

Intestinal schistosomiasis in pre school-aged children of Lake Albert, Uganda: diagnostic accuracy of a rapid test for detection of anti-schistosome antibodies

Emily M. Dawson^{a,*}, José C. Sousa-Figueiredo^{b,c}, Narcis B. Kabatereine^d,
Michael J. Doenhoff^a and J. Russell Stothard^c

^aSchool of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK; ^bDepartment of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 6HT, UK; ^cParasitology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK; ^dVector Control Division, Ministry of Health, P.O. Box 1661, Kampala, Uganda

*Corresponding author: Tel: +44 7535 671404; E-mail: plxed2@nottingham.ac.uk

Received 28 March 2013; revised 29 May 2013; accepted 5 July 2013

Background: A sensitive and reliable rapid diagnostic test (RDT) which should have comparable diagnostic performance against reference host serological methods is urgently needed for use in point-of-care (POC) diagnosis of intestinal schistosomiasis in pre school-aged children.

Methods: The diagnostic accuracy of a RDT incorporating *Schistosoma mansoni* cercarial transformation fluid (SmCTF) for anti-schistosome antibody detection was evaluated with serum samples from a cohort of children from Uganda: 42 children aged under the age of 3 years and 40 children aged between 3 and 5 years. The infection status of these children had been previously determined by inspection of quadruplicate Kato-Katz faecal smears, a single urine circulating cathodic antigen (CCA) dipstick and antibody titres to *S. mansoni* soluble egg antigen (SmSEA) with a commercially available ELISA.

Results: Upon comparison with quadruplicate Kato-Katz the sensitivity and specificity of the RDT were 75.7% and 31.1%, respectively. When using the SmSEA-ELISA as an alternate reference test, the RDT achieved 81.3% sensitivity and 61.1% specificity. Sensitivity and specificity compared to the urine-CCA test was 74.5% and 32.3% respectively. Sensitivity differed significantly according to age group.

Conclusions: The performance of the RDT within this study appeared favourable when compared with the currently-available SmSEA-ELISA. Looking to the future a serological POC test would be particularly promising for use in disease mapping in younger children especially in guiding administration of praziquantel treatment in selective treatment settings.

Keywords: Schistosomiasis, Serodiagnosis, Cercariae, Mapping, Sensitivity, Specificity

Introduction

According to WHO guidelines, field-diagnosis of schistosomiasis is based upon parasitological methods:¹ the Kato-Katz faecal smear (41.7 mg) for intestinal disease² and 10 ml urine filtration for urogenital schistosomiasis.³ Typically, 50 children from each of 5–10 schools in a given area are tested by these methods for presence of schistosome eggs, prevalence is estimated and the strategy for preventive chemotherapy using praziquantel decided upon this estimate.⁴ These egg-detection methods are, however, relatively slow and laborious, and trained laboratory technicians are required to examine slides microscopically. They are also known to be insensitive, with infections often missed, especially when

infections are light or recently acquired.^{5–7} Whilst microscopy is appropriate for detection of infection in school-aged children, serological methods of detection are better for identifying infections in younger children, i.e., infants and pre school-aged children.⁸

With the expansion and acceleration of schistosomiasis control programmes in order to meet the WHO's 2020 goals for neglected tropical diseases,⁹ more accurate and rapid methods of diagnosis are needed for mapping prevalence prior to initiation of preventive chemotherapy, particularly in areas of low-endemicity or infections in pre school-aged children. A rapid diagnostic test (RDT) or point-of-care (POC) assay would be most useful for mapping on a large-scale. A POC assay based on detection of circulating cathodic antigen (CCA) has been shown to be useful for the diagnosis of

intestinal schistosomiasis,^{10–16} but less so for urogenital schistosomiasis.^{15,17}

Detection of host antibodies to schistosome antigens is recognised as the most sensitive method of diagnosis, but is limited to ELISA formats and not yet to POC settings. A novel *Schistosoma mansoni* cercarial antigen preparation – cercarial transformation fluid (SmCTF) – has recently been shown to perform equivalently to schistosome soluble egg antigens (SEA) in an ELISA format for the detection of anti-*S. mansoni*, *S. haematobium* and *S. japonicum* antibodies.^{18–20} Following these results, SmCTF (BioGlab Ltd., Nottingham, UK) has been incorporated into a RDT format for detection of anti-schistosome antibodies in human fingerprick blood (Vision Biotech, Cape Town, South Africa). Recent work in Côte d'Ivoire shows promising results for the SmCTF-RDT for detection of intestinal and urogenital schistosomiasis at the POC.²¹ Here we further evaluated the diagnostic accuracy of the SmCTF-RDT for the diagnosis of *S. mansoni* infections in a subset of pre school-aged children from Lake Albert, Uganda.

Materials and methods

Fieldwork

The study described here was part of a wider study carried out during May/June 2011 in the villages of Walukuba and Piida (Buliisa District) on the shoreline of Lake Albert, Uganda.²²

Kato-Katz faecal smears

Duplicate Kato-Katz thick smears (41.7mg) were made from each of the first and second day stool samples.²³ The four faecal smears were each examined under the microscope at x100, schistosome eggs were counted and the result expressed as eggs per gram (epg) of faeces. Infection intensity was classified as light (1–100 epg), medium (101–399 epg) and heavy (≥ 400 epg) infections according to WHO guidelines.⁴ Prevalence of intestinal worms was also investigated as part of the larger project.

POC-CCA cassettes

Using a commercially available lateral flow immuno-chromatographic urine dipstick (Rapid Medical Diagnostics, Pretoria, South Africa) 50 μ l of urine was tested for the presence of schistosome CCA. Test results were graded as either trace, single (+), double (++) and triple (+++) positive reactions. When creating binomial variables to depict infection status according to CCA, trace results were considered to be positive.²⁴

S. mansoni soluble egg antigens-ELISA

A commercially available ELISA kit (Scimedx, Denville NJ, USA) was used to test for host antibodies (IgG/M) to *S. mansoni* soluble egg antigens (SmSEA) according to the manufacturer's instructions. Briefly, approximately 75 μ l of finger-prick blood was taken from each child and sera harvested therefrom diluted 1:40 with specimen dilution buffer before loading a total of 100 μ l into each ELISA microwell. ELISA reactions were read using a portable ELISA plate reader (Labtech International Ltd., East Sussex, UK) to quantify optical density (OD) at 450nm. Samples with OD₄₅₀ readings >0.2 were considered positive.

SmCTF-RDTs

SmCTF was prepared as described earlier²⁰ by BioGlab Ltd, freeze-dried and sent to Vision Biotech for incorporation into the RDT format. A total of 88 SmCTF-RDTs were used retrospectively with randomly selected sera from the above-described study at the Liverpool School of Tropical Medicine. Ten μ l of sera was added to the sample well of the RDT, followed by a few drops of buffer. After 15 minutes results were read independently by three different readers. Readers of the SmCTF-RDTs did not know the results from other diagnostic tests used previously. Test results were scored according to the colour intensity of the SmCTF band on the RDT: negative, trace, single (+), double (++) and triple (+++) positive reactions. Where scores were discordant between readers, tests were read again and the results discussed until a final decision was made. When creating binomial variables to depict infection status according to the SmCTF-RDT, trace results were considered to be positive.

Inclusion criteria

To be included in the study subjects had to meet the following criteria: aged below 6 years at recruitment; no history of previous treatment for schistosomiasis; and had provided one sera, one urine and two stool samples. For retrospective testing with the SmCTF-RDTs, 88 serum samples were randomly selected from the collection of available samples. However, only those pre school-aged children with complete datasets (i.e., results from four Kato-Katz thick smears, one SEA-ELISA, one POC-CCA and one SmCTF-RDT) were included in the final analysis: a roughly similar number of children aged under 3 years and between 3 and 5 years were selected and results from these two age groups were considered separately on the basis that infections in children under the age of 3 years were likely to be less prevalent and lighter than those aged 3–5 years.²⁵ Thus 46 children in the study were aged under 3 years and 42 children were aged between 3 and 5 years (see Supplementary Figure 1: STARD flow-chart).

Statistical analysis

In the field, data were collected from each individual using pre-format data sheets, which were then entered into an electronic format using Microsoft Excel[®] spreadsheet software (Microsoft Corp., Redmond, WA, USA). Figures and statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, USA) and Stata version 12 (StataCorp LP, College Station, TX, USA). Univariate logistic regression analyses were performed to look for associations between test results and population demographics.

Diagnostic accuracy was assessed using sensitivity and specificity, negative predictive values and positive predictive values, which were calculated as described previously.²⁶ Results from quadruplicate Kato-Katz were used as the primary reference standard for diagnostic comparisons, but given the shortcomings of stool tests the SmSEA-ELISA was used as a second alternative reference test.¹⁰ The SmSEA-ELISA has previously been shown to be a potentially powerful tool for diagnosis of schistosomiasis in preschool-age children.²⁵ Diagnostic test results across the different age groups were compared using Fisher's exact test.

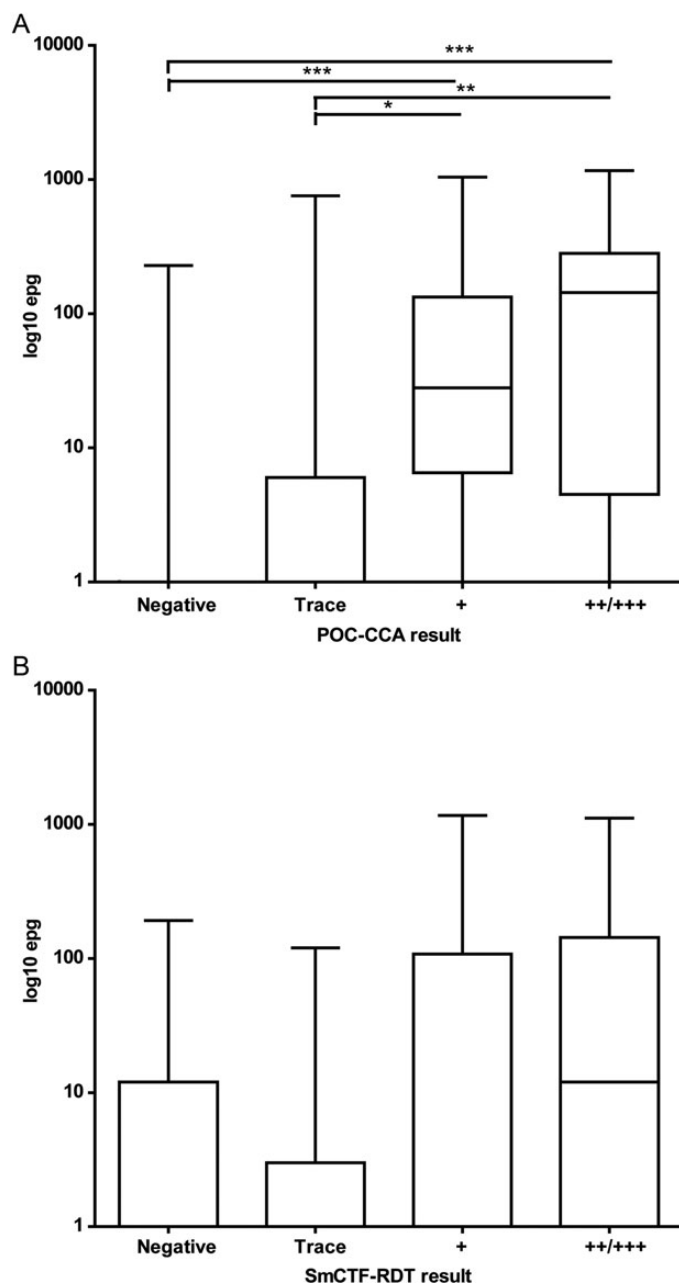


Figure 1. Box-and-whisker plot of faecal egg count against the reaction intensity of (A) the point-of-care circulating cathodic antigen (POC-CCA) cassette; (B) the *Schistosoma mansoni* cercarial transformation fluid-rapid diagnostic test (SmCTF-RDT).

Differences between test reaction intensities according to *S. mansoni* epg or SmSEA-ELISA OD₄₅₀ were analysed using Kruskal-Wallis tests with Dunn's multiple comparison.

Results

Table 1 shows the characteristics of both the under 3 year-old and 3–5 year-old age groups, as well as overall. No associations were found between schistosome infection status (as determined by

Table 1. Characteristics of study participants

	Under 3 years (n = 42)	3–5 years (n = 40)	All children (n = 82)
Mean age in years	1.4	4.3	2.8
Female:male ratio	0.5:1	1.22:1	0.78:1
Intestinal helminths			
Number of <i>Ascaris lumbricoides</i> infections	0	0	0
Number of <i>Trichuris trichuria</i> infections	2	0	2
Number of hookworm infections	0	1	1
Quadruplicate Kato-Katz			
Total number of positives	13	24	37
Mean epg of positives	66	249	185
Light (1–100 epg)	9	13	22
Medium (101–399 epg)	4	6	10
Heavy (400+ epg)	0	5	5
SmSEA-ELISA			
Total number of positives	28	36	64
Strong positives (>2.0 OD/450nm)	5	10	15
POC-CCA cassette			
Total number of positives ^a	20	31	51
Trace	7	10	17
+	7	13	20
++	0	1	1
+++	6	7	13
SmCTF-RDT			
Total number of positives ^a	24	35	59
Trace	4	5	9
+	12	14	26
++	5	11	16
+++	3	5	8

Epg: eggs per gram of faeces.

^aTest results were graded as either trace, single (+), double (++) and triple (+++) positive reactions.

any of the diagnostic tests employed) and age, sex, presence of other helminths or infection status of the mother in either age group.

Diagnostic performance for point-of-care

The diagnostic performance of each test employed in this study compared to either quadruplicate Kato-Katz faecal smears or the SmSEA-ELISA as the reference standard is presented in Table 2.

In the under 3 year-old group there was little agreement between the results from the different diagnostic tests. Agreement between the SmCTF-RDT and four Kato-Katz slides was the poorest of all in this age group (53.9% sensitivity and 41.4% specificity). Sensitivity was however equivalent to that achieved by one Kato-Katz slide. Compared with the SmSEA-ELISA, the SmCTF-RDT

Table 2. The diagnostic performance of each test employed in this study

Studied population	Diagnostic parameter	Quadruplicate Kato–Katz as the reference standard				
		One Kato–Katz	Two Kato–Katz	SmSEA–ELISA	POC–CCA	SmCTF–RDT
Under 3 year olds	Sensitivity (95% CI)	53.9 (25.1–80.8)	69.2 (38.6–90.9)	76.9 (46.2–95.0)	69.2 (38.6–90.9)	53.9 (25.1–80.8)
	Specificity (95% CI)	100 (88.1–100)	100 (88.1–100)	37.9 (20.7–57.7)	62.1 (42.3–79.3)	41.4 (23.5–61.1)
	PPV (95% CI)	100 (59.0–100)	100 (66.4–100)	35.7 (18.6–55.9)	45.0 (23.1–68.5)	29.2 (12.6–51.1)
	NPV (95% CI)	82.9 (66.4–93.4)	87.9 (71.8–96.6)	78.6 (49.2–95.3)	81.8 (59.7–94.8)	66.7 (41.0–86.7)
3–5 year olds	Sensitivity (95% CI)	66.7 (44.7–84.4)	75.0 (53.3–90.2)	87.5 (67.6–97.3)	95.8 (78.9–99.9)	87.5 (67.6–97.3)
	Specificity (95% CI)	100 (79.4–100)	100 (79.4–100)	6.3 (0.2–30.2)	50.0 (24.7–75.4)	12.5 (1.6–38.4)
	PPV (95% CI)	100 (79.4–100)	100 (81.5–100)	58.3 (40.8–74.5)	74.2 (55.4–88.1)	60.0 (42.1–76.1)
	NPV (95% CI)	66.7 (44.7–84.4)	72.7 (49.8–89.3)	25.0 (0.6–80.6)	88.9 (51.8–99.7)	40.0 (5.3–85.3)
Studied population	Diagnostic parameter	SmSEA–ELISA as the reference standard				
		One Kato–Katz	Two Kato–Katz	Four Kato–Katz	POC–CCA	SmCTF–RDT
Under 3 year olds	Sensitivity (95% CI)	25.0 (10.7–44.9)	32.1 (15.9–52.4)	35.7 (18.6–55.9)	53.6 (33.9–72.5)	64.3 (44.1–81.4)
	Specificity (95% CI)	100 (76.8–100)	100 (76.8–100)	78.6 (49.2–95.3)	64.3 (35.1–87.2)	57.1 (28.9–82.3)
	PPV (95% CI)	100 (59.0–100)	100 (66.4–100)	76.9 (46.2–95.0)	75.0 (50.9–91.4)	75.0 (53.3–90.2)
	NPV (95% CI)	40.0 (23.9–57.9)	42.4 (25.5–60.8)	37.9 (20.7–57.7)	40.9 (20.7–63.7)	44.4 (21.5–69.2)
3–5 year olds	Sensitivity (95% CI)	36.1 (20.8–53.8)	41.7 (25.5–59.2)	58.3 (40.8–74.5)	75.0 (57.8–87.9)	94.4 (81.3–99.3)
	Specificity (95% CI)	25.0 (0.6–80.6)	25.0 (0.6–80.6)	25.0 (0.6–80.6)	0 (0–60.2)	75.0 (19.4–99.4)
	PPV (95% CI)	81.3 (54.4–96.0)	83.3 (58.6–96.4)	87.5 (67.6–97.3)	87.1 (70.2–96.4)	97.1 (85.1–99.9)
	NPV (95% CI)	4.2 (0.1–21.1)	4.5 (0.1–22.8)	6.3 (0.2–30.2)	0 (0–33.6)	60.0 (14.7–94.7)

NPV: negative predictive value; POC-CCA: point-of-care circulating cathodic antigen; PPV: positive predictive value; SmCTF-RDT: *S. mansoni* cercarial transformation fluid-rapid diagnostic test; SmSEA-ELISA: *Schistosoma mansoni* soluble egg antigen-ELISA.

showed the highest sensitivity at 64.3% in the under 3 year-old group and specificity was 57.1%. The POC-CCA test showed 53.6% sensitivity and 64.3% specificity. Relative to SmSEA-ELISA sensitivity of the Kato-Katz technique was lower than both the POC-CCA cassette and the SmCTF-RDT (25%, 32.1% and 35.7% for one, two and four Kato-Katz slides respectively; please refer to Table 2 for 95% CIs).

The sensitivity of the SmCTF-RDT was much higher in the 3–5 year-old age group than in the under 3 year-old group when using either four Kato-Katz or the SmSEA-ELISA as the reference standard ($p = 0.0425$ and $p = 0.0031$ respectively). Sensitivity increased to 87.5% and 94.4% in the 3–5 year-olds using quadruplicate Kato-Katz or the SmSEA-ELISA as the reference standard respectively. Sensitivity of the RDT was thus comparable to that achieved by the SmSEA-ELISA. All other diagnostic tests employed also showed a better sensitivity when using either quadruplicate Kato-Katz or the SmSEA-ELISA as the reference standard in the 3–5 year-old age group compared with under 3 year-olds. However only the sensitivity of the POC-CCA compared to quadruplicate Kato-Katz showed a significantly higher difference in the older age group ($p = 0.042$). Specificity of both antibody-detection assays compared to quadruplicate Kato-Katz was poor in this age group. That of the SmSEA-ELISA was significantly less than seen in the under 3 year-old group ($p = 0.033$). The POC-CCA test had a 50% rate of false-positive results. It was difficult to calculate specificity of other diagnostic tests compared to the SmSEA-ELISA since there were only four children negative by this assay. It is worth noting that three of these were egg-positive by the Kato-Katz method.

Across both age groups combined, the sensitivity and specificity of the RDT compared to quadruplicate Kato-Katz were 75.7% and 31.1%, respectively. When using the SmSEA-ELISA as an alternate reference test, the SmCTF-RDT achieved 81.3% sensitivity and 61.1% specificity. Sensitivity and specificity compared to the urine-CCA test was 74.5% and 32.3% respectively. If 'trace' SmCTF-RDT results are considered to be negative, sensitivity and specificity compared to quadruplicate Kato-Katz were 70.3% and 46.7% respectively. When using the SmSEA-ELISA as an alternate reference test, the RDT achieved 70.3% sensitivity and 72.2% specificity.

Figure 1A shows the relationship between *S. mansoni* epg and the reaction intensity of the POC-CCA test for all of the 82 children employed in this study. Figure 1B shows the relationship between epg and the reaction intensity of the SmCTF-RDT. Significant differences between groups were only found with the POC-CCA test, where a positive increasing association between the results of the two tests was observed.

Figure 2 shows the relationship between the anti-SmSEA ELISA OD₄₅₀ readings and infection intensity as determined by Kato-Katz (A), reaction intensity of the POC-CCA test (B) and reaction intensity of the SmCTF-RDT (C) for all of the 82 children employed in this study. Significant differences between groups were only found with the SmCTF-RDT, where a slight positive increasing association between the results of the two tests was observed.

Diagnostic performance as mapping tools

Estimated prevalence according to each diagnostic assay in the under 3 year-old and 3–5 year-old age groups is presented in

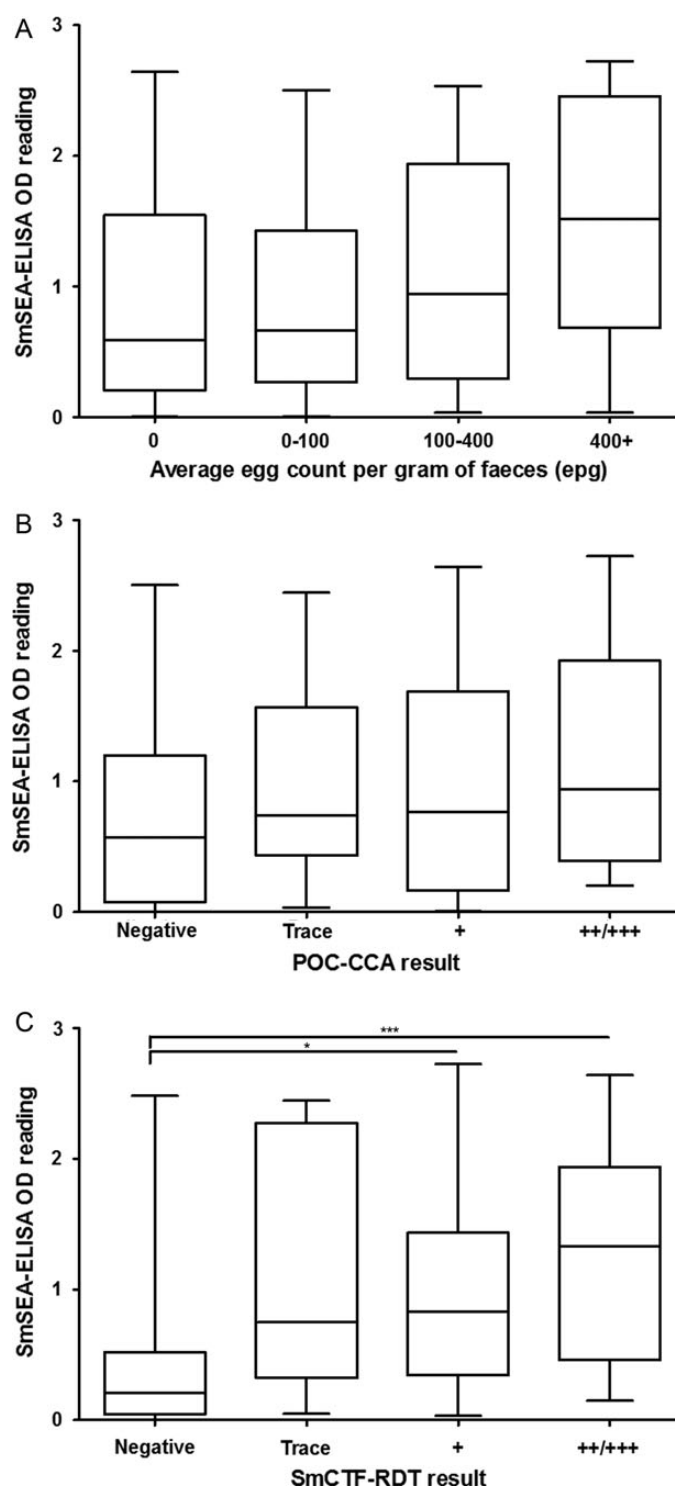


Figure 2. Box-and-whisker plot of the anti-*Schistosoma mansoni* soluble egg antigen (SmSEA) OD₄₅₀ reading in an ELISA against (A) infection intensity as determined by quadruplicate Kato-Katz, (B) the reaction intensity of the point-of care circulating cathodic antigen (POC-CCA) test, (C) the reaction intensity of the *S. mansoni* cercarial transformation fluid-rapid diagnostic test (SmCTF-RDT).

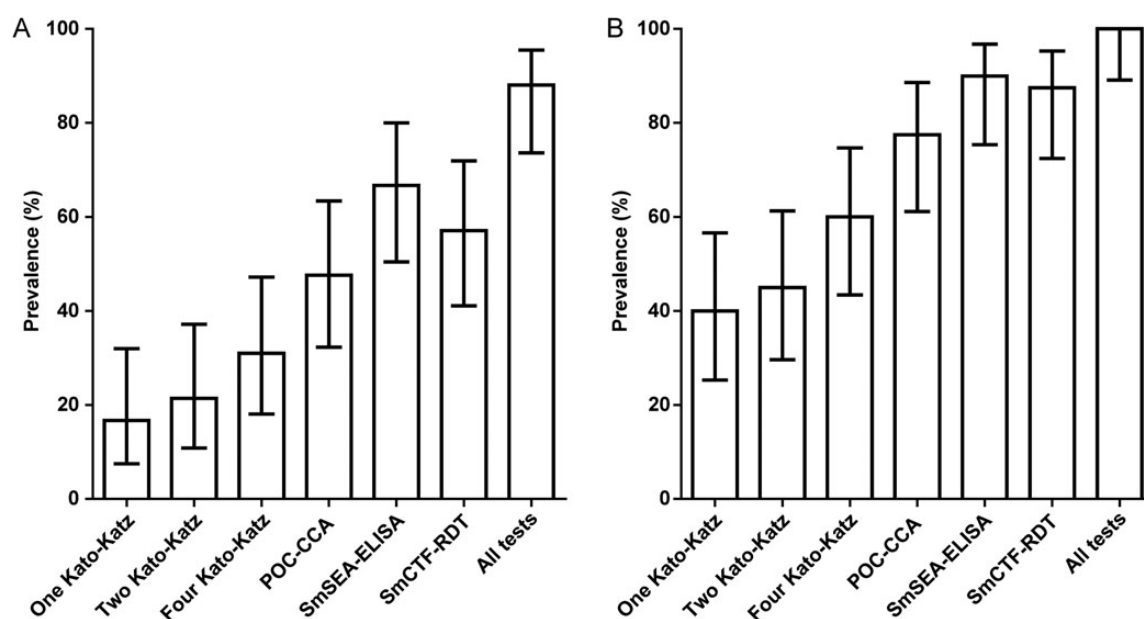


Figure 3. Prevalence as estimated by each diagnostic assay employed in a study of pre school-aged children of Lake Albert, Uganda (one, two and four Kato-Katz faecal smears; a point-of-care circulating cathodic antigen (POC-CCA) test; an ELISA incorporating *Schistosoma mansoni* soluble egg antigen (SmSEA); *S. mansoni* cercarial transformation fluid rapid diagnostic test (SmCTF-RDT)), as well as prevalence when the results of all diagnostic tests are taken to be true. (A) Estimated prevalence in a subset of 42 children under the age of 3 years. (B) Estimated prevalence in a subset of 40 children aged between 3 and 5 years.

Figures 3A and 3B respectively. As indicated in the figure when all diagnostic test results were considered to be true prevalence was estimated at 100% in the 3–5 year-old age group.

Supplementary Figure 2 shows the agreement between the different diagnostic assays used in this study by use of a Venn diagram. Agreement between the different tests was poorer in the under 3 year-old age group (Supplementary Figure 2A): only three children were positive by all diagnostic tests, 13 were positive by a total of three tests, 13 were positive by two tests, eight were positive by only one and five children were negative by all. Nineteen children were positive by all diagnostic tests in the 3–5 year-old age group (Supplementary Figure 2B), 10 were positive by three tests, nine positive by two tests and two positive by only one test.

Discussion

Antibody-detection methods of diagnosis have the merit of high sensitivity and remain the best available techniques for diagnosis in areas of low-endemicity.^{27,28} As infection intensities decrease with the success of schistosomiasis control programmes, it is thought that they will become increasingly useful.²⁸ They are already incorporated into the national control program for schistosomiasis japonica in the People's Republic of China,²⁹ and are the method of choice for diagnosis in travel medicine clinics, where often only light infections are encountered.³⁰ Antibody-detection methods have proven useful for diagnosis in endemic areas prior to initiation of control,¹⁵ and also for detection of infections in areas with very low transmission rates (such as those approaching elimination).^{31,32} Whilst sensitive and specific antibody-detection assays are available, they do not meet all of the remaining ASSURED criteria for diagnostic tests that will allow them to be

useful for testing at the community level in low-income countries, i.e., in terms of affordability, user-friendliness, rapidity and being equipment-free.³³

Here, we evaluated the diagnostic accuracy of a recently developed anti-schistosome antibody-detection RDT (SmCTF-RDT) in two subsets of pre school-aged children: under 3 year-olds and 3–5 year-olds. This assay is not yet commercially available but it is estimated that it will become available for less than US\$1/test, making it a cheaper alternative to both Kato-Katz and the POC-CCA cassette.^{16,34} The test works with whole blood as well as serum, obviating the need for a laboratory or even electricity, and therefore allows for diagnosis at the POC in remote locations. Although use of finger-prick blood is more invasive than use of alternatives, such as urine for the POC-CCA test, there are some advantages. A test that works on whole blood for example could be readily integrated into programmes already in place for malaria. Malaria RDTs are formulated for use with whole blood and are widely used for diagnosis in children under the age of five years.³⁵

It has recently been suggested that preschool-aged children should be included in treatment programmes for schistosomiasis.³⁶ Since these younger children have been shown to harbour mainly light infections,³⁷ which are more likely to be missed by insensitive parasitological techniques, they provide a good cohort for evaluation of new, potentially more sensitive, diagnostic tests. Quadruplicate Kato-Katz faecal smears were primarily used as the reference standard test in this study, but given this method's shortcomings with regard to sensitivity the SmSEA-ELISA was used as a second alternative reference standard.^{10,37}

When using either four Kato-Katz or the SmSEA-ELISA as the reference standard, the sensitivity of the SmCTF-RDT was poorer in the under 3 year-old age group (53.9% and 64.3% respectively).

Two Kato-Katz, the POC-CCA test and the SmSEA-ELISA all showed a higher sensitivity than the SmCTF-RDT compared to four Kato-Katz. However the RDT was the most sensitive assay compared to the SmSEA-ELISA. The sensitivity of the RDT compared with both Kato-Katz and the SmSEA-ELISA improved significantly in the 3–5 year-old group (87.5% and 94.4% respectively). Sensitivity compared with quadruplicate Kato-Katz was more than that achieved by duplicate Kato-Katz (75.0%), comparable to that achieved with the SmSEA-ELISA (87.5%), but not as high as that achieved by the POC-CCA test (95.8%).

In a previous study no significant difference has been seen between the diagnostic accuracy of the SmCTF-RDT in under 3 year-olds compared with older age groups.²¹ Further investigation into the reliability of the RDT for diagnosis in children under the age of three years is therefore warranted. Given the under 3 year-old group were harbouring low-intensity infections, further studies should also take place in areas of low transmission. Higher sensitivity and poorer specificity of the SmCTF-RDT observed here in the 3–5 year-old group when using parasitology as the reference standard is in agreement with a previous study.²¹ Whilst antibody-detection methods are often criticised for lacking specificity, at the pre-treatment stage this apparent lack of specificity may be attributed to the insensitivity of parasitological techniques, particularly in situations such as this where infections were mostly light.⁶ When another antibody-detection method (i.e., the SmSEA-ELISA) was used as the reference standard, specificity was found to be much higher, however only four children aged between 3 and 5 years were negative by the SmSEA-ELISA, so CIs are very wide.

It has been shown previously that the results of different diagnostic tests do not correlate well in young children.²¹ The lack of agreement between the different diagnostic tests employed in this study, particularly in the younger sub-set of preschool-aged children, further illustrates how difficult it is to obtain accurate estimates of prevalence of schistosome infections, particularly in lightly infected populations. The diagnosis of schistosomiasis in young children may be challenging, particularly for antibody-detection tests, for a number of reasons such as worm populations having been only recently established, the immune systems of children being immature and maybe still unable to distinguish between self and non-self epitopes, and the possibility that the child's immune response to schistosome antigens has not developed and/or has been maternally modulated.^{22,25} The lack of a 'gold-standard' diagnostic test for schistosomiasis makes the situation even more difficult.

As a mapping tool, the SmCTF-RDT showed very promising results here. Prevalence estimated by the RDT was similar to that estimated by the SmSEA-ELISA, and almost as high as all tests combined, particularly in the 3–5 year-old age group (Figure 3). Although the SmCTF-RDT was less sensitive and specific than the POC-CCA test using four Kato-Katz as the reference standard, it showed similar sensitivity and specificity to the SmSEA-ELISA. Given that the RDT has the ability to provide rapid results, is cheaper than current alternatives (<US\$1/test) and is relatively user-friendly, it may be useful for large-scale mapping of disease prevalence prior to treatment interventions.

Limitations of the study

Due to lack of resources, the sample size used for this study was smaller than ideal and CIs are therefore relatively wide (Table 1).

In future studies aimed at evaluating the performance of the SmCTF-RDT, larger sample sizes should be used to gain greater precision in the estimates of sensitivity, specificity, PPV and NPV. Future studies should also focus on older subjects with more established disease since to date all published studies have focussed on young children.²¹

Since it is known that schistosome antigens cross-react to some extent with antibodies against other infections^{18,19} studies should also be carried out in areas non-endemic for schistosomiasis to determine the specificity of the RDT for human schistosome infections.

It is also important to note that in this study 'trace' results were taken to be positive. If 'trace' was taken to be negative, less egg-positive and SmSEA-ELISA-positive cases would be identified as positive by the SmCTF-RDT, although there is an increase in specificity. Future studies employing more accurate reference standard tests should aim to resolve the issue of whether 'trace' results are taken to be positive or negative. In trials using European negative sera, however, there has never been a report of a 'trace' result (Dawson and Doenhoff, unpublished results).

Conclusions

For the diagnosis of *S. mansoni* infections, the SmCTF-RDT showed the most agreement with the SmSEA-ELISA in both under 3 year-olds and those aged 3–5 years compared with other diagnostic techniques (i.e., Kato-Katz and the POC-CCA test). Sensitivity was significantly worse in the under 3 year-old group, but was still comparable to that achieved by the current field standard test, i.e., one Kato-Katz faecal smear. Further investigation into the reliability of the RDT for diagnosis in children under the age of three and in areas of low transmission, using larger sample sizes, is therefore warranted. As a mapping tool, the sensitivity of the SmCTF-RDT appeared greater than that of duplicate Kato-Katz and similar to that given by an ELISA test for antibody and a POC test for detecting antigen. Specificity was similar to that achieved by the SmSEA-ELISA. The SmCTF-RDT may be a useful tool for affordable and rapid mapping of schistosome infections prior to intervention with chemotherapy.

Supplementary data

Supplementary data are available at Transactions Online (<http://trstmh.oxfordjournals.org/>).

Authors' contributions: JCSF, JRS and NBK carried out the original field trials and testing; EMD, JCSF, JRS and MJD conceived and designed the retrospective study; EMD, JRS and JCSF carried out the retrospective testing with SmCTF-RDTs; EMD analysed and interpreted the data. EMD drafted the manuscript; MJD, JRS and JCSF critically revised the manuscript. All authors read and approved the final manuscript. EMD is the guarantor of this paper.

Funding: Field-based sample collection was conducted under the SIMI project funded by the Wellcome Trust, a fund supporting JRS, JCSF and NBK.

SmCTF-RDTs were provided free-of-charge by Vision Biotech (Cape Town, South Africa). SmCTF incorporated in the RDTs was provided free-of-charge by BioGlab Ltd. (Nottingham, UK).

EMD is funded by the Sir Halley Stewart Trust (<http://www.sirhalleystewart.org.uk/>). The Trust had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

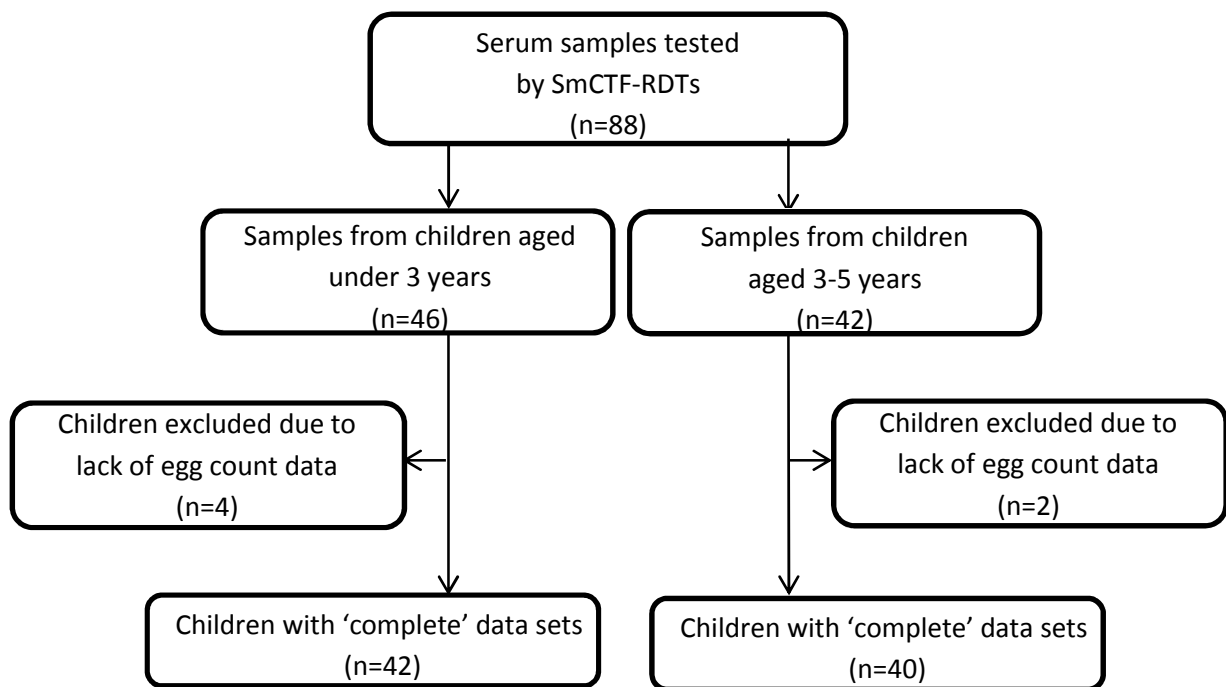
Competing interests: MJD is the owner of BioGlab Ltd.

Ethical approval: The London School of Hygiene and Tropical Medicine, London, UK (application no. LSHTM 5538.09) and the Ugandan National Council of Science and Technology approved this study. For more details please see Sousa-Figueiredo et al. (2013).¹⁰ All children received treatment for both schistosomiasis and intestinal worm infections (praziquantel and albendazole respectively).

References

- WHO. Preventive Chemotherapy in Human Helminthiasis. Geneva: World Health Organization; 2006.
- Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop* 1972;14:397–400.
- Mott KE. A reusable polyamide filter for diagnosis of *S. haematobium* infection by urine filtration. *B Soc Pathol Exot* 1983;76:101–4.
- WHO. Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis. Report of a WHO Expert Committee. Geneva: World Health Organization; 2002. Technical Report Series 912.
- De Vlas SJ, Gryseels B. Underestimation of *Schistosoma mansoni* prevalences. *Parasitol Today* 1992;8:274–7.
- Doenhoff MJ, Chiodini PL, Hamilton JV. Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? *Trends Parasitol* 2004;20:35–9.
- Feldmeier H, Poggensee G. Diagnostic techniques in schistosomiasis control: a review. *Acta Trop* 1993;52:205–20.
- Stothard JR, Sousa-Figueiredo JC, Betson M et al. Schistosomiasis in African infants and preschool children: let them now be treated! *Trends Parasitol* 2013;29:197–205.
- WHO. Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases: a Roadmap for Implementation. Geneva: World Health Organization; 2012.
- Sousa-Figueiredo JC, Betson M, Kabatereine NB, Stothard JR. The urine circulating cathodic antigen (CCA) dipstick: a valid substitute for microscopy for mapping and point-of-care diagnosis of intestinal schistosomiasis. *PLoS Negl Trop Dis* 2013;7:e2008.
- Standley CJ, Lwambo NJS, Lange CN et al. Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria. *Parasite Vector* 2010;3:7.
- Coulbaly JT, Knopp S, N'Guessan NA et al. Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire. *PLoS Negl Trop Dis* 2011;5:e1384.
- Tchuem Tchuenté L-A, Kueté Fouodo CJ, Kamwa Ngassam RI et al. Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of *Schistosoma mansoni* infection in Cameroon. *PLoS Negl Trop Dis* 2012;6:e1758.
- Shane HL, Verani JR, Abudho B et al. Evaluation of urine CCA assays for detection of *Schistosoma mansoni* infection in Western Kenya. *PLoS Negl Trop Dis* 2011;5:e951.
- Stothard JR, Kabatereine NB, Tukahebwa EM et al. Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. *Acta Trop* 2006;97:219–28.
- Colley DG, Binder S, Campbell C et al. A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. *Am J Trop Med Hyg* 2013;48:426–32.
- Stothard JR, Sousa-Figueiredo JC, Standley C et al. An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta Trop* 2009;111:64–70.
- Chand MA, Chiodini PL, Doenhoff MJ. Development of a new assay for the diagnosis of schistosomiasis, using cercarial antigens. *Trans R Soc Trop Med Hyg* 2010;104:255–8.
- El Aswad BEDW, Doenhoff MJ, El Hadidi AS et al. Use of recombinant calreticulin and cercarial transformation fluid (CTF) in the serodiagnosis of *Schistosoma mansoni*. *Immunobiology* 2011;216:379–85.
- Smith H, Doenhoff M, Aitken C et al. Comparison of *Schistosoma mansoni* soluble cercarial antigens and soluble egg antigens for serodiagnosing schistosome infections. *PLoS Negl Trop Dis* 2012;6:e1815.
- Coulbaly JT, N'Goran EK, Utzinger J et al. A new rapid diagnostic test for detection of anti-*Schistosoma mansoni* and anti-*Schistosoma haematobium* antibodies. *Parasit Vectors* 2013;6:29.
- Stothard JR, Sousa-Figueiredo JC, Betson M et al. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. *Parasitology* 2011;138:1593–606.
- Berhe N, Medhin G, Erko B et al. Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. *Acta Trop* 2004;92:205–12.
- Navaratnam AM, Mutumba-Nakalembe MJ, Stothard JR et al. Notes on the use of urine-CCA dipsticks for detection of intestinal schistosomiasis in preschool children. *Trans R Soc Trop Med Hyg* 2012;106:619–22.
- Stothard JR, Sousa-Figueiredo JC, Betson M et al. *Schistosoma mansoni* infections in young children: when are schistosome antigens in urine, eggs in stool and antibodies to eggs first detectable? *PLoS Negl Trop Dis* 2011;5:e938.
- Harper R, Reeves B. Reporting of precision of estimates for diagnostic accuracy: a review. *Brit Med J* 1999; 318: 1322–3.
- McCarthy JS, Lustigman S, Yang G-J et al. A research agenda for helminth diseases of humans: diagnostics for control and elimination programmes. *PLoS Negl Trop Dis* 2012;6:e1601.
- Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol* 2009;25:151–6.
- Zhu YC. Immunodiagnosis and its role in schistosomiasis control in China: a review. *Acta Trop* 2005;96:130–6.
- Clerinx J, Van Gompel A. Schistosomiasis in travellers and migrants. *Travel Med Infect Dis* 2011;9:6–24.
- WHO. Report. Inter-Country Meeting on Strategies to Eliminate Schistosomiasis from the Eastern Mediterranean Region. Muscat, Oman 6–8 November 2007. http://www.who.int/schistosomiasis/resources/EMRO_report_Schistosomiasis.pdf [accessed 4 March 2013].

- 32 Zhou XN, Xu J, Chen HG et al. Tools to support policy decisions related to treatment strategies and surveillance of schistosomiasis japonica towards elimination. *PLoS Negl Trop Dis* 2011;5:e1408.
- 33 Pai NP, Vадnais C, Denkinger C et al. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Med* 2012;9:e1001306.
- 34 Speich B, Knopp S, Mohammed KA et al. Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. *Parasite Vector* 2010;3:71.
- 35 WHO. Universal Access to Malaria Diagnostic Testing. An Operational Manual. Geneva: World Health Organization; 2011.
- 36 WHO. Report of a Meeting to Review the Results of Studies on the Treatment of Schistosomiasis in Preschool-Age Children. Geneva: World Health Organization; 2011.
- 37 Sousa-Figueiredo JC, Pleasant J, Day M et al. Treatment of intestinal schistosomiasis in Ugandan preschool children: best diagnosis, treatment efficacy and side-effects, and an extended praziquantel dosing pole. *Int Health* 2010;2:103–13.



Supplementary Figure 1. STARD Flowchart.

Children were considered to have 'complete' data sets if there was data from four Kato-Katz slides (two from each of two faecal samples), one SmSEA-ELISA, one POC-CCA cassette and one SmCTF-RDT.

SmSEA-ELISA: *Schistosoma mansoni* soluble egg antigen- ELISA; OD: optical density; POC-CCA: point-of-care circulating cathodic antigen; SmCTF-RDT: *S. mansoni* cercarial transformation fluid-rapid diagnostic test.