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Validation of a Point-of-Care Circulating Cathodic Antigen Urine Cassette Test for *Schistosoma mansoni* Diagnosis in the Sahel, and Potential Cross-Reaction in Pregnancy

Helena Greter, Stefanie J. Krauth, Bongo N. R. Ngandolo, Idriss O. Alfaroukh, Jakob Zinsstag, and Jürg Utzinger*

Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire; Institut de Recherche en Elevage pour le Développement, N'Djamena, Chad

Abstract. On the shores of Lake Chad, schistosomiasis among mobile pastoralists was investigated in a field laboratory. Point-of-care circulating cathodic antigen (POC-CCA) cassette test, reagent strip, and filtration were conducted on urine samples. Fresh stool samples were subjected to the Kato-Katz technique, and fixed samples were examined with an ether-concentration method at a reference laboratory. POC-CCA urine cassette tests revealed a *Schistosoma mansoni* prevalence of 6.9%, compared with only 0.5% by stool microscopy. Three pregnant women with otherwise negative urine and stool testing had positive POC-CCA. This observation raises concern of cross-reactivity in pregnancy. Hence, two pregnant women in Switzerland with no history of schistosomiasis were subjected to POC-CCA and one tested positive. Our data suggest that POC-CCA can be performed under extreme Sahelian conditions (e.g., temperatures > 40°C), and it is more sensitive than stool microscopy for *S. mansoni* diagnosis. However, potential cross-reactivity in pregnancy needs further investigation.

INTRODUCTION

Schistosomiasis is listed among the neglected tropical diseases[1] and specifically mentioned in the World Health Assembly resolution 65.21a that calls for global elimination by 2025.[2] In Africa, schistosomiasis is mainly caused by chronic infection with *Schistosoma haematobium* and *Schistosoma mansoni*.[3] The most widely used diagnostic methods for *S. haematobium* are urine filtration with microscopy and reagent strip testing for microhematuria, whereas the diagnosis of *S. mansoni* heavily relies on Kato-Katz thick smear examination under a microscope.[4] In rural areas of Africa, where most of the worldwide *Schistosoma* infections occur, the capacity of health centers is insufficient to provide even these basic laboratory diagnostics. Hence, the World Health Organization (WHO)'s plan for global elimination of schistosomiasis will require additional diagnostic tools, characterized by high sensitivity, high specificity, ease of use at the point-of-care (POC), and low costs.[4] First products developed within this spirit are already available on the market. One of them is a POC circulating cathodic antigen (CCA) urine cassette test for the diagnosis of *S. mansoni*. The test is based on a lateral flow principle and detects adult *Schistosoma* worm CCA in the hosts' urine when a colloid carbon conjugate of a monoclonal antibody is added to the sample.[5] Although CCA is excreted by adult worms of different *Schistosoma* species, the test is most sensitive to CCA excreted from adult *S. mansoni* worms. The test also allows detection of *S. mansoni* infection before the excretion of parasite eggs in the stool. According to the manufacturer's guidelines, heavy infections with *S. haematobium* might also be detected. Hence, the test reveals *Schistosoma* infections by indirect depiction of the presence of adult worms (or young developing stages of the parasite) in the host body and has therewith a higher sensitivity than stool microscopy, since *S. mansoni* egg excretion shows considerable intraspecimen and day-to-day variation.[6] Of note, the POC-CCA urine cassette test has been successfully validated in a multicountry study and showed an overall specificity of 94%.[6] The POC-CCA is now being recommended by WHO for the rapid mapping of community prevalence in *S. mansoni*-endemic settings,[4] and might be used for POC diagnosis of migrants and returning travelers from endemic to non-endemic regions.[7]

With regard to the WHO goal of schistosomiasis elimination, it is important to gain a deeper insight into the disease situation in endemic areas. In Chad, nationwide data on schistosomiasis date back to the 1970s.[8,9] More recent studies reflect the infection status of the urban population in the vicinity of the capital city of N'Djamena.[10–13] Yet, to attempt elimination, a more detailed picture of the infection status in the rural Chadian population is required and specific at-risk groups must be identified.

Here, we report findings from a cross-sectional survey among mobile pastoralists from the Lake Chad region placing particular emphasis on the use of the POC-CCA urine cassette test under extreme environmental conditions (e.g., aridness and air temperature up to 48°C). Our findings are compared with the standard Kato-Katz technique and an ether-concentration method employed to fixed stool samples for *S. mansoni* diagnosis. As we found a potential cross-reaction of the POC-CCA test among pregnant women, we invited two pregnant women from Switzerland with no history of schistosomiasis to participate.

METHODS

Ethical considerations. The validation of the POC-CCA urine cassette test for the diagnosis of *S. mansoni* was embedded in a larger epidemiologic study pertaining to helminth infections in mobile pastoralists and their livestock in the southeastern Lake Chad area in Chad. The study was approved by the ethics committee in Basel, Switzerland (EKBB; reference no. 64/13). In Chad, research permission, including ethical approval, was obtained from the "Direction Générale des Activités Sanitaires" (reference no. 343/MSP/SE/SG/DGAS/2013). The aim and procedures of the study were explained to each group of mobile pastoralists. Informed consent was signed by group leaders after discussion within the groups. Because of high illiteracy rates, randomly selected individuals within each group provided oral consent. All participants found with a positive test result for *S. haematobium* by urine filtration and *S. mansoni* on the spot (Kato-Katz thick smear and POC-CCA urine cassette test) were treated with

*Address correspondence to Jürg Utzinger, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland. E-mail: juerg.utzinger@unibas.ch

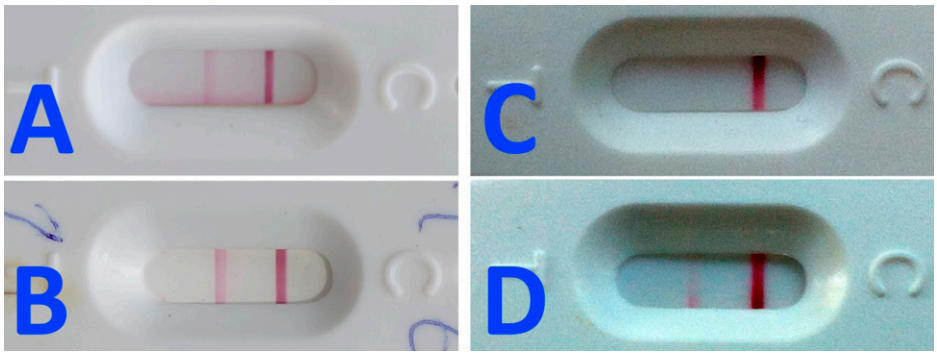

FIGURE 1. Point-of-care circulating cathodic antigen (POC-CCA) urine cassette test results obtained from samples in Chad (**A**, **B**) and from pregnant women in Switzerland (**C**, **D**). (**A**) Faintly positive test band from Chad, (**B**) strong positive test band from Chad, (**C**) negative test result from a pregnant Swiss woman, and (**D**) positive test result from another pregnant Swiss woman.

a single 40 mg/kg oral dose of praziquantel.[14] In addition, two pregnant women in Switzerland participated on a voluntary basis. Both provided written informed consent.

Procedures. The data were collected between April and May 2014 in 13 camps of mobile pastoralists on the southeastern shores of Lake Chad. A mobile field laboratory was set up in the shade of a tree in close proximity to camps. Study participants were given two collection containers: one for urine and one for stool. Urine samples were collected between 10 AM and 9 PM, with 60% of the urine specimens sampled between 10 AM and 2 PM. Urine samples were first tested for microhematuria, a proxy for *S. haematobium* infection,[15] using reagent strips (Hemastix; Siemens Healthcare Diagnostics GmbH; Eschborn, Germany). Second, a POC-CCA cassette test (Rapid Medical Diagnostics; Pretoria, South Africa) was used for *S. mansoni* diagnosis. Third, 10 mL of urine was filtered using a syringe pressed through a 13-mm diameter filter holder containing a 20-μm wire-mesh filter (Sefar AG; Heiden, Switzerland). Filters were examined under a solar-powered light microscope by a trained laboratory technician and the first author (Helena Greter).

From each stool sample, a single Kato-Katz thick smear using 41.7 mg standard templates was prepared and examined under a microscope on the spot by a trained laboratory technician.[16] In addition, approximately 1 g of stool was fixed in a Falcon tube containing 20 mL sodium acetate–acetic acid–formalin (SAF) solution. The SAF-fixed stool samples were forwarded to the Swiss National Reference Laboratory for Imported Parasitic Infections at the Swiss Tropical and Public Health Institute (Basel, Switzerland) and subjected to an ether-concentration method for the diagnosis of *S. mansoni* and other helminths.[17]

RESULTS

Urine and stool samples were obtained from a random sample of 193 individuals in 13 mobile camps. Complete parasitologic data (i.e., reagent strip, urine filtration, POC-CCA urine cassette test, Kato-Katz thick smear, and SAF-fixed stool samples examined by an ether-concentration technique) were available from 187 participants (96 females [47 girls aged < 14 years] and 91 males [51 boys aged < 14 years]). There were 13 positive POC-CCA urine cassette tests, owing to a *S. mansoni* prevalence of 6.9%. Stool microscopy, using Kato-Katz and ether-concentration, detected eggs of *S. mansoni* in only one individual (0.5%). There were more than twice as many positive POC-CCA urine cassette tests in females ($N = 9$) compared with males ($N = 4$), whereas the only positive stool microscopy was in a male participant. Among the 13 positive POC-CCA urine cassette tests, eight showed a faintly positive test line, whereas the remaining five showed a strong positive test line (Figure 1).[18]

According to the manufacturer's guidelines for the POC-CCA assay, the intensity of the test line is correlated with the intensity of *S. mansoni* infection. It is also mentioned in the guidelines that heavy infections with *S. haematobium* and microhematuria may produce positive test results in the POC-CCA cassette test. Positive reagent strip or urine filtration results were present in all four male participants who were POC-CCA cassette test positive, but coprology negative. In females, on the other hand, six individuals with positive POC-CCA cassette tests were found negative with all other tests used (Kato-Katz, ether-concentration, reagent strip, and urine filtration) (Table 1).

Those six females who had a positive POC-CCA urine cassette test that could not be explained because of an *S. haematobium* infection or microhematuria were adults at reproductive age (14–49 years) (Table 2). We found that three of the six women were pregnant. For all pregnant women, POC-CCA urine cassette tests were repeated and positive tests were confirmed.

TABLE 1

Comparison between POC-CCA urine cassette test and stool microscopy, urine filtration, and reagent strip results for total number of participants, stratified by sex

	Overall	Males	Females
Participants with complete data	187	91	96
POC-CCA urine cassette test negative	174	87	87
POC-CCA urine cassette test positive	13	4	9
Schistosoma mansoni egg positive (Kato-Katz and/or ether-concentration)	1	1	0
Schistosoma haematobium egg positive (urine filtration) and microhematuria positive (reagent strip)	3	2	1
Microhematuria positive (reagent strip) and *S. haematobium* egg negative (urine filtration)	3	1	2
POC-CCA urine cassette test positive alone	6	0	6

POC-CCA = point-of-care circulating cathodic antigen.

TABLE 2

Comparison between POC-CCA urine cassette test and stool microscopy, urine filtration, and reagent strip results for female participants, stratified by age group

	Females, all ages	Females aged < 14 years	Females aged 14–49 years	Females aged > 49 years
Female participants with complete data	96	47	41	8
POC-CCA urine cassette test negative	87	46	33	8
POC-CCA urine cassette test positive	9	1	8	0
Schistosoma mansoni egg positive (Kato-Katz and/or ether-concentration)	0	0	0	0
Schistosoma haematobium egg positive (urine filtration) and microhematuria positive (reagent strip)	1	0	1	0
Microhematuria positive (reagent strip) and *S. haematobium* egg negative (urine filtration)	2	1	1	0
POC-CCA urine cassette test positive alone	6	0	6	0

POC-CCA = point-of-care circulating cathodic antigen.

The aforementioned positive POC-CCA urine cassette test results, coupled with negative coprologic examinations, raised concern about a potential cross-reaction of POC-CCA in pregnancy. Hence, we invited two pregnant women from Switzerland without any history of schistosomiasis to provide urine samples that were subjected to duplicate POC-CCA urine cassette testing. As shown in Figure 1, in one of the two pregnant Swiss women, the POC-CCA consistently revealed a faintly positive test line.

DISCUSSION

POC-CCA urine cassette tests were used among 187 mobile pastoralists, aged 5–77 years, under extreme environmental conditions in Chad. In contrast, most of the previously published studies that validated the POC-CCA urine cassette test for the diagnosis of *S. mansoni* in schistosomiasis-endemic areas focused on school-aged children.[5,19–27] Positive POC-CCA urine cassette test results in males and females with negative stool microscopy may be explained by the fact that we only examined a single stool sample per participant, and hence, we might have missed individuals with low infection intensity.[28] Interestingly, we found three positive POC-CCA urine cassette tests among pregnant women with negative stool microscopy. These results were confirmed when repeating POC-CCA urine cassette tests. Moreover, one of two pregnant Swiss women without history of schistosomiasis had a positive POC-CCA result. Hence, our results raise concern about a potential cross-reaction with certain non-*Schistosoma*-related metabolites in pregnant women's urine. These observations warrant further research to assess the reliability and accuracy of POC-CCA urine cassette tests before wider use in healthcare practice.

Treatment with praziquantel—be it in the frame of preventive chemotherapy or targeted to positive individuals—is safe,[29] and WHO guidelines established in 2002 explicitly recommend its administration also to pregnant or lactating women.[30] Yet, most guidelines discourage intake of medicines during pregnancy to minimize risk of adverse events. In the endgame of reaching the goal of schistosomiasis elimination, a test-and-treat strategy is likely to gain traction. It will be important to dispose of rapid, easy to handle diagnostic tools at the POC that are highly accurate and whose performance remains reliable even under extreme environmental conditions such as in the Sahel.

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Authors' addresses: Helena Greter, Stefanie J. Krauth, Jakob Zinsstag, and Jürg Utzinger, Swiss Tropical and Public Health Institute, and University of Basel, Basel, Switzerland, E-mails: helena.greter@unibas.ch, stefanie.krauth@unibas.ch, jakob.zinsstag@unibas.ch, and juerg.utzinger@unibas.ch. Stefanie J. Krauth, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire. Bongo N. R. Ngandolo and Idriss O. Alfaroukh, Institut de Recherche en Elevage pour le Développement, N'Djamena, Chad, E-mails: bongo_nov@yahoo.fr and aolidriss@yahoo.fr.

REFERENCES

1. Hotez PJ, Alvarado M, Basáñez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fèvre EM, Fürst T, Halasa YA, Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SDS, Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA, Utzinger J, Wang M, Murray CJL, Naghavi M, 2014. The Global Burden of Disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis 8:* e2865.
2. WHO, 2013. *Schistosomiasis: Progress Report 2001–2011 and Strategic Plan 2012–2020*. Geneva: World Health Organization.
3. Colley DG, Bustinduy AL, Secor WE, King CH, 2014. Human schistosomiasis. *Lancet 383:* 2253–2264.
4. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S, 2015. New diagnostic tools in schistosomiasis. *Clin Microbiol Infect 21:* 529–542.
5. van Dam GJ, Wichers JH, Ferreira TM, Ghati D, van Amerongen A, Deelder AM, 2004. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *J Clin Microbiol 42:* 5458–5461.
6. Colley DG, Binder S, Campbell C, King CH, Tchuem Tchuenté LA, N'Goran EK, Erko B, Karanja DM, Kabatereine NB, van Lieshout L, Rathbun S, 2013. 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 88:* 426–432.
7. Becker SL, Marti H, Zimmermann S, Vidacek D, Herrmann M, Utzinger J, Schnabel PA, Bohle RM, 2015. Application in Europe of a urine-based rapid diagnostic test for confirmation

of *Schistosoma mansoni* infection in migrants from endemic areas. *Euro Surveill 20:* 21151.
8. Becquet R, Saout J, Pascal JM, 1970. La bilharziose intestinale à *Schistosoma intercalatum* en Republique du Tchad (à propos de deux observations). *Bull Soc Pathol Exot 63:* 343–350.
9. Delpy P, Sirol J, Troncy J, Bono O, 1972. Problèmes diagnostiques posés à Fort-Lamy (Tchad) par la présence d'oeufs de schistosome à éperon terminal dans les selles de malades indemnes de bilharziose urinaire. *Bull Soc Pathol Exot 65:* 417–430.
10. Massenet D, Abakar D, Karifene R, 1995. Prévalence de la bilharziose urinaire en milieu scolaire à N'Djamena (Tchad). *Bull Soc Pathol Exot 88:* 35–37.
11. Hamit MA, Tidjani MT, Bilong Bilong CF, 2008. Recent data on the prevalence of intestinal parasites in N'Djamena, Chad Republic. *Afr J Environ Sci Technol 2:* 407–411.
12. Massenet D, Gamougam K, Djerane L, Toukour A, 2012. Cinquante années de diagnostics coprologiques parasitaires au Tchad et au Nord-Cameroun (1963–2009). *Med Sante Trop 22:* 330–331.
13. Moser W, Greter H, Schindler C, Allan F, Ngandolo BNR, Moto DD, Utzinger J, Zinsstag J, 2014. The spatial and seasonal distribution of *Bulinus truncatus*, *Bulinus forskalii* and *Biomphalaria pfeifferi*, the intermediate host snails of schistosomiasis, in N'Djamena, Chad. *Geospat Health 9:* 109–118.
14. WHO, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee. *WHO Tech Rep Ser 921:* 1–57.
15. Savioli L, Hatz C, Dixon H, Kisumku UM, Mott KE, 1990. Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and hematuria as indicators of infection. *Am J Trop Med Hyg 43:* 289–295.
16. Katz N, Chaves A, Pellegrino J, 1972. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop São Paulo 14:* 397–400.
17. Utzinger J, Botero-Kleiven S, Castelli F, Chiodini PL, Edwards H, Köhler N, Gulletta M, Lebbad M, Manser M, Matthys B, N'Goran EK, Tannich E, Vounatsou P, Marti H, 2010. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories. *Clin Microbiol Infect 16:* 267–273.
18. Coulibaly JT, N'Gbesso YK, Knopp S, N'Guessan NA, Silué KD, van Dam GJ, N'Goran EK, Utzinger J, 2013. 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 7:* e2109.
19. Stothard JR, Kabatereine NB, Tukahebwa EM, Kazibwe F, Rollinson D, Mathieson W, Webster JP, Fenwick A, 2006. Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. *Acta Trop 97:* 219–228.
20. Legesse M, Erko B, 2007. 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 101:* 668–673.
21. Ayele B, Erko B, Legesse M, Hailu A, Medhin G, 2008. Evaluation of circulating cathodic antigen (CCA) strip for diagnosis of urinary schistosomiasis in Hassoba school children, Afar, Ethiopia. *Parasite 15:* 69–75.
22. Midzi N, Butterworth AE, Mduluza T, Munyati S, Deelder AM, van Dam GJ, 2009. Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis. *Trans R Soc Trop Med Hyg 103:* 45–51.
23. Coulibaly JT, Knopp S, N'Guessan NA, Silué KD, Fürst T, Lohourignon LK, Brou JK, N'Gbesso YK, Vounatsou P, N'Goran EK, Utzinger J, 2011. Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Côte d'Ivoire. *PLoS Negl Trop Dis 5:* e1384.
24. Tchuem Tchuenté LA, Kuete Fouodo CJ, Kamwa Ngassam RI, Sumo L, Dongmo Noumedem C, Kenfack CM, Gipwe NF, Nana ED, Stothard JR, Rollinson D, 2012. Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of *Schistosoma mansoni* infection in Cameroon. *PLoS Negl Trop Dis 6:* e1758.
25. Adriko M, Standley CJ, Tinkitina B, Tukahebwa EM, Fenwick A, Fleming FM, Sousa-Figueiredo JC, Stothard JR, Kabatereine NB, 2014. 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 136:* 50–57.
26. Foo KT, Blackstock AJ, Ochola EA, Matete DO, Mwinzi PN, Montgomery SP, Karanja DM, Secor WE, 2015. Evaluation of point-of-contact circulating cathodic antigen assays for the detection of *Schistosoma mansoni* infection in low-, moderate-, and high-prevalence schools in western Kenya. *Am J Trop Med Hyg 92:* 1227–1232.
27. Mwinzi PN, Kittur N, Ochola E, Cooper PJ, Campbell CH Jr, King CH, Colley DG, 2015. Additional evaluation of the point-of-contact circulating cathodic antigen assay for *Schistosoma mansoni* infection. *Front Public Health 3:* 48.
28. Utzinger J, Booth M, N'Goran EK, Müller I, Tanner M, Lengeler C, 2001. Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology 122:* 537–544.
29. Utzinger J, Keiser J, 2004. Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother 5:* 263–285.
30. Allen HE, Crompton DWT, de Silva N, LoVerde PT, Olds GR, 2002. New policies for using anthelmintics in high risk groups. *Trends Parasitol 18:* 381–382.