Tropical Medicine and International Health doi:10.1111/tmi.12117

VOLUME 18 NO 8 PP 1029–1035 AUGUST 2013

Evaluation of urine-circulating cathodic antigen (Urine-CCA) cassette test for the detection of *Schistosoma mansoni* infection in areas of moderate prevalence in Ethiopia

Berhanu Erko, Girmay Medhin, Tilahun Teklehaymanot, Abraham Degarege and Mengistu Legesse

Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Abstract

OBJECTIVE To evaluate the diagnostic performance of antigen detecting urine-CCA cassette test for the detection of *Schistosoma mansoni* infection in areas of moderate prevalence in Ethiopia.

METHODS Stool specimens were collected from 620 schoolchildren on three consecutive days. The samples were microscopically examined using double Kato slides; midstream urine specimens were also collected for three consecutive days and tested for *S. mansoni*. The sensitivity of the urine-CCA cassette test was determined using combined results of six Kato–Katz thick smears and three urine-CCA cassette tests as gold standard. The specificity of the urine-CCA cassette test was evaluated in an area where schistosomiasis is not endemic.

RESULTS Prevalence of *S. mansoni* infection as determined by single urine-CCA cassette test was 65.9%, by single Kato–Katz smear 37.3% and by six Kato–Katz thick smears 53.1% ($P < 0.001$). A single urine-CCA cassette test was significantly ($P < 0.001$) more sensitive (89.1%), had a lower negative predictive value (78.2%), was more accurate (92.6%) and agreed better with the gold standard ($k = 0.83$) than one or six Kato–Katz thick smears. However, both the Kato–Katz and urine-CCA cassette test showed 100% specificity in endemic settings.

CONCLUSIONS In moderate and high prevalence areas, urine-CCA cassette test is more sensitive than the Kato–Katz method and can be used for screening and mapping of *S. mansoni* infection.

keywords *Schistosomiasis mansoni*, rapid diagnosis, urine-circulating cathodic antigen cassette, Ethiopia

Introduction

Schistosomiasis is a chronic, parasitic disease caused by blood flukes of the genus *Schistosoma*. It ranks second behind malaria among the parasitic diseases in terms of socio-economic and public health importance in tropical and subtropical areas (Waknine-Grinberg *et al.* 2010). The current strategy for the control of schistosomiasis is the reduction of morbidity through regular treatment of all people in at-risk groups with praziquantel (WHO 2012). Proper diagnosis of *Schistosoma* infection enables accurate prevalence estimation for deciding about the necessity of regular drug administration to communities at risk of infection with the parasite, evaluation of drug efficacy and control programs and better patient management (Sturrock 2001).

Currently, Kato–Katz is the method recommended for diagnosis of *S. mansoni* infection by WHO for its high specificity, handiness and cost effectiveness (WHO 2002). However, the Kato–Katz technique has limited sensitivity in situations where light intensity infection is common (Berhe *et al.* 2004), and the procedure is also relatively time consuming, requiring well-trained personnel and heavy equipment (Speich *et al.* 2010; Shane *et al.* 2011). Although its sensitivity can be improved by increasing the number of stool samples tested, getting stool specimens from individuals on different days would be challenging (Barreto *et al.* 1990). In addition, Kato–Katz method exposes laboratory workers to potentially harmful fresh stools which can contain infectious agents (Shane *et al.* 2011).

The concern that conventional Kato–Katz method misses *Schistosoma mansoni* infection in a proportion of young people in areas of low prevalence and intensity (Allam *et al.* 2009) has a very important implication, particularly when it is used in monitoring the impact of preventive chemotherapy. The few missed light infections constitute a potential source of continued schistosomiasis transmission.

The single urine-CCA cassette test is more sensitive than Kato–Katz thick smear (Standley *et al.* 2010;

Coulibaly *et al.* 2011; Shane *et al.* 2011; Tchuem Tchuenté *et al.* 2012), but some investigators reported that urine-CCA cassette test has several limitations such as low sensitivity in cases of light intensity of infection (Stothard *et al.* 2006). Furthermore, high genetic variability of *S. mansoni* and its potential impact on cathodic antigens might contribute to differences in test diagnostic performance (Stothard *et al.* 2009). Hence, it is crucial to evaluate commercially available urine-CCA tests in different endemic regions (Stothard 2009) and at levels of endemicity.

The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) funded evaluation of the performance of point-of-care circulating cathodic antigen (POC/CCA) cassette test among school children in five African countries including Ethiopia (http://score.uga.edu/). This multi-country study has recommended the POC-urine-CCA cassette test for community-level prevalence mapping of *S. mansoni* infection (Colley *et al.*2013). As a part of this multicountry study, we evaluated the diagnostic performance and accuracy of the urine-CCA cassette test for the diagnosis of *S. mansoni* infection among school children in two areas of moderate prevalence in Ethiopia.

Materials and methods

Study area and population

A cross-sectional study involving 320 school children from Jiga and 300 from Harbu Elementary Schools (ages ranged between 8 and 12) was conducted between 2010 and 2011. Jiga is a town located 375 km north-west of Addis Ababa on the road to Bahir Dar city. Jiga has been known to be endemic for *Schistosomiasis mansoni*, and recent survey showed that the prevalence of the disease was 34% (Teklemariam 2007). Harbu Town is located some 355 km to the north-east of Addis Ababa and is also known to be endemic for *Schistosomiasis mansoni* (Erko *et al.* 2002). The specificity of the test was evaluated in school children from Gedo Elementary School in western Ethiopia, an area where human schistosomiasis is not endemic.

Stool collection and examination

Stool samples of about 2 g were collected from each child for three consecutive days. The specimens were processed using a 41.7 mg Kato–Katz template (WHO 1991), preparing duplicate slides per stool sample. The slides were quantitatively examined for intestinal schistosomiasis independently by two experienced laboratory technicians. The third technician checked the reading of the two technicians for discrepancy, and in case of discrepancies, the slides were re-examined. The results were recorded as eggs count per slide and multiplied by 24 to convert them into eggs per gram (epg) of stool before analysis.

Urine specimen collection and testing

Midstream urine specimens were collected for three consecutive days from the same children who provided stool specimens. The urine POC/CCA (point-of-care circulating cathodic antigen) cassette test was performed according to the protocol and procedures described by the manufacturer (http://www.rapid-diagnostics.com/downloads/RMD%20Pamphlet%202011_06_13%20.pdf).

Ethical considerations

The study was approved by the Institutional Review Board (IRB) of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. Permission to conduct the study was also obtained from local government administration and school directors. The children were included in the study after obtaining informed consent from their parents/guardians. The objective of the study was also clearly explained to the children, and their assent was sought. Children found positive for intestinal schistosomiasis, and soil-transmitted helminthiasis were treated with a single dose of PZQ (40 mg/kg body weight) and a single dose of albendazole (400 mg) after the third day of specimen collection and examination.

Data analysis

Data were analysed using STATA (version 10). As there has been no perfect diagnostic technique for *S. mansoni* infection, we considered combined results of six Kato–Katz and triple urine-CCA cassette tests as a 'gold standard' in determining the sensitivities, specificities and negative predictive value (NPV) and accuracy of both urine-CCA cassette test and Kato–Katz thick smear. Accordingly, results which were positive in six Kato–Katz and/or triple urine-CCA cassette were considered as 'true positive'. Results based on the six Kato–Katz method alone were also used as 'gold' standard to evaluate the performance of CCA cassette. Agreement of both Kato–Katz and urine-CCA cassette test with the gold standard was evaluated using Kappa statistics. Results of the urine-CCA cassette test as a categorical variable (weak and strong intensity of the urine-CCA cassette test band colour) and egg counts as a continuous

explanatory variable were used in a logistic regression model to evaluate the association of *S. mansoni* intensity based on egg count and the intensity of the urine-CCA cassette test band colour. The urine-CCA cassette test band colour with very weak intensity results was considered as negative during analysis. McNemar's chi-squared test was used to evaluate differences in the prevalence of *S. mansoni* infection based on six Kato–Katz and one or triple urine-CCA cassette test. The arithmetic mean egg count was calculated as the average egg counts (epg) of six Kato–Katz thick smears, and classes of intensity of *S. mansoni* were determined as light (1–99 EPG), moderate (100–399 EPG) and heavy ($\geq$400 EPG). Ninety-five percentage of confidence interval (CI) was estimated for each diagnostic performance parameter. Values were considered significant when $P < 0.05$.

Results

Prevalence and intensity of *Schistosoma mansoni* infection

Prevalence of *S. mansoni* infection as determined by Kato–Katz method and urine-CCA cassette test is summarised in Tables 1 and 2. A total of 620 school children (mean age in years $\pm$ SD = 9.9 $\pm$ 1.3 females = 306, males = 314) were examined for *S. mansoni* infection, of whom 73.4% tested positive based on combined six Kato–Katz method and three urine-CCA cassette tests. Prevalence of *S. mansoni* infection was significantly higher as diagnosed using six Kato–Katz thick smears than by double or single Kato–Katz thick smears ($P < 0.001$). Similarly, there was an increase in the prevalence of *S. mansoni* infection as diagnosed using three urine-CCA cassette tests compared to one urine-CCA cassette test. When trace positive results were included in the analysis, the prevalence of *S. mansoni* infection was 70.8% based on single urine-CCA cassette test and 81.1% based on triple urine-CCA cassette tests.

Table 1 Prevalence of *Schistosoma mansoni* infection as diagnosed by Kato–Katz and urine-CCA cassette test among 620 school children in Tikur Wuha and Harbu Elementary Schools

Method	Number positive	Per cent positive
Single Kato–Katz	231	37.3
Double Kato–Katz	267	43.1
Six Kato–Katz	329	53.1
Single CCA	409	65.9
Triple CCA	439	70.8

Of 329 children found positive for *S. mansoni* [mean egg per gram (epg) = 110.7 $\pm$ 187.8] using six Kato–Katz thick smears, 229 (69.6%), 80 (24.6%) and 18 (5.8%) showed light, moderate and heavy classes of intensity, respectively. Of 439 children found positive for *S. mansoni* using triple urine-CCA cassette test, 360 showed strong intensity of the urine-CCA cassette test band colour, while 79 showed weak intensity. In all children who had moderate and heavy intensity of infection, the urine-CCA cassette test band colour was intense. The odds of strong intensity of the urine-CCA cassette test

Table 2 Prevalence of *Schistosoma mansoni* infection among school children by age groups and gender among Tikur Wuha and Harbu Elementary Schools, Ethiopia, based on six Kato–Katz and triplicate urine-CCA cassette test

	Number examined	Number positive (%) based on six Kato–Katz	Number positive (%) based on three CCA	*P* value	Number positive (%) based on combined six Kato–Katz and three CCA
Age (years)					
5–9	237	124 (52.3)	166 (70.0)	$P < 0.001$	173 (73.0)
10–12	383	205 (53.5)	273 (71.2)	$P < 0.001$	282 (73.6)
P*		0.770	0.742		
Sex					
Male	314	179 (58.9)	235 (77.3)	$P < 0.001$	239 (78.6)
Female	306	150 (47.5)	204 (64.6)	$P < 0.001$	216 (68.4)
P*		0.004	$P < 0.00$		
School					
Jiga	320	158 (49.4)	200 (62.5)	$P < 0.001$	205 (64.1)
Harbu	300	171 (57.0)	239 (79.7)	$P < 0.001$	250 (83.3)
Total	620	329 (53.1)	439 (70.8)	$P < 0.001$	455 (73.4)
P*		0.057	$P < 0.001$		

*P value = two sample test of proportion (McNemar's chi-squared test).

band colour increased with an increase in 1 epg (OR = 1.07, 95% CI = 1.04, 1.10). There was also a significant increase in the prevalence of *S. mansoni* infection as determined using single or triple urine-CCA cassette tests with an increase in 1 epg (OR = 1.06, 95% CI = 1.04, 1.08). Prevalence of *S. mansoni* infection based on single urine-CCA cassette test was 90.8% (206/227), 97.5% (78/80) and 100% (18/18) among children who had light, moderate and heavy intensity of infection, respectively based on six Kato–Katz thick smear. Among children who had light (227), moderate (80) and heavy (18) *S. mansoni* infections based on estimates from six Kato–Katz thick smears, 93.4%, 98.8% and 100% were positive for the parasite based on triple urine-CCA cassette tests, respectively.

Results of stool examinations of school children in Gedo town showed that the prevalence of any soil-transmitted helminthiasis (ascariasis and trichuriasis only) was 50%. Of 100 urine specimens tested using urine-CCA cassette test, only one child tested positive (1%) for *S. mansoni*, the intensity of urine-CCA cassette colour band being weak. The specificity of the urine-CCA cassette test based on this result was 99%.

Information about performance of both the Kato–Katz method and urine-CCA cassette test in diagnosing *S. mansoni* infection using combined six Kato–Katz and three urine-CCA cassette test as a 'gold standard' is summarised in Tables 3 and 4. Six Kato–Katz thick smears showed higher sensitivity, NPV and accuracy compared to double and single Kato–Katz methods. The three urine-CCA cassette tests showed higher sensitivity, negative predictive value and accuracy for detecting *S. mansoni* infection than single urine-CCA cassette test, and the single urine-CCA cassette test was better in its performance than the six Kato–Katz thick smears. Both Kato–Katz (based on single, double or six slides) and urine-CCA cassette test (single or triple) showed 100% specificity for diagnosing *S. mansoni* infection. The agreement between Kato–Katz/urine-CCA cassette test and the 'gold standard' for detecting *S. mansoni* infection was higher in the case of six Kato–Katz than double or single Kato–Katz and three urine-CCA cassette tests than single urine-CCA cassette test. Single urine-CCA cassette test (weak and strong positive) agreed much more than six Kato–Katz with the 'gold standard' in diagnosing *S. mansoni* infection. Of 291 children tested negative for *S. mansoni* egg by six Kato–Katz method, 126 were found positive for *S. mansoni* antigen by urine-CCA cassette test (95 showed strong band colour and 31 showed weak band colour intensity). On the other hand, only 16 children (of 181) who tested negative by triple urine-CCA cassette test were diagnosed as positive by six Kato–Katz method.

Discussion

This study was conducted to evaluate the performance and accuracy of urine-CCA cassette test for diagnosis of *Schistosoma mansoni* infection in relation to combined results of six Kato–Katz thick smears and three urine-CCA cassette tests as a 'gold standard'. The prevalence of *S. mansoni* infection as determined by single or triple urine-CCA cassette test was greater than the prevalence determined by single and six Kato–Katz thick smears. According to WHO (2002), the outlined treatment strategies to guide preventive chemotherapy of schistosomiasis is based on prevalence of the diseases. In accordance with these strategies, the urine-CCA cassette test seems to be more appropriate candidate tool for screening *S. mansoni* infection for preventive chemotherapy in endemic communities. The observation of lower prevalence of *S. mansoni* infection among the study participants when determined even by six Kato–Katz compared to single urine-CCA test could be attributed to variations in the number of eggs released in stool (Berhe *et al.* 2004). Had the number of stool samples examined in the present study increased to 9, as in the study carried out by Coulibaly *et al.* (2011), comparable results would have been obtained for Kato–Katz and urine-CCA cassette test. In the present study, single urine-CCA cassette test also showed greater sensitivity, negative predictive value and accuracy in detecting *S. mansoni* infection than six, double or single Kato–Katz thick smears. Sensitivity, NPV and accuracy of both the Kato method and urine-CCA cassette rose with an increase in the number of samples tested.

Previous studies also reported better performance of single urine-CCA cassette test for diagnosis of *S. mansoni* infection compared to single or more Kato–Katz thick smears (Standley *et al.* 2010; Coulibaly *et al.* 2011; Shane *et al.* 2011; Stothard *et al.* 2011; Tchuem Tchuenté *et al.* 2012; Colley *et al.*2013). These previous studies have also indicated the possible use of urine-CCA cassette test for diagnosis and mapping of *S. mansoni* infection. Findings from the present study can also corroborate the previous recommendation that the urine-CCA cassette test (batch 32727) can be used for the mapping of schistosomiasis and guiding preventive chemotherapy.

Both Kato–Katz method and urine-CCA cassette test are claimed to be insensitive to assess low intensity of *S. mansoni* infection (Berhe *et al.* 2004; Stothard *et al.* 2006). The present study corroborates this previous claim to some degree. Of 291 children found negative for *S. mansoni* egg by six Kato–Katz thick smears, 126 tested positive for *S. mansoni* antigen by urine-CCA cassette

Table 3 Performance of Kato–Katz method and urine-CCA cassette test in diagnosing *Schistosoma mansoni* infection among school children in Tikur Wuha and Harbu Elementary Schools, Ethiopia

	Six Kato–Katz as gold standard		Combined six Kato–Katz and three urine-CCA cassette test as gold standard	
Methods	Positive	Negative	Positive	Negative
Single Kato–Katz				
Positive	231	0	231	0
Negative	98	291	224	165
Sensitivity (95% CI)	70.2 (65.3, 75.1)		50.8 (46.2, 55.3)	
Specificity (95% CI)	100		100	
NPV(95% CI)	74.8 (70.4, 79.1)		42.4 (37.5, 47.3)	
Accuracy (95% CI)	84.2 (81.1, 87.3)		63.9 (60.1, 67.7)	
Kappa (95% CI)	0.69 ($P < 0.001$) (0.65, 0.70)		0.35 (0.32, 0.36)	
Double Kato–Katz				
Positive	267	0	267	0
Negative	62	291	188	165
Sensitivity (95% CI)	81.2 (76.9, 85.4)		58.7 (54.2, 63.2)	
Specificity (95% CI)	100		100	
NPV (95% CI)	82.4 (78.4, 86.3)		46.7 (40.5, 52.8)	
Accuracy (95% CI)	90 (87.6, 92.3)		69.7 (66.1, 73.3)	
Kappa (95% CI)	0.80 (0.77, 0.81)		0.43 (0.40, 0.44)	
Six Kato–Katz				
Positive	N/A	N/A	329	0
Negative	N/A	N/A	126	165
Sensitivity (95% CI)	N/A	N/A	72.3 (68.2, 76.4)	
Specificity (95% CI)	N/A	N/A	100 (0.973, 1.00)	
NPV (95% CI)	N/A	N/A	56.7 (51.0, 62.4)	
Accuracy (95% CI)	N/A	N/A	79.7 (76.5, 82.9)	
Kappa (95% CI)	N/A	N/A	0.58 (0.55, 0.59)	
Single urine-CCA				
Positive	306	103	409	0
Negative	23	188	46	165
Sensitivity (95% CI)	93.0 (90.2, 95.7)		89.9 (87.1, 92.6)	
Specificity (95% CI)	64.6 (59.1, 70.1)		100	
NPV (95% CI)	89.1 (84.9, 93.3)		78.2 (72.6, 83.7)	
Accuracy (95% CI)	79.7 (76.5, 82.9)		92.6 (90.5, 94.6)	
Kappa (95% CI)	0.59 (0.52, 0.63)		0.83 (0.79, 0.84)	
Triple urine-CCA				
Positive	313	126	439	0
Negative	16	165	16	165
Sensitivity (95% CI)	95.1 (92.8, 97.4)		96.5 (94.8, 98.1)	
Specificity (95% CI)	56.7 (51.0, 62.4)		100 (0.96, 1.00)	
NPV (95% CI)	91.2 (87.1, 95.3)		91.2 (87.1, 95.3)	
Accuracy (95% CI)	77.1 (73.8, 80.4)		97.4 (96.1, 98.7)	
Kappa (95% CI)	0.53 (0.47, 0.57)		0.94 (0.89, 0.95)	

NPV, Negative predictive value; CCA, circulating cathodic antigen.

test, of whom 95 showed strong band colour and 31 showed weak band colour intensity. On the other hand, only 16 children (of 181) who tested negative by triple urine-CCA cassette test were diagnosed as positive by six Kato–Katz thick smears. From among 16 children who tested positive for *S. mansoni* egg but negative for *S. mansoni* antigen, 15 had light infection intensity, and only one child had moderate infection intensity of *S. mansoni*. Hence, it is preferable to use a combination of both methods in areas of low *S. mansoni* infection prevalence.

Failure to detect eggs in stool by Kato method while children tested positive for *S. mansoni* by urine-CCA cassette test could be due to the reduction in the number of

Table 4 Performance of six Kato–Katz thick smear and triplicate urine-CCA cassette test in diagnosing *Schistosoma mansoni* infection by age and sex among school children in Tikur Wuha and Harbu Elementary Schools, Ethiopia

	Six Kato–Katz			Triplicate urine-CCA cassette test		
Variable	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)
Age						
5–9	71.7 (64.9, 78.4)	100	56.4 (47.6, 65.7)	95.9 (92.9, 98.8)	100	90.1 (83.1, 97.0)
10–12	72.7 (67.5, 77.8)	100	56.7 (49.4, 63.9)	96.8 (9, 4.7, 98.8)	100	91.8 (86.7, 96.9)
Sex						
Female	69.4 (63.2, 75.5)	100	60.2 (52.7, 67.6)	94.4 (91.3, 97.4)	100	89.3 (83.6, 95.0)
Male	74.9 (69.4, 80.3)	100	52.0 (43.2, 60.7)	98.3 (96.7, 99.9)		94.2 (88.7, 99.7)
School						
Jiga	77.1 (71.3, 82.8)	100	70.9 (63.9, 77.8)	97.6 (95.5, 99.6)	100	95.8 (92.2, 99.3)
Harbu	68.4 (62.6, 74.1)	100	38.7 (30.3, 47.1)	95.6 (93.0, 98.1)	100	81.9 (72.2, 91.5)

NB, Combined six Kato–Katz and triplicate urine-CCA cassette test used as gold standard; NPV, negative predictive value

egg-laying females releasing eggs below the detection level by Kato–Katz method (Berhe *et al.* 2004) or the presence of light, single sex or pre-patent infections (Mutapi *et al.* 2011). Detection of *S. mansoni* by urine-CCA cassette test could also be false positive results that may arise due to non-specific cross reaction of the urine-CCA test with Lewis-X tri-saccharide epitopes of inflammatory biomarkers (van Dam *et al.* 1996). Nevertheless, the present study showed that of 100 urine specimens tested using urine-CCA cassette test in an area non-endemic for schistosomiasis, only one child tested positive for *S. mansoni*, giving the specificity of 99%. On the other hand, the reasons why urine-CCA cassette test revealed negative results when eggs are present in stool as confirmed by the Kato–Katz method is not clear.

The urine-CCA cassette test band colour was intense in all children who had moderate and heavy intensity of infection suggesting strong colour reaction in heavy intensity of *S. mansoni* infection. Previous studies also reported increased positive rate and band colour strength of urine-CCA cassette test with an increase in egg counts (Stothard *et al.* 2006; Legesse & Erko 2008; Standley *et al.* 2010; Coulibaly *et al.* 2011; Shane *et al.* 2011; Tchuem Tchuenté *et al.* 2012). This suggests that the urine-CCA cassette test could be used as an alternative to indicate the intensity of *S. mansoni* infection. However, the urine-CCA cassette test needs to be formulated for semi-quantitative detection of *S. mansoni* infection with limits representing classes of intensity.

The characteristic that circulating cathodic antigens (CCAs) are released by viable adult worms that are absent after treatment and the resolution of infection (van Lieshout *et al.* 1993; Agnew *et al.* 1995) would make the urine-CCA cassette test suitable for the evaluation of drug efficacy trials. However, the effectiveness of the urine-CCA cassette test needs to be evaluated after drug treatment before using it for large scale follow-up studies.

In conclusion, the urine-CCA cassette test showed much better sensitivity, NPV and accuracy compared to Kato–Katz in diagnosing *S. mansoni* infection in moderate and heavy intensity of infection. The test could be used for screening of *S. mansoni* infection in moderate and high prevalence area to guide preventive chemotherapy if its present cost of about 1.98 USD/casette test were substantially reduced. Evaluation of the performance of the test in areas of low prevalence and intensity of infection is indicated.

Acknowledgements

The study was funded by the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE). We gratefully acknowledge the technical assistance by the technical staff of Medical Parasitology Research Unit of Aklilu Lemma Institute of Pathobiology. We are also grateful to district education and health bureaus, school directors and teachers, parents/guardians and school children for their cooperation.

References

Agnew A, Fulford AJ, De Jonge N *et al.* (1995) The relationship between worm burden and levels of a circulating antigen (CAA) of five species of Schistosoma in mice. *Parasitology* **111** (Pt 1), 67–76.

Allam AF, Kader O, Zaki A, Shehab AY & Farag HF (2009) Assessing the marginal error in diagnosis and cure of

Schistosoma mansoni in areas of low endemicity using Percoll and PCR techniques. *Tropical Medicine & International Health* **14**, 316–321.

Barreto ML, Smith DH & Sleigh AC (1990) Implication of faecal egg count variation when using the Kato—Katz method to assess *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 554–555.

Berhe N, Medhin G, Erko B *et al.* (2004) Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. *Acta Tropica* **92**, 205–212.

Colley DG, Binder S, Campbell C *et al.* (2013) A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **88**, 426–432.

Coulibaly JT, Knopp S, N'Guessan NA *et al.* (2011) Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire. *PLOS Neglected Tropical Diseases* **5**, e1384.

van Dam GJ, Claas FHJ, Yazdanbakhsh M *et al.* (1996) *Schistosoma mansoni* excretory circulating cathodic antigen shares Lewis-x epitopes with a human granulocyte surface antigen and evokes host antibodies mediating complement-dependent lysis of granulocytes. *Blood* **88**, 4246–4251.

Erko B, Abebe A, Berhe N *et al.* (2002) Control of *Schistosomiasis mansoni* by local production and use of the soapberry Endod (*Phytolacca dodecandra*) in Wollo, northeastern Ethiopia: post-intervention prevalence. *East African Medical Journal* **79**, 198–201.

Legesse M & Erko B (2008) Field-based evaluation of a reagent strip test for diagnosis of *Schistosomiasis mansoni* by detecting circulating cathodic antigen (CCA) in urine in low endemic area in Ethiopia. *Parasite* **15**, 151–155.

van Lieshout L, De Jonge N, Mansour MM, Bassily S, Krijger FW & Deelder AM (1993) Circulating cathodic antigen levels in serum and urine of schistosomiasis patients before and after chemotherapy with praziquantel. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 311–312.

Mutapi F, Rujeni N, Bourke C *et al.* (2011) *Schistosoma haematobium* treatment in 1-5 year old children: safety and efficacy of the antihelminthic drug praziquantel. *PLOS Neglected Tropical Diseases* **5**, e1143.

Shane HL, Verani JR, Abudho B *et al.* (2011) Evaluation of Urine CCA Assays for detection of *Schistosoma mansoni* infection in Western Kenya. *PLOS Neglected Tropical Diseases* **5**, e951.

Speich B, Knopp S, Mohammed KA *et al.* (2010) Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. *Parasite & Vectors* **3**, 71.

Standley C, Lwambo N, Lange C, Kariuki H, Adriko M & Stothard J (2010) Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria. *Parasites & Vectors* **3**, 7.

Stothard JR (2009) Improving control of African schistosomiasis: towards effective use of rapid diagnostic tests within an appropriate disease surveillance model. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 325–332.

Stothard JR, Kabatereine NB, Tukahebwa EM *et al.* (2006) Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. *Acta Tropica* **97**, 219–228.

Stothard JR, Sousa-Figueiredoa JC, Standleya C *et al.* (2009) An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta Tropica* **111**, 64–70.

Stothard JR, Sousa-Figuereido JC, Betson M *et al.* (2011) *Schistosoma mansoni* infections in young children: when are schistosome antigens in urine, eggs in stool and antibodies to eggs first detectable? *PLOS Neglected Tropical Diseases* **5**, e938.

Sturrock RF (2001) Schistosomiasis epidemiology and control: how did we get here and where should we go? *Memorias Do Instituto Oswaldo Cruz* **96**(Suppl.), 17–27.

Tchuem Tchuenté LA, Kueté Fouodo CJ, Kamwa Ngassam RI *et al.* (2012) Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of *Schistosoma mansoni* infection in Cameroon. *PLOS Neglected Tropical Diseases* **6**, e1758.

Teklemariam Z (2007) Current status of *Schistosomiasis mansoni* in Jiga and Bahir Dar, Northwestern Ethiopia. Master thesis, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa.

Waknine-Grinberg JH, Gold D, Ohayon A *et al.* (2010) *Schistosoma mansoni* infection reduces the incidence of murine cerebral malaria. *Malaria Journal* **9**, 5.

WHO (1991) Basic Laboratory Methods in Medical Parasitology. WHO, Geneva.

WHO (2002) The Prevention and Control of Schistosomiasis and Soil Transmitted Helminthiasis. World Health Organization, Geneva, Technical Report Series No. 912.

WHO (2012) *Schistosomiais*. Fact sheet No. 115. Available at: http://www.who.int/mediacentre/factsheets/fs115/en/index.html (accessed 1 October 2012).

Corresponding Author **Berhanu Erko**, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia. E-mail: berhanu.erko@aau.edu.et