies had indicated that reagent strip/dipstick based assays are highly sensitive and specific in detecting CCA in urine in active *S. mansoni*-infected individuals (Legesse and Erko, 2008). Indeed, in all endemic settings, results from this study showed higher *S. mansoni* prevalence scores by CCA1 assay than those obtained when double, quadruple or sextuple Kato-Katz thick smears were applied. Our results were consistent with recently published data collected elsewhere as part of this multi-country study (Colley et al., 2013; Coulibaly et al., 2011; Shane et al., 2011; Tchuem Tchuente et al., 2012). Generally, the prevalence of *S. mansoni* increased with increasing number of examined Kato-Katz smears from two through four to six smears and this pattern was maintained in all epidemiological settings.

Similar to earlier studies (Colley et al., 2013; Tchuem Tchuente et al., 2012), our results showed that CCA sensitivity increased with increasing prevalence of infection. These results seem to indicate that CCA1 is an appropriate test for diagnosis of *S. mansoni* particularly in moderate and high transmission zones, as earlier reported (Tchuem Tchuente et al., 2012), but may also have some utility in low transmission settings. In addition, the strong association between the intensity of the colouration of the CCA1 band and *S. mansoni* infection intensities according to EPGs by the Kato-Katz method in our study is in line with previous reports (Coulibaly et al., 2011; Standley et al., 2010a). Our results further showed that when trace scores were considered negative, the CCA1 sensitivity drastically declined and it was no longer a suitable alternative test to the Kato-Katz method. Hence, trace CCA results should always be

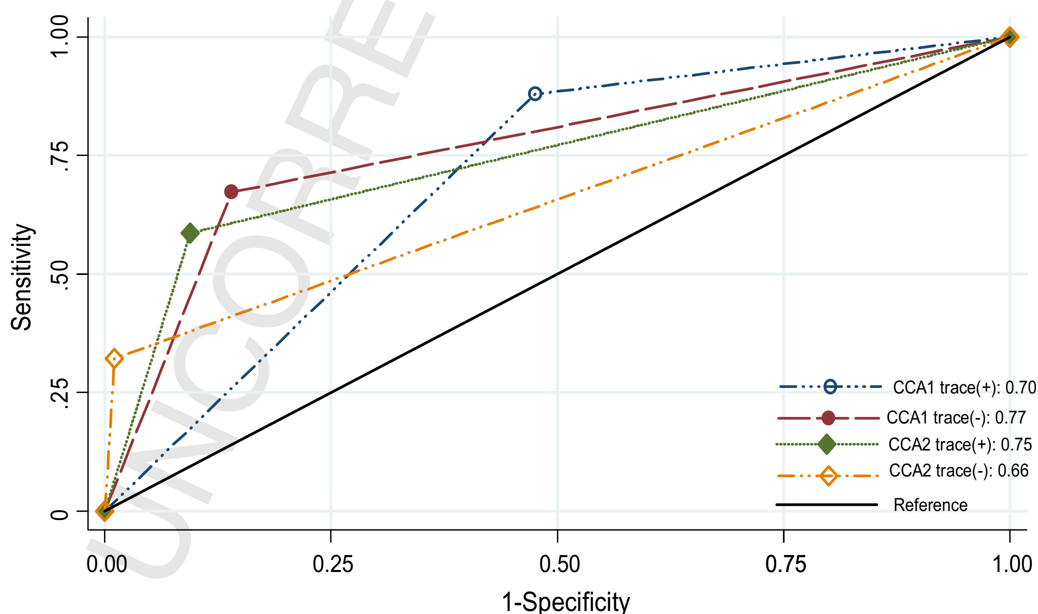

Fig. 3. Receiver operating characteristic (ROC) curves and area under curve (AUC) of CCA results using Kato Katz as reference tests. The ROC and AUC of CCA1and CCA2 when trace are considered positive and negative regardless of setting are presented using six Kato-Katz thick smears as reference tests.

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considered positive. A similar conclusion had been reached through earlier research (van Dam et al., 2004).

One may suggest that the low specificity of the CCA test could imply that the test produces many false *S. mansoni* positives. However, this suggestion is not likely to be true because even with the Kato-Katz method, the prevalence of infection continues to increase with increasing number of smears (de Vlas and Gryseels, 1992) implying that the true underlying prevalence of infection is higher than the cumulative results of the 6 Kato-Katz slides. Indeed in Cameroon, it was shown that a single urine CCA1 assay produced similar or even higher prevalence results than those obtained with 9 Kato-Katz slides (Tchuem Tchuente et al., 2012), implying that the 6 smears are an imperfect gold standard test.

However, it has also been reported that CCA1 can produce false *S. mansoni* negatives (Colley et al., 2013; Stothard et al., 2006). Indeed our results showed that 21 (12%) pupils were positive by Kato-Katz method but negative by the CCA assay. Eighteen of these children (86%) had very light infections and had eggs in only one slide. However, 3 children had heavy egg load (mean EPG of 903) and all their 6 slides were *S. mansoni* positive. One may argue that like the Kato-Katz, CCA test is inappropriate in very light infections as earlier reported (Tchuem Tchuente et al., 2012), and t there might be day to day variation in urine CCA as has been suggested (Disch et al., 1997). It was surprising however that the CCA assay also failed to detect the intense *S. mansoni* infections in the three children who shed eggs daily. The sharing of stool or urine samples by study participants (Colley et al., 2013) or mixing of data during data entry or physiological processes unique to these children might explain these discordant results. Our results also included a large number of CCA positive cases who were negative by the Kato-Katz test but such results were expected since the CCA assay is a more sensitive tool (Colley et al., 2013; Stothard et al., 2006; Standley et al., 2010a; Ashton et al., 2011; Sousa-Figueiredo et al., 2013).

In conformity with many previous studies (Tchuem Tchuente et al., 2012; Shane et al., 2011), our results indicated that the diagnostic accuracy of the CCA assays were not influenced by the presence or absence of STH. Even in an area known to be non-endemic to *S. mansoni* in Ethiopia (Legesse and Erko, 2008), rigorous CCA examinations in a large sample of children produced negative CCA results further confirming that indeed the CCA test cannot produce false results due to the presence of STH infections.

One of our primary objectives in this study was to asses if the diagnostic accuracy of the experimental CCA2 assay was better than that of the CCA1 test. In all endemic settings, the prevalence of *S. mansoni* decreased drastically whenever the CCA2 assay was applied and the results were worse when traces were regarded as negative. Thus even though the CCA2 specificity was high, the diagnostic performance of this version of CCA is poor and cannot be recommended as an alternative test to the Kato-Katz method. However, when the CCA2 performance was evaluated against fewer Kato-Katz slides, its sensitivity was consistently better than that of the highly applauded double Kato-Katz test (WHO, 2012) implying that the CCA2 assay would be a better mapping tool for *S. mansoni* than the single stool double slide Kato-Katz method.

One of the major limitations of the CCA1 test is its high cost currently estimated at US $ 1.75 per test (Colley et al., 2013). If the production of the alternative CCA2 is cheaper, it can be a fair alternative to the Kato-Katz method which is largely claimed to be cheap. However, if operational costs are considered, the Kato Katz method is not a good value and it may even be more expensive than the CCA test (Colley et al., 2013). To supplement this claim, compound microscopes are expensive currently at US$ 1500 each (Colley et al., 2013). Furthermore, training of adequate numbers of competent technicians is also costly, whereas accurate application of CCA tests requires minimal training.

From our experience, we have observed that the main expenditure during schistosomiasis prevalence surveys is due to personnel costs. To examine 50 school age children using the double Kato-Katz test, a single technician, a driver, a teacher and an auxiliary worker are required to complete the job in a single day, yet if CCA method is applied, the same team excluding an auxiliary worker can survey 4–6 schools each day. Night allowance of a technician in Uganda is currently at US $ 43. A driver and an auxiliary worker each costs US $ 23 while participation of a teacher costs US $ 2.3. Other Kato-Katz costs include reagents such as malachite green and glycerine, the Kato Katz kit and consumables including stool containers, detergents, soap, adsorbent paper, gloves and insecticide all estimated to cost US $10 per school. Thus excluding the cost of a microscope, the overall cost per school using the Kato-Katz method is estimated at US $102 vs. US $23 for the CCA assay. The cost using the Kato Katz method is even higher if 3 stool samples collected over 3 days are examined to improve the accuracy of the test (de Vlas et al., 1993; Utzinger et al., 2001; Engels et al., 1997).

This rapid, highly sensitive and convenient CCA test became available as early as 2003 (Stothard et al., 2006), yet to date, it has not yet been applied for large scale mapping in any national control programme (Colley et al., 2013). However, with the recent resolution on schistosomiasis elimination by the 65th World Health Assembly (WHO, 2012), the urine POC CCA test is a convenient and attractive tool to replace the Kato Katz test. Its potential benefits could include substantial savings in time for specimen collection and processing with the results available within 5–20 min. In agreement with past studies (Tchuem Tchuente et al., 2012), there is urgent need to review the current WHO MDA guidelines to suit results obtained using CCA test before it is recommended for wide use.

Resources for mapping are now available from various donors particularly DFID and USAID and WHO has set a timeline for completing the mapping exercise in all remaining endemic countries by the end of 2013. Thus as the next crucial step on this road map towards elimination of schistosomiasis, it is important to urgently evaluate all potential candidate tests for their diagnostic accuracy.

Conflict of interest

None of the authors have a conflict of interest.

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