Trans R Soc Trop Med Hyg 2018; **00**: 1–7
doi:10.1093/trstmh/try014

ORIGINAL ARTICLE

Innovative methodology for point-of-care circulating cathodic antigen with rapid urine concentration for use in the field for detecting low *Schistosoma mansoni* infection and for control of cure with high accuracy

Rafaella F. Q. Grenfell[a,b,*], Diana Taboada[a], Lucélia A. Coutinho[a], Maria Luysa C. Pedrosa[a], Jéssica V. Assis[a], Matheus S. P. Oliveira[a], Renata R. Cruz[a], Aureo Almeida[a], Vanessa Silva-Moraes[a], Naftale Katz[a] and Paulo Marcos Z. Coelho[a]

[a]*Schistosomiasis Laboratory, Rene Rachou Research Center, Oswaldo Cruz Foundation (Fiocruz), Belo Horizonte, Minas Gerais, 30190-002, Brazil;* [b]*Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602-7387, USA*

*Corresponding author: Tel: +55 31 33497868; E-mail: rafaella@cpqrr.fiocruz.br

Received 20 July 2017; revised 27 December 2017; editorial decision 5 February 2018; accepted 5 February 2018

Background: Prior to eliminating schistosomiasis, efforts must address accurate and fast individual diagnosis. Diagnosis is still inaccurate by parasitological and point-of-care circulating cathodic antigen (POC-CCA) in areas of low endemicity.

Methods: Our group has optimized POC-CCA with a 30 min urine concentration step with no need for specialized technicians or equipment and with high accuracy. We evaluated this new method, called POC-CCA filter (FLT), in two Brazilian endemic areas with distinct profiles.

Results: At baseline, POC-CCA had a poor performance with several false results and undefined trace readings, revealing a prevalence rate of 10% against a rate of 23% for POC-CCA FLT, which was similar to the parasitological rates. Accuracy increased from as low as 0.36 to 0.96 after urine concentration in one area. POC-CCA properly diagnosed only half of the cases at three post-treatment time points, while POC-CCA FLT was able to diagnose 96, 83 and 100%, respectively.

Conclusions: The improvement of conventional POC methodology by a fast and simple urine concentration step provided not only an increase in its accuracy before and after praziquantel treatment, but also preserved its applicability in low-prevalence endemic areas, allowing the definition of trace readings as negative cases.

Keywords: Control of cure, Cross-reactivity, Fast urine concentration, POC-CCA improvement, Schistosomiasis mansoni, Trace readings definition

Introduction

The strategy for schistosomiasis control aims to prevent morbidity through regular treatment with praziquantel, which is currently the only drug available for infection and disease caused by *Schistosoma mansoni*.[1] The goal is for elimination of schistosomiasis in Brazil by 2020, according to the last World Health Organization (WHO) bulletin.[2] In order to reach this goal, better control strategies are needed for diagnosis, for subsequent treatment of positive individuals and for morbidity control. Individual diagnosis is often inaccurate when one to two slides of the Kato-Katz technique are performed as the reference methodology in low-endemicity areas, which is the current Brazilian endemic profile[3–7] according to the National Prevalence Survey (2011–2014).[8] The new alternative method, the point-of-care circulating cathodic antigen (POC-CCA), has presented satisfactory performance where moderate and high endemicity are found[9–16] but presents several problems in low-endemicity areas.[3,17]

A recent publication showed a typical low-prevalence area (1–80 epg) called Estreito de Miralta, in southeast Brazil, as a reference for POC-CCA evaluation.[17] The accuracy of the POC test was revealed to be poor and the results fragile when a significant number of trace readings was obtained (33/84 individuals).

© *The Author(s) 2018. Published by Oxford University Press on behalf of Royal Society of Tropical Medicine and Hygiene. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.*

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

Among these, 17 were negative and 16 were positive after an intensive investigation was performed by two parasitological techniques (1 g of faeces each, 24 Kato-Katz slides and 2 saline gradient analyses), revealing several false-negative results (25/84 individuals). Additionally, the POC-CCA sensitivity was extremely reduced, showing a 2% prevalence rate when one to two cassettes were used vs a 30% rate obtained by the Kato-Katz and saline gradient parasitological methods.

Considering the low sensitivity of POC-CCA for the identification of individuals living in areas where the prevalence of *S. mansoni* is >25%, and therefore the hardest level of detection, Coelho et al.[17] proposed a new step of urine concentration before using the POC-CCA conventional methodology (POC-CCA Lyo). The 10-fold lyophilized/concentrated urine improved POC sensitivity and preserved its strong specificity. The Estreito de Miralta prevalence rate was then increased from 2% to 32%. Conventional POC-CCA reached a sensitivity of 6% vs 56% for POC-CCA Lyo. Although effective, this new step took 34 h until the diagnosis was complete. Lyophilizer complicates its use in the field, where the infrastructure is limited and sample transportation to a laboratory would be time consuming.

Since Brazil and Africa present different prevalence profiles in their endemic areas and it is not acceptable to have false-negative and double trace reading rates as high as 30% and 39%, respectively, leading to untreated patients,[17] we have now optimized a urine concentration step to incorporate in the POC-CCA. This results in a 50-min diagnostic methodology. The test, called POC-CCA filter (FLT), eliminates the need for specialized technicians and maintains the cost savings of the conventional POC-CCA. This work addresses the performance of the POC-CCA and POC-CCA FLT tests compared with rigorous parasitological screening in two Brazilian endemic areas that have very distinct profiles: one with a low endemicity rate and one with no schistosomiasis transmission, although located in a region with similar epidemiology conditions. Comparison was done with the Kato-Katz and saline gradient methodologies to determine POC-CCA and POC-CCA FLT applications in Brazil and define the significance of all trace readings. In this work we also tested the performance of the POC-CCA and POC-CCA FLT tests for the control of cure after praziquantel administration on Kato-Katz-positive individuals and the cross-reactivity with other intestinal helminths.

Materials and methods

Community prospective survey

Residents from two areas were selected to participate on this prospective survey. Both are located in the rural region of Montes Claros, Minas Gerais, approximately 500 km from the state capital, Belo Horizonte, in southeast Brazil. This population has a low migration index. The survey was conducted in 2015–2016.

Area 1

Estreito de Miralta (EM) is a village with 163 residents. The schistosomiasis prevalence rate was determined to be 10.34% by the Montes Claros Zoonosis Control Centre in 2008. Through Kato-Katz stool examination, we found a prevalence rate of 30% in 2016 after an intensive search for eggs performed by using 1 g of faeces for Kato-Katz and saline gradient tests.[17] These participants had not received treatment for schistosomiasis in the previous 2 y. Infected patients were treated with praziquantel (a single oral dose of 60 mg/kg for children and 50 mg/kg for adults) and submitted to the same procedure as the baseline performed at 30, 90 and 180 days post-treatment. The period of 30 days after treatment was for evaluation of drug efficacy and the period of 180 days was established to detect reinfections. Retreatment was done, when needed. The total number of residents donating urine samples for this specific survey was 84 individuals (46 females and 38 males, 1–86 years old).

Area 2

Samambaia (SB) is a village with 179 inhabitants (79 females and 100 males, 2–84 years old) that was chosen at random (99 for the study). The prevalence rate was never determined in this area and participants of this study had never being treated before. Infected patients were treated as described for area 1.

Parasitological diagnosis

Each resident participating in the study provided a stool sample that was used to make 24 slides for Kato-Katz thick smear examination,[18] with a total of 1 g of faeces (24×41.7 mg of faeces) (Helm-Test, Bio-Manguinhos/Fiocruz, Rio de Janeiro, Brazil). The same sample was analysed by the saline gradient technique (two analyses of 500 mg, also with a total of 1 g of faeces).[19] The results were expressed as eggs per gram (epg) of faeces for both methods. These two parasitological methods were used as diagnostic tools for the detection of other helminths.

POC-CCA

Residents were also asked to provide one midstream urine sample. POC-CCA tests were performed according to the manufacturer's instructions (Rapid Medical Diagnostics, Pretoria, South Africa) and the test is performed on one cassette for all samples. In order to confirm the result, it is repeated on two or three cassettes. Briefly, one drop of urine is added to the well of the testing cassette. After absorption, a drop of the kit buffer is added to the well. The results are read after 20 min. The test is considered invalid if the control band does not appear. Tests were scored as 'negative' if the result revealed no test line, 'trace' if a very light test line appeared and 'positive' if a test line appeared. Four trained laboratory technicians read the results of each test in blind conditions.

POC-CCA FLT

In order to simplify the urine concentration step described earlier,[17] 0.5 ml of each sample was inserted in a 30-kDa filter (MRCF0R030, Merck Millipore, Darmstadt, Germany) and centrifuged (PMC880; Gilson, Middleton, WI, USA) at 2000 *g* for 30 min. The pellet was resuspended in 0.05 ml of distilled water. Once again, one drop of the 10 times concentrated sample was used in the conventional POC-CCA test.

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

Data analyses

Data collected from the parasitological diagnosis were used to identify the infection intensity of schistosomiasis-positive individuals. POC-CCA and POC-CCA FLT data were entered into an Excel database (Microsoft, Redmond, WA, USA) and analysed in comparison with the parasitological results. The reference was defined as any positive slide performed for each individual stool sample by the Kato-Katz and/or saline gradient techniques. Minitab statistical software (Minitab, State College, PA, USA) was used for statistical analysis. Accuracy was determined with Prism 4.0 (GraphPad Software, La Jolla, CA, USA) by comparing positive and negative POC-CCA FLT results with the standard diagnostic method defined.

Ethical approval

This work was approved by the Ethical Research Committee of the Rene Rachou Research Center (CEPSH/CPqRR 03/2008). Study objectives were explained to the participants who signed the written informed consent form before admission to the study. Parents/guardians provided written consent on behalf of all participants younger than 18 years of age. After parents/guardians had signed the informed consent, children received an explanation about the procedure and had the right to express their opinion. Procedures were performed in the presence of parents/guardians. Faecal and urine samples were coded by numbers and the results treated confidentially. All infected participants (by *S. mansoni* and other helminths defined by the parasitological methods) were clinically examined by physicians and treated with praziquantel (60 mg/kg for children and 50 mg/kg for adults) and albendazole (400 mg), respectively, in a single oral dose, as recommended by the Brazilian Health Ministry.

Results

Positivity estimates the prevalence rates for parasitological methods, POC-CCA and POC-CCA FLT

Parasitological analysis revealed 53/74 negative individuals in EM and 98/99 individuals in SB, while POC-CCA revealed 41/74 and 36/99, respectively, for EM and SB. In contrast, 21/74 individuals presented with eggs in their stool samples (1–76 epg) in EM; among those, POC-CCA properly diagnosed 7 individuals. It is important to point out that POC-CCA revealed a high number of trace results (26/74 in EM and 56/99 in SB). Among the 26 trace results in EM, 7 presented with eggs in their stool samples and 19 were negative. Only one individual presented with eggs in their stool sample (8 epg) and was diagnosed as a trace result by POC-CCA. A total of 55 negative individuals in SB also presented traces (Table 1). For the 21/74 individuals from EM that had eggs in their stools, only 7 were positive for POC-CCA while 17 were positive for POC-CCA FLT. POC-CCA showed 26 trace results from which no diagnosis could be concluded, as there is no definition about double trace results for conventional POC-CCA tests. The POC-CCA FLT presented 57/74 negative individuals in EM (31 trace and 26 negative results), among whom 47 were negative for parasitological tests and 10 had eggs in their stools (1–76 epg). The POC-CCA test diagnosed 36/99 as negative individuals in SB, with 56 trace readings and 7 positive individuals, although only 1 individual had eggs in his/her stool and this particular case was diagnosed as trace. The POC-CCA test diagnosed the unique positive individual as trace, together with 55 negative individuals, and revealed 7 individuals as false positive. The POC-CCA FLT showed 94/99 negative individuals (20 negative and 74 traces) and 5 positive results, among whom 1 had eggs in his/her stool and 4 were considered as false-positive results. Among the seven individuals in SB presenting false-positive results for POC-CCA, all were negative for parasitological analysis and POC-CCA FLT. Together, the four individuals in SB with false-positive results for POC-CCA FLT were diagnosed as two traces and two negative results for POC-CCA.

The Kato-Katz and saline gradient tests separately showed similar prevalence rates of 28% and 26% for EM, respectively. This prevalence rate was substantially higher than the 10% shown by POC-CCA (one to two cassettes). This 10% rate turned into 23% for POC-CCA FLT when urine samples were concentrated, reaching similar rates as both parasitological techniques. Only one positive individual for schistosomiasis was identified in SB by the parasitological methods, giving a prevalence rate of 1%. When prevalence was estimated by POC-CCA and POC-CCA FLT, the prevalence rate was 7% and 6%, respectively. The relation between the intensity of the line and the epg was not seen in any of the POC tests. POC-CCA FLT results when two or three cassettes were used were reproducible, while POC-CCA had a 75% rate of reproducibility, revealing positives and negatives, positives and traces or negatives and traces for same sample.

Post-treatment diagnosis evaluation for POC-CCA and POC-CCA FLT

All treated patients presented negative results for the faeces examination (Kato-Katz and saline gradient) at 30, 90 and 180 d post-treatment. POC-CCA presented poor performance for the control of cure, with 13/22, 13/24 and 11/19 (equivalent to 55, 54 and 56%) true-negative results, respectively, for 30, 90 and 180 d post-treatment. In contrast, POC-CCA FLT was shown to be a sensitive tool for the control of cure, with 21/22, 20/24 and 19/19 true-negative results (equivalent to 96, 83 and 100%), respectively, for 30, 90 and 180 d after praziquantel treatment, as shown in Table 2.

Accuracy of POC-CCA and POC-CCA FLT

The accuracy of both POC tests was estimated with the 95% confidence interval (CI), shown in Table 3. Once again, analyses were done against the combined parasitological techniques (1 g of faeces each). In EM, POC-CCA accuracy was 0.51, whereas POC-CCA FLT reached 0.86, showing it to be superior to the conventional methodology. Similar results were observed in SB, where POC-CCA had an accuracy of 0.36 vs 0.96 obtained by POC-CCA FLT.

Cross-reactivity evaluation for POC-CCA and POC-CCA FLT

Twenty individuals in EM presented no *S. mansoni* eggs in their stools but were positive for the presence of eggs from other helminths (hookworms, *Hymenolepis nana*, *Enterobius vermicularis*

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

Table 1. Diagnosis for *Schistosoma mansoni* using Kato-Katz and saline gradient tests for faeces samples and POC-CCA for urine samples from Estreito de Miralta and Samambaia individuals, Minas Gerais, Brazil

	Kato-Katz (2 slides)	Kato-Katz (24 slides, 1 g of faeces)+saline gradient (1 g of faeces)	POC-CCA	POC-CCA FLT
Estreito de Miralta				
Negative	63	53	41	26
Positive	11	21	7	17
Trace	—	—	26 (33)*	31[a] (48)*
Total		74		
Samambaia				
Negative	98	98	36	20
Positive	1	1	7	5
Trace	—	—	56 (63)*	74[a] (79)*
Total		99		

[a]Traces after urine concentration by POC-CCA FLT were all negative individuals, totalling 57/74 negative individuals in EM and 94/99 negative individuals in SB. *Traces as positive.

Table 2. Post-treatment diagnosis with POC-CCA and POC-CCA FLT in positive individuals from Estreito de Miralta

	30 dpt (N=22)	90 dpt (N=24)	180 dpt (N=19)
POC-CCA			
Negative	13	13	11
Trace	5	8	8
Positive	4 (9)*	3 (11)*	0 (8)*
POC-CCA FLT			
Negative	1	7	2
Trace	20	13	17
Positive	1 (21)*	4 (17)*	0 (17)*
Kato-Katz (24 slides, 1 g of faeces)			
Negative	22	24	18
Positive	0	0	0
Saline gradient (1 g of faeces)			
Negative	22	24	18
Positive	0	0	0

dpt: days post-treatment. All the individuals received the same treatment. Not all the individuals provided faecal and urine samples on the three timelines. *Trace as positive.

Table 3. Accuracy of POC-CCA and POC-CCA FLT evaluated against a combined gold standard of 24 Kato-Katz slides and 2 saline gradient analyses

	Estreito de Miralta	Samambaia
	Accuracy	
POC-CCA	0.51	0.36
POC-CCA FLT	0.86	0.96

and *Ascaris lumbricoides*). Urine samples of these individuals were used for evaluation of the cross-reactivity between POC-CCA and POC-CCA FLT. As shown in Table 4, no significant differences were noticed when comparing the methods, as both POC tests presented false-positive results. The number of available samples for this cross-reactivity analysis was small and future studies should address the same tests with a greater number of patient samples.

Discussion

The introduction of POC-CCA, a technique for the detection of CCA present in the urine of schistosomiasis-infected individuals, represents an important improvement for epidemiology and disease control in endemic areas. The rapid identification of infected individuals and treatment implementation makes this technique a valuable tool for the control of *S. mansoni*. However, recent studies have shown that POC-CCA presents low sensitivity and specificity resulting in high levels of false-positive and false-negative results, especially for patients with low parasite burden living in areas of low prevalence. A high number of trace readings have been observed when diagnosing these patients, leading to dubious interpretations and frequent mistaken drug treatment.[3,9–17] Our group has been working for several years to evaluate and improve the diagnosis in low-prevalence areas with low endemicity, and same results have been noticed when POC-CCA is used: double results are frequently obtained and no pattern is identified for trace readings when infected and non-infected individuals commonly present traces in their POC exams. It is important to point out that a rigorous quantitative

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

Table 4. Cross-reactivity analysis of POC-CCA and POC-CCA FLT detected by the combined gold standard parasitological diagnosis in Estreito de Miralta individuals

	POC-CCA	POC-CCA FLT
Hookworms (N=8)		
Negative	5	6
Positive	3	2
Hymenolepis nana (N=3)		
Negative	1	2
Positive	2	1
Enterobius vermicularis (N=8)		
Negative	2	2
Positive	6	6
Ascaris lumbricoides (N=1)		
Negative	0	0
Positive	1	1

parasitological examination using two parasitological techniques (Kato-Katz and saline gradient) using 1 g of faeces to identify the real positive individuals is frequently done by our group in Brazil. In addition, cross-reactivity and post-treatment analysis are beginning to show that some helminths can cross-react with CCA detection on POC, resulting in patients remaining 'positive' even after drug treatment.[17,20] These analyses confirm the limitations of POC-CCA and the need for improvement prior to its introduction in endemic areas as a diagnostic choice, especially when trace readings have no definition on the conventional methodology.

Aiming to improve the POC-CCA technique, especially its capacity to detect low parasite burdens, our group recently demonstrated that by adding a concentrated urine sample through lyophilization, for a 10-fold urine concentration, traces and false-negative results of individuals with eggs in their faeces were converted to POC-positive results.[17] This study clarifies an important issue about the uncertainty of trace readings that still leads authors to determine prevalence by considering trace as positive and negative cases in the same work.[9–12,14,20–24] Although greatly promising, presenting results superior to the available POC test and the POC under study, the urine lyophilization process is time consuming, taking up to 34 h until the diagnosis is ready to be reported, and it is not suitable for use in the field. Moreover, operational costs are increased when compared with the POC-CCA test.

In this study, our group was able to improve the urine concentration step with a fast and simple methodology for even hard-to-detect individuals (with 1–76 epg) from low-endemicity areas. This new methodology includes a filter to separate specific proteins and concentrates CCA in a small amount of urine. The antigen resuspension step in water proved to be especially easy and the resultant solution is 10-fold more concentrated. When applied in the conventional POC-CCA test, results showed an increase of 60% in its accuracy when compared with the conventional methodology. This is a method that is easy to use in the field, where operational conditions are limited, as well as in laboratories, taking a total of 50 min (30 min for urine concentration and 20 min for POC development) for the diagnosis to be reported to the patient. And finally, it does not need any complex equipment or technical training.

The present study highlights the results found for conventional POC-CCA and the new POC-CCA FLT test in two areas with distinct profiles. The first area is Estreito de Miralta, located in southeast Brazil. It is an endemic area with a prevalence rate in 2008 of approximately 10% as determined by one Kato-Katz slide and a rate of 30% reported by our group in 2016 when 24 Kato-Katz slides and 2 saline gradient analyses were used. A significant difference in the identification of negative and positive individuals for schistosomiasis was noticed when comparing the POC-CCA and POC-CCA FLT test. The prevalence rates determined by each diagnostic method used here are Kato-Katz (2 slides) 15%, Kato-Katz (24 slides) 28%, saline gradient 26%, POC-CCA 10% and POC-CCA FLT 23%. A second area called Samambaia, also in southeast Brazil, was investigated. We found that the socio-economic and environmental conditions in this area were similar to all the schistosomiasis endemic areas nearby, but only 1 individual among 99 residents was positive for *S. mansoni*, with 8 EPG of faeces. Interestingly, this individual had recently moved to Samambaia from a schistosomiasis endemic area. Considering this scenario and the rigorous diagnosis performed by faeces examination, plus the rapid treatment intervention, the possible development of a new schistosomiasis focus was eliminated. When both methods were performed in Samambaia, POC-CCA FLT showed superior performance, with 96% of cases correctly diagnosed vs 63% diagnosed by POC-CCA.

POC-CCA had poor performance in Brazilian areas, as shown previously,[17] revealing several false results and undefined trace readings. Recent publications have shown that when trace readings are considered as negative or positive cases, prevalence rates are significantly unrelated in different endemic areas.[9–12,14,20–24] These findings agree with data found in Brazilian areas.[3–7,17] Although conventional POC-CCA provides no definition of trace results, we were able to establish a pattern for traces obtained after the urine concentration step. Therefore, traces obtained by POC-CCA FLT, as for POC-CCA Lyo,[17] are well defined as negative cases and no unnecessary treatment should be performed on these individuals. We also analysed the relationship between the band intensity and the epg in each positive case, however, none was seen in any of the POC tests. Inversely, POC-CCA FLT reproduced all the results when two or three cassettes were used with the same sample and at the same time. POC-CCA reproduced 75% of the results, revealing meaningful variations as negative, trace and positive results for the same urine samples. Post-treatment analysis revealed an improved specificity by POC-CCA FLT vs POC-CCA, as one was able to properly diagnose up to 100% of the cases and the other was only effective in half of the cases, agreeing with data found by others.[20] Remaining questions include whether POC tests can determine if a person is cured after treatment,[9] considering that it is vital for a program to define the control of cure rate after mass or individual drug treatments. Finally, no significant differences were seen for the POC tests on the cross-reaction analysis since both presented false-positive results. Considering the lack of cross-reaction studies for POC-CCA

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

evaluation and the small number of samples used here, new analyses must be performed in other areas for consistent data.

Conclusions

The WHO has established goals for schistosomiasis transmission control worldwide.[2] For countries on the African continent, transmission control should be accomplished by 2025 and for other countries, including Brazil, by the end of 2020. It is urgent to have improved diagnostic methods with high accuracy and ease of diagnosis in endemic areas to accomplish the goals set by the WHO. POC tests for diagnosing schistosomiasis include methods based on circulating antigen detection and urine reagent strip tests. If POC-CCA had sufficient diagnostic accuracy in endemic areas with distinct profiles it could be used as an essential tool in control programs, as it provides fast results.[24] The innovative POC-CCA FLT test described in this study introduces a new and fast method for field application by improving POC-CCA sensitivity and accuracy with a single urine concentration step.

Authors' contributions: All authors contributed significantly to the experimental design, implementation, analysis and interpretation of the data. All authors were involved in the writing of the manuscript at draft and any revision stages and have read and approved the final version. RFQG and PMZC made major contributions to the writing of the manuscript and are the guarantors of the paper.

Acknowledgements: We thank Beak Sanchoi for the English editing.

Funding: This work was supported by the National Council for Scientific and Technological Development (ProepPclin 401942/2012-0) and the Oswaldo Cruz Foundation.

Competing interests: None declared.

Ethical approval: Not required.

References

1 World Health Organization. Schistosomiasis: Strategy in 2011: Switzerland. http://www.who.int/schistosomiasis/strategy/ [accessed 15 May 2017].

2 World Health Organization. Defining a road map toward verification of elimination of schistosomiasis transmission in Latin America and the Caribbean by 2020 in 2014. http://www.paho.org/hq/index.php?option=com_topics&view=article&id=50&Itemid=40770 [accessed 20 May 2017].

3 Siqueira LM, Couto FF, Taboada D et al. Performance of POC-CCA® in diagnosis of schistosomiasis mansoni in individuals with low parasite burden. Rev Soc Bras Med Trop 2016;49(3):341–7.

4 Siqueira LM, Gomes LI, Oliveira E et al. Evaluation of parasitological and molecular techniques for the diagnosis and assessment of cure of schistosomiasis mansoni in a low transmission area. Mem Inst Oswaldo Cruz 2015;110(2):209–14.

5 Grenfell RF, Martins W, Enk M et al. *Schistosoma mansoni* in a low-prevalence area in Brazil: the importance of additional methods for the diagnosis of hard-to-detect individual carriers by low-cost immunological assays. Mem Inst Oswaldo Cruz 2013;108(3):328–34.

6 Grenfell RF, Silva-Moraes V, Taboada D et al. Immunodiagnostic methods: what is their role in areas of low endemicity? Sci World J 2012;2012:593947.

7 Enk MJ, Lima AC, Drummond SC et al. The effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. Acta Trop 2008;108(2–3):222–8.

8 Katz N, Rocha RS, Simões Barbosa C et al. National survey on schistosomiasis mansoni and geohelminths in Brazil (2011–2014). XIII International Congress of Parasitology ICOPA. Mexico City, 2014.

9 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;88(3):426–32.

10 Coulibaly JT, Knopp S, N'Guessan NA et al. Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Cote d'Ivoire. PLoS Negl Trop Dis 2011;5(11):e1384.

11 Coulibaly JT, N'Gbesso YK, Knopp S et al. Accuracy of urine circulating cathodic antigen test for the diagnosis of *Schistosoma mansoni* in preschool-aged children before and after treatment. PLoS Negl Trop Dis 2013;7(3):e2109.

12 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(2):219–28.

13 Legesse M, Erko B. Field-based evaluation of a reagent strip test for diagnosis of *Schistosoma mansoni* by detecting circulating cathodic antigen in urine before and after chemotherapy. Trans R Soc Trop Med Hyg 2007;101(7):668–73.

14 Tchuem Tchuente LA, Kuete 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(7):e1758.

15 Casacuberta M, Kinunghi S, Vennervald BJ et al. Evaluation and optimization of the Circulating Cathodic Antigen (POC-CCA) cassette test for detecting *Schistosoma mansoni* infection by using image analysis in school children in Mwanza Region, Tanzania. Parasite Epidemiol Control 2016;1(2):105–15.

16 Kittur N, Castleman JD, Campbell CH Jr et al. Comparison of *Schistosoma mansoni* prevalence and intensity of infection, as determined by the circulating cathodic antigen urine assay or by the Kato-Katz fecal assay: a systematic review. Am J Trop Med Hyg 2016;94(3):605–10.

17 Coelho PM, Siqueira LM, Grenfell RF et al. Improvement of POC-CCA interpretation by using lyophilization of urine from patients with *Schistosoma mansoni* low worm burden: towards an elimination of doubts about the concept of trace. PLoS Negl Trop Dis 2016;10(6):e0004778.

18 Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 1972;14(6):397–400.

19 Coelho PM, Jurberg AD, Oliveira AA et al. Use of a saline gradient for the diagnosis of schistosomiasis. Mem Inst Oswaldo Cruz 2009;104(5):720–3.

20 Lamberton PH, Kabatereine NB, Oguttu DW et al. Sensitivity and specificity of multiple Kato-Katz thick smears and a circulating cathodic antigen test for *Schistosoma mansoni* diagnosis pre- and post-repeated-praziquantel treatment. PLoS Negl Trop Dis 2014;8(9):e3139.

21 Foo K, Blackstock AJ, Ochola EA et al. Evaluation of point-of-contact circulating cathodic antigen assays for the detection of *Schistosoma*

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018

mansoni infection in low-, moderate-, and high-prevalence schools in western Kenya. Am J Trop Med Hyg 2015;92(6):1227–32.

22 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(1):e591.

23 Adriko M, Standley CJ, Tinkitina B et al. Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for *Schistosoma mansoni* in different transmission settings within Bugiri District, Uganda. Acta Trop 2014;136:50–7.

24 Ochodo EA, Gopalakrishna G, Spek B et al. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database Syst Rev 2015;3: CD009579.

Downloaded from https://academic.oup.com/trstmh/advance-article-abstract/doi/10.1093/trstmh/try014/4924493
by Jacob Heeren user
on 29 March 2018