Acta Tropica 141 (2015) 198–203

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Evaluation of banked urine samples for the detection of circulating anodic and cathodic antigens in *Schistosoma mekongi* and *S. japonicum* infections: A proof-of-concept study

Govert J. van Dam[a,*], Peter Odermatt[b,c], Luz Acosta[d], Robert Bergquist[e], Claudia J. de Dood[f], Dieuwke Kornelis[a], Sinuon Muth[g], Jürg Utzinger[b,c], Paul L.A.M. Corstjens[f]

[a] Department of Parasitology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[b] Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland
[c] University of Basel, P.O. Box, CH-4003 Basel, Switzerland
[d] Research Institute for Tropical Medicine, Alabang, Muntinlupa City 1781, The Philippines
[e] Ingerod 407, S-45494 Brastad, Sweden
[f] Department of Molecular Cell Biology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[g] National Centre of Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh 12302, Cambodia

ARTICLE INFO

Article history:
Available online 16 September 2014

Keywords:
Asian schistosomiasis
Diagnosis
Circulating anodic antigen (CAA)
Circulating cathodic antigen (CCA)
Up-converting phosphor
Lateral flow strip test
Cambodia
Philippines

ABSTRACT

In Asia, *Schistosoma japonicum* is the predominant schistosome species, while *Schistosoma mekongi* is confined to limited foci in Cambodia and Lao People's Democratic Republic. While the People's Republic of China has been successful in controlling schistosomiasis, the disease remains a major public health issue in other areas. In order to prioritise intervention areas, not only accurate diagnosis is important but also other factors, such as practicality, time-efficiency and cost-effectiveness, since they strongly influence the success of control programmes. To evaluate the highly specific urine-based assays for the schistosome circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA), banked urine samples from Cambodia ($n = 106$) and the Philippines ($n = 43$) were examined by the upconverted phosphor lateral flow (UCP-LF) CAA assay and the point-of-care (POC)-CCA urine assay. Based on 250 μl urine samples, UCP-LF CAA sensitivity outcomes surpassed a single stool examination by the Kato-Katz technique. The banked urine samples in the current study did not allow the evaluation of larger volumes, which conceivably should deliver considerably higher readings. The sensitivity of a single urine POC-CCA was in the same order as that of a single Kato-Katz thick smear examination, while the sensitivity approached that of triplicate Kato-Katz when a combination of both CAA and CCA assays was used. The promising results from the current proof-of-concept study call for larger investigations that will determine the accuracy of the urine-based CCA and CAA assays for *S. mekongi* and *S. japonicum* diagnosis.

1. Introduction

An estimated 230 million people are infected with one of the six *Schistosoma* species that cause schistosomiasis, a chronic and debilitating disease of the tropics and subtropics (Colley et al., 2014). More than 90% of the global schistosomiasis burden is concentrated in Africa, where the infections are mainly due to *Schistosoma haematobium* and *Schistosoma mansoni* (Murray et al., 2012; Utzinger et al., 2009). In Asia, *Schistosoma japonicum* is found in the People's Republic of China (P.R. China), the Philippines (Olveda et al., 2014; Zhou et al., 2010) and Indonesia (Satrija et al., 2015), while the infection is caused by *Schistosoma mekongi* in Cambodia and Lao People's Democratic Republic (Lao PDR) (Muth et al., 2010).

Accurate determination of prevalence and intensity of infection are important for prioritising areas for interventions (Brooker et al., 2009). As long as the prevalence is medium or high (>10%), microscopy works reasonably well (Bergquist et al., 2009; Knopp et al., 2013; Utzinger et al., 2011), although multiple specimens may need to be tested. However, areas characterised by low prevalence (<5%) and low intensity of infection present a challenge, particularly when the overall prevalence falls below 1%, as many positive

* Corresponding author at: Department of Parasitology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 71 526 4400; fax: +31 71 526 6907.
E-mail address: G.J.van_Dam@lumc.nl (G.J. van Dam).

http://dx.doi.org/10.1016/j.actatropica.2014.09.003
0001-706X/

cases are missed if not diagnostics of considerably higher sensitivity are applied (van Dam et al., 2015). Reflecting this need, diagnostics development has concentrated on techniques based on the detection of the pathogen DNA. Such molecular methods have successfully been used for the detection of DNA from various schistosome species, including *S. japonicum* and *S. mekongi* (Clerinx et al., 2013; Wang et al., 2011). Additionally, similar levels of sensitivity have also been achieved with reagents active against the schistosome circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) in the blood and urine of infected hosts (Colley et al., 2013; Corstjens et al., 2014; van Dam et al., 2014). While the latter tests have been widely applied for the diagnosis of African schistosomiasis (*S. mansoni* and *S. haematobium*) as well as for *S. japonicum* infections (van Lieshout et al., 2000), detection of CAA and CCA in *S. mekongi* infections has not yet been studied. Besides the diagnostic shortcomings of stool microscopy, compliance to provide (multiple) faecal samples is a challenge, specifically in areas of lower prevalence and morbidity (Bergquist, 2013). Using urine as a diagnostic specimen would greatly enhance the possibility of reliable case finding.

While P.R. China has been uniquely successful in reducing prevalence and disease burden of *S. japonicum* infections over the past 60 years (Utzinger et al., 2005; Xu et al., 2015; Zhou et al., 2010), schistosomiasis is still a major problem in the Philippines (Bergquist and Tanner, 2010; Olveda et al., 2014). Presence of *S. mekongi* infections is limited to a few foci in northern Cambodia and southern Lao PDR (Muth et al., 2010).

Ultrasensitive diagnosis is a prerequisite for delineating the spatial distribution of *S. mekongi* and *S. japonicum* infections. Furthermore, sensitivity is imperative in the elimination stage, where the role of low-level infections play a critical role (Knopp et al., 2013; Rollinson et al., 2013; van Dam et al., 2014). This has convincingly been shown in Africa (Colley et al., 2013) and recently in P.R. China (van Dam et al., 2015). In order to extend the proof-of-principle not only by investigating various intensities of *S. japonicum* infection but also exploring the distribution of *S. mekongi* more exactly, sets of banked urine samples were used to evaluate the sensitivity of assays based on circulating antigens in areas where presently available diagnostic tools have proved inadequate.

2. Materials and methods

2.1. Study area

The current study was facilitated by banked urine samples collected some 20 years ago in schistosome-endemic areas of longstanding foci in Cambodia and the Philippines. In brief, urine samples from Cambodia were collected in 1995 from school-aged children in Krakor, a village situated approximately 5 km north of Kratié (northern Cambodia). Krakor is one of 20 villages along the Mekong River in the districts of Kratié and Sambo of Kratié province that had been subjected to *S. mekongi* risk profiling and subsequent control by the Cambodian Ministry of Health (MoH), in collaboration with Médecins Sans Frontières (Stich et al., 1999).

Urine samples from the Philippines were collected as part of a cohort study pertaining to human water contact patterns performed in 1993–1995 in the island of Leyte. The study was originally designed to investigate exposure and infection patterns in combination with cellular immunological mechanisms and evaluation of diagnostics (Acosta et al., 2002a,b; Webster et al., 1997).

Both studies were approved by the responsible authorities and consent was obtained according to the prevailing and acceptable procedures at that time, as detailed in the cited papers (Stich et al., 1999; Acosta et al., 2002b).

2.2. Sample collection and storage

In Cambodia, stool and urine samples were collected from 126 schoolchildren in the primary school of Krakor. Children were invited to provide three faecal samples over a period of five consecutive days. Additionally, on the first examination day, children provided a urine sample. Stool and urine samples were transferred to a central laboratory in Kratié. Faecal samples were subjected to the Kato-Katz technique using a single 41.7 mg thick smear (Katz et al., 1972). *S. mekongi* and other helminth eggs were counted and recorded separately for each sample with the geometric means calculated for egg counts from multiple samples. A small aliquot of urine (5–10 ml) was frozen and kept at −20 °C in the hospital laboratory in Kratié prior to transfer to the Department of Parasitology, Leiden University Medical Center (LUMC) in the Netherlands pending further analysis. Complete data records (i.e. at least 1 faecal sample and 1 urine sample examination) were finally available from 106 children.

In the Philippines, the stool samples were transferred to central laboratories and processed the same day according to Katz et al. (1972) using 2 slides of 50 mg faeces per sample. Urine samples were obtained from a sub-cohort from the larger study, frozen and transported to the LUMC for circulating antigen testing. Overall, a panel of 43 *S. japonicum* egg-positive individuals (according to Kato-Katz results) was selected for diagnostic evaluation by the CAA and CCA assays. Additionally, 10 urine samples for negative endemic controls were evaluated for specificity.

2.3. Urine examination

All urine samples were frozen and transferred to LUMC in Leiden, where they were stored at −20 °C until detailed follow-up. Overall, 106 urine samples from Cambodia and 43 urine samples from the Philippines were examined by an upconverted phosphor lateral flow (UCP-LF) assay for schistosome CAA. Samples were investigated both by the conventional as well as a concentration assay (UCAA10 and UCAA250 with wet reagents, as described elsewhere (Corstjens et al., 2014). Briefly, samples were diluted with an equal volume of 4% (w/v) tri-chloro-acetic acid (TCA) and centrifuged and assayed as such (UCAA10). Alternatively, the clear supernatants were reduced to amounts of 20–30 μl using a Millipore Ultra-0.5 device (UCAA250). The latter was only applied if, in the UCAA10, CAA levels were below 30 pg/ml (i.e. low or negative). After incubation with the UCP-antibody conjugate, strips were added and the samples allowed to run as described previously (van Dam et al., 2013). Following drying overnight, the strips were scanned for bound UCP using a Packard FluoroCount microtiter plate reader adapted with an IR laser (980 nm) modified to scan LF strips (Niedbala et al., 2001).

All samples were examined in one series, including two standard curves of serial dilutions of *S. mansoni* in a TCA-soluble fraction of adult worm antigen (AWA) containing 3% CAA (AWA-TCA) added to negative urine to allow quantitative CAA determination (pg/ml urine) as described elsewhere (Polman et al., 2000). The assay cut-offs were decided in accordance with Corstjens et al. (2014) using 10 pg/ml urine (UCAA10) and 1 pg/ml urine (UCAA250) as the lower limits of detection (LOD), and 5 pg/ml and 0.5 pg/ml urine as the LOD if the assay would have been performed with multiple samples under ideal laboratory conditions. The region between the two LODs was designated as 'indecisive', indicating that samples were suspected to be positive but that retesting would be required to truly ascertain the score, preferable using a higher sample volume.

Additionally, the commercially available point-of-care (POC)-CCA urine assay (Rapid Medical Diagnostics; Pretoria, South Africa) was performed, according to the manufacturer's instructions. The scoring of the test lines was done similarly to other groups who

have worked with the POC-CCA for the diagnosis of *S. mansoni* in different African settings (Colley et al., 2013; Coulibaly et al., 2011) with negative, 'trace', and three increasing intensity grades of positivity.

2.4. Statistical analysis

Data were entered in an Excel 2010™ spreadsheet. Statistical analysis was done with Excel and SPSS version 20 (IBM Corp.; Armonk, USA). Non-parametric statistics were used for correlation between test results. The sensitivity of the diagnostic tests was calculated and used for indication of test performance. For this reason, we applied a combined infection positive ('gold') standard, defined as being positive if a sample from an individual presented with *Schistosoma* spp. eggs at least once out of three faecal samples and/or found positive in the UCP-LF CAA assay and/or the POC-CCA. This approach implied that, by definition, the specificities of the individual tests were 100%. This was considered valid because of the very high specificities of the circulating antigen assays used (Midzi et al., 2009). Following the approach described previously (Coulibaly et al., 2013), an 'indecisive' or 'trace' category was defined and analysed separately, both for the UCAA and the POC-CCA results.

3. Results

3.1. Banked urine samples for S. mekongi diagnosis

Among the 106 children (age range 9–16 years, median 13 year) with complete data records (i.e. at least one stool and one urine sample examined), 62 were found positive for *S. mekongi* eggs in stool (58%); the infection intensity ranged between 0 and 480 eggs per 1 g of stool (EPG) with a median of 4 EPG. On the three consecutive days, the positive rates were 40%, 43% and 44%, respectively (although not all individuals supplied a stool sample each day). In addition to the 62 egg-positive cases, six positives were found by CAA and one by POC-CCA, resulting in a final positive rate of 65%. The sensitivities of the various assays, including the separate analysis with trace/indecisive results being considered as negative or positive *versus* our pre-defined 'gold standard', are shown in Table 1. There were 58 cases with complete egg count data and out of these the sensitivities of a single stool examination with one Kato-Katz thick smear *versus* triplicate Kato-Katz ranged between 38% and 45%. The 'true' prevalence, based on a readily available pocket-chart model would have been about 73% (de Vlas et al., 1993).

Three stool examinations with single Kato-Katz thick smears each could detect 90% of the positive individuals. The POC-CCA sensitivity was 39% (including trace positives, the sensitivity was 49%), the UCAA250 sensitivity was 57% (including indecisive positives, the sensitivity was 70%) and POC-CCA and/or CAA 68% (including trace positives, the sensitivity was 80%). In those corresponding to egg counts >100 EPG ($n = 18$), one case was missed by POC-CCA (trace positivity only) and two cases by the UCP-LF CAA test (sensitivity 94% and 89%, respectively). However, the combined POC-CCA/CAA results detected all cases with more than 100 EPG. Based on the 10 endemic negatives, the UCP-LF CAA high specificity cut-off was 0.23 pg/ml.

Fig. 1 depicts the correlations between the CAA concentrations and egg counts (panel A, Spearman's rho = 0.68, $p < 0.001$) and POC-CCA intensity scores (panel B, Spearman's rho = 0.60, $p < 0.001$) as well as between egg counts and POC-CCA (panel C, Spearman's rho = 0.64, $p < 0.001$). Panel A shows that the eight samples with CAA concentrations in the indecisive range from 0.5 (dotted line in Fig. 1) to 1 pg/ml were confirmed by positive egg counts. The data

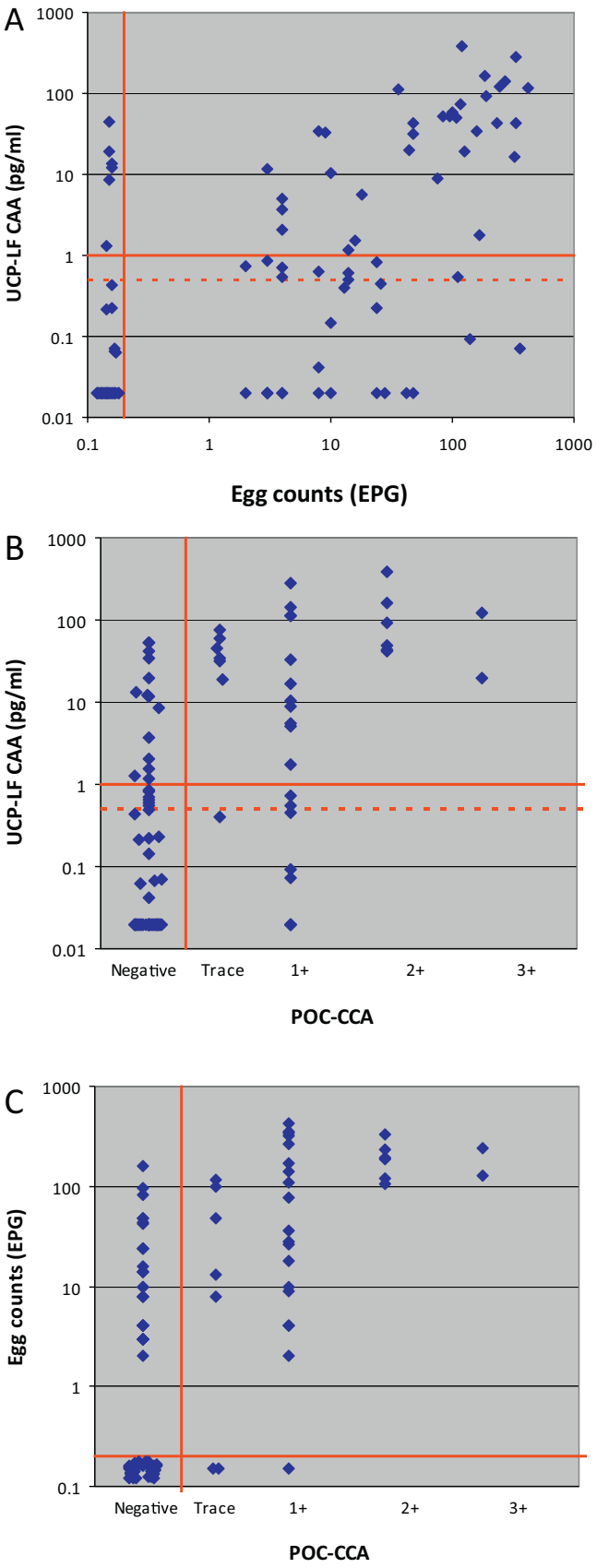

Fig. 1. Correlations of *S. mekongi* urine CAA levels (pg/ml) determined by the UCP-LF CAA test with (panel A) stool egg counts (mean EPG of 3 slides, 1 slide per stool), and with (panel B) POC-CCA intensity scores; panel C shows correlations between EPG and POC-CCA.
The solid line represents the high specificity cut-off for the UCP-LF CAA assay, while the region between the dotted line and the solid line is classified as 'indecisive'.

Table 1
Sensitivity of triplicate Kato-Katz and urine CCA and CAA assays against a standard of infection-positivity by either egg and/or CAA and/or CCA (assuming 100% specificity of all tests), in patient urine samples from Krakor, Cambodia ($n = 69$ positives).

Diagnostic test	No. pos	Sensitivity (%) (95% confidence interval)	No. pos	Sensitivity (%) (95% confidence interval)
	Trace/indecisive considered negative		Trace/indecisive considered positive	
Kato-Katz (3×)	62	90 (83–97)	62	90 (83–97)
POC-CCA	27	39 (28–51)	34	49 (37–61)
UCAA10	27	39 (28–51)	29	42 (30–54)
UCAA250	39	57 (45–68)	48	70 (59–80)
CCA and/or CAA[a]	47	68 (57–79)	55	80 (70–89)

[a] Considered positive as either POC-CCA or UCAA250 or both were positive, and trace if both or only one of the two tests scored trace; negative if both tests were negative.

also show that the UCP-LF CAA test in the currently used 250 μl format still renders 20 egg-positive cases undetected, but finds an additional six positive individuals. Panels B and C show that six, respectively five of the seven POC-CCA tests with 'trace' signals were confirmed positive by the UCP-LF CAA test or by the egg counts (all seven were confirmed positive by either one of the other two tests).

3.2. Banked urine samples for S. japonicum diagnosis

Of the 43 egg-positive cases (age range 3–69 years, median 13 year), 39 and 35 showed *S. japonicum* eggs in one of the duplicate Kato-Katz thick smears (sensitivity 91% and 81%, respectively), as shown in Table 2. The samples originated from a high-intensity area with egg counts ranging from 10 to 3,670 EPG (median 100 EPG). The sensitivities of the POC-CCA, UCAA10, UCAA250 and the CAA/CCA combined results (with trace and indecisive scores considered as positive), were 65%, 44%, 70% and 84%, respectively. The correlations between the antigen assays among themselves and with the egg counts were all statistically significant (Spearman, $p < 0.05$). With the combined POC-CCA/CAA results (trace/indecisive '+') all cases with >100 EPG were detected. All 10 negative endemic controls were negative in the POC-CCA and the UCAA assays.

4. Discussion

This proof-of-concept study highlights the potential usefulness of urine samples for a CCA and CAA diagnostic approach for Asian schistosomiasis. With respect to *S. mekongi*, this is the first time that the presence and detectability of CCA and CAA in patient samples has been demonstrated. The UCP-LF CAA assay, based on 250 μl urine samples, showed a satisfactory sensitivity level as its outcomes surpassed a single faecal sample examination using the widely used Kato-Katz technique. Laboratory studies indicate that also larger volumes of urine can be concentrated with significantly decreasing LOD levels and thus increasing sensitivities (Corstjens et al., 2014). Indeed, in an accompanying article (van Dam et al., 2015), a 2 ml urine concentration assay has been applied in a Chinese setting characterised by a very low prevalence of *S. japonicum*, resulting in a five-fold increase in prevalence. The banked urine samples in the current study did not allow the evaluation of larger volumes, which conceivably would have led to sensitivities at least equally high as those shown by the Kato-Katz results. While the sensitivity of the UCAA250 method was lower than that of triplicate Kato-Katz thick smears, the test identified an additional six positive cases. It has repeatedly been shown by studies carried out in different African settings, where *S. mansoni* is endemic, that the POC-CCA is capable of detecting many additional positive cases compared to Kato-Katz thick smears (Colley et al., 2013; Coulibaly et al., 2011; Shane et al., 2011). The UCP-LF CAA assay has not yet been largely evaluated in endemic settings but, based on results on the former (less sensitive) CAA-ELISA (enzyme-linked immunosorbent assay), considerably more positive cases should be expected (Van Lieshout et al., 1995).

Table 2
Sensitivity of single Kato-Katz and urine CCA and CAA assays against a standard of duplicate Kato-Katz (50 mg), in patient urine samples from Leyte, the Philippines ($n = 43$ positives).

	No. pos	Sensitivity (%) (95% confidence interval)
Kato-Katz slide 1	39	91 (82–99)
Kato-Katz slide 2	35	81 (70–93)
POC-CCA[a]	28	65 (51–79)
UCAA10[a]	19	44 (29–59)
UCAA250[a]	30	70 (56–83)
CCA and/or CAA[b]	36	84 (73–95)

[a] With traces and indecisives considered as positive.
[b] Considered positive as either POC-CCA or UCAA250 or both were positive, and trace if both or only one of the two tests scored trace; negative if both tests were negative.

The sensitivity of POC-CCA was in the same order as that of a single Kato-Katz and therefore relatively low. However, this is the first time that the commercially available POC-CCA test, which is already advocated as a mapping tool for the determination of *S. mansoni* prevalence in school-aged children in Africa (Colley et al., 2013), has been employed and validated in Asian settings. Based on the current results it holds promise as a simple, non-invasive screening tool in areas with medium to high *S. mekongi* or *S. japonicum* prevalence rates. Improved compliance can be expected because of urine rather than stool collection.

By combining the POC-CCA with the UCP-LF CAA assay, the sensitivity approached that of triplicate Kato-Katz thick smears. As it would consider feasible to develop a true, field-applicable CAA-based LF assay with the same qualifications as that of the UCAA250 assay, the approach to evaluate both assays for a urine-based assessment in control programmes of Asian schistosomiasis deserves further attention.

Visually read, LF tests in general suffer from the common appearance of 'trace' signals, i.e. very light lines. Therefore, in a thorough performance analysis, the results are usually analysed with both interpretations of the trace signals. The same can be applied to the indecisive range as defined for the UCP-LF CAA assay. Of note, our results showed that the "trace" and "indecisive" test results were confirmed to primarily originate from *Schistosoma* egg-positive individuals. This was also suggested by calculating a cut-off limit based on 10 endemic negative samples.

Given these promising observations, a larger study should be conducted with the aim to provide more evidence on the potential for the CAA and POC-CCA assays *versus* currently used diagnostic approaches in areas endemic for *S. mekongi* and *S. japonicum*. First, the study's sample size would need to be higher in order to increase the precision of the measured sensitivities. Second, population-based samples from different endemic settings would provide a better base for decision-making. This is of crucial importance with respect to *S. mekongi* infections, since prevalence rates vary considerably. For example, in Cambodia the current infection rates

(measured by Kato-Katz stool examination) in most endemic villages are below 5%, which differs from the endemic villages in Lao PDR where infection rates of 20% and more are frequently observed (Lovis et al., 2012; Sayasone et al., 2012). Furthermore, co-infection with other trematode species might influence test performance. In particular, the fish-borne trematode *Opisthorchis viverrini* is highly endemic in *S. mekongi*-endemic areas in Lao PDR and might therefore influence test outcomes (Lovis et al., 2012; Sayasone et al., 2011, 2012).

In conclusion, an adequate diagnostic approach is required with 'surveillance and response' approaches taking over from extensive monitoring and evaluation (Tambo et al., 2014). Highly sensitive diagnostic tools must be in place to confirm elimination or the potential beginning of resurgence of infection (Bergquist et al., 2015). These tools should follow the ASSURED characteristics as defined by the World Health Organization (i.e. affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to users). This proof-of-concept study, although performed on a limited number of samples collected some 20 years ago, underscores that CCA- and CAA-based assays would hold high expectations as promising candidates fulfilling these needs and requirements.

Acknowledgements

This study was mainly supported by the authors' individual institutions, while part of the UCP-LF CAA assay development received financial support from the University of Georgia Research Foundation, which was funded by the Bill & Melinda Gates Foundation for the SCORE project (http://score.uga.edu; subaward no. RR374-053/4692608).

References

Acosta, L.P., Aligui, G.D., Tiu, W.U., McManus, D.P., Olveda, R.M., 2002a. Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte. Philippines: I. Assessment of 'resistance' versus 'susceptibility' to *S. japonicum* infection. Acta Trop. 84, 127–136.

Acosta, L.P., Waine, G., Aligui, G.D., Tiu, W.U., Olveda, R.M., McManus, D.P., 2002b. Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte, Philippines: II. Cellular immune responses to *S. japonicum* recombinant and native antigens. Acta Trop. 84, 137–149.

Bergquist, R., 2013. Good things are worth waiting for. Am. J. Trop. Med. Hyg. 88, 409–410.

Bergquist, R., Tanner, M., 2010. Controlling schistosomiasis in Southeast Asia: a tale of two countries. Adv. Parasitol. 72, 109–144.

Bergquist, R., Johansen, M.V., Utzinger, J., 2009. Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol. 25, 151–156.

Bergquist, R., Yang, G.J., Knopp, S., Utzinger, J., Tanner, M., 2015. Surveillance and response: tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141, 229–234.

Brooker, S., Kabatereine, N.B., Gyapong, J.O., Stothard, J.R., Utzinger, J., 2009. Rapid mapping of schistosomiasis and other neglected tropical diseases in the context of integrated control programmes in Africa. Parasitology 136, 1707–1718.

Clerinx, J., Cnops, L., Huyse, T., Tannich, E., Van Esbroeck, M., 2013. Diagnostic issues of acute schistosomiasis with *Schistosoma mekongi* in a traveler: a case report. J. Travel. Med. 20, 322–325.

Colley, D.G., Binder, S., Campbell, C., King, C.H., Tchuem Tchuenté, L.A., N'Goran, E.K., Erko, B., Karanja, D.M., Kabatereine, N.B., 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.

Colley, D.G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. Lancet 383, 2253–2264.

Corstjens, P.L.A.M., de Dood, C.J., Kornelis, D., Tion Kon Fat, E.M., Wilson, R.A., Kariuki, T.M., Nyakundi, R.K., LoVerde, P.T., Abrams, W.R., Tanke, H.J., van Lieshout, L., Deelder, A.M., van Dam, G.J., 2014. Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow-based assays and up-converting phosphor labels. Parasitology. 141, 1841–1855.

Coulibaly, J.T., Knopp, S., N'Guessan, N.A., Silué, K.D., Fürst, T., Lohourignon, L.K., Brou, J.K., N'Gbesso, Y.K., Vounatsou, P., N'Goran, E.K., 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.

Coulibaly, J.T., N'Gbesso, Y.K., Knopp, S., N'Guessan, N.A., Silué, K.D., van Dam, G.J., N'Goran, E.K., 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.

de Vlas, S.J., Gryseels, B., van Oortmarssen, G.J., Polderman, A.M., Habbema, J.D., 1993. A pocket chart to estimate true *Schistosoma mansoni* prevalences. Parasitol. Today 9, 305–307.

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.

Knopp, S., Becker, S.L., Ingram, K.J., Keiser, J., Utzinger, J., 2013. Diagnosis and treatment of schistosomiasis in children in the era of intensified control. Expert Rev. Anti-Infect. Ther. 11, 1237–1258.

Lovis, L., Mak, T.K., Phongluxa, K., Aye Soukhathammavong, P., Vonghachack, Y., Keiser, J., Vounatsou, P., Tanner, M., Hatz, C., Utzinger, J., Odermatt, P., Akkhavong, K., 2012. Efficacy of praziquantel against *Schistosoma mekongi* and *Opisthorchis viverrini*: a randomized, single-blinded dose-comparison trial. PLoS Negl. Trop. Dis. 6, e1726.

Midzi, N., Butterworth, A.E., Mduluza, T., Munyati, S., Deelder, A.M., van Dam, G.J., 2009. Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. 103, 45–51.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., et al., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2197–2223.

Muth, S., Sayasone, S., Odermatt-Biays, S., Phompida, S., Duong, S., Odermatt, P., 2010. *Schistosoma mekongi* in Cambodia and Lao People's Democratic Republic. Adv. Parasitol. 72, 179–203.

Niedbala, R.S., Feindt, H., Kardos, K., Vail, T., Burton, J., Bielska, B., Li, S., Milunic, D., Bourdelle, P., Vallejo, R., 2001. Detection of analytes by immunoassay using up-converting phosphor technology. Anal. Biochem. 293, 22–30.

Olveda, D.U., Li, Y.S., Olveda, R.M., Lam, A.K., McManus, D.P., Chau, T.N.P., Harn, D.A., Williams, G.M., Gray, D.J., Ross, A.G.P., 2014. Bilharzia in the Philippines: past, present, and future. Int. J. Infect. Dis. 18, 52–56.

Polman, K., Diakhate, M.M., Engels, D., Nahimana, S., Van Dam, G.J., Falcao Ferreira, S.T., Deelder, A.M., Gryseels, B., 2000. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. Trop. Med. Int. Health 5, 534–537.

Rollinson, D., Knopp, S., Levitz, S., Stothard, J.R., Tchuem Tchuenté, L.A., Garba, A., Mohammed, K.A., Schur, N., Person, B., Colley, D.G., Utzinger, J., 2013. Time to set the agenda for schistosomiasis elimination. Acta Trop. 128, 423–440.

Satrija, F., Ridwan, Y., Jastal, Samarang, Rauf, A., 2015. Current status of schistosomiasis in Indonesia. Acta Trop. 141, 349–353.

Sayasone, S., Mak, T.K., Vanmany, M., Rasphone, O., Vounatsou, P., Utzinger, J., Akkhavong, K., Odermatt, P., 2011. Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasak Province, Lao People's Democratic Republic. PLoS Negl. Trop. Dis. 5, e1037.

Sayasone, S., Rasphone, O., Vanmany, M., Vounatsou, P., Utzinger, J., Tanner, M., Akkhavong, K., Hatz, C., Odermatt, P., 2012. Severe morbidity due to *Opisthorchis viverrini* and *Schistosoma mekongi* infection in Lao People's Democratic Republic. Clin. Infect. Dis. 55, e54–e57.

Shane, H.L., Verani, J.R., Abudho, B., Montgomery, S.P., Blackstock, A.J., Mwinzi, P.N., Butler, S.E., Karanja, D.M., Secor, W.E., 2011. Evaluation of urine CCA assays for detection of *Schistosoma mansoni* infection in western Kenya. PLoS Negl. Trop. Dis. 5, e951.

Stich, A.H., Biays, S., Odermatt, P., Men, C., Saem, C., Sokha, K., Ly, C.S., Legros, P., Philips, M., Lormand, J.D., Tanner, M., 1999. Foci of *Schistosomiasis mekongi*, northern Cambodia: II. Distribution of infection and morbidity. Trop. Med. Int. Health 4, 674–685.

Tambo, E., Ai, L., Zhou, X., Chen, J.H., Hu, W., Bergquist, R., Guo, J.G., Utzinger, J., Tanner, M., Zhou, X.N., 2014. Surveillance-response systems: the key to elimination of tropical diseases. Infect. Dis. Poverty 3, 17.

Utzinger, J., Zhou, X.N., Chen, M.G., Bergquist, R., 2005. Conquering schistosomiasis in China: the long march. Acta Trop. 96, 69–96.

Utzinger, J., Raso, G., Brooker, S., de Savigny, D., Tanner, M., Ørnbjerg, N., Singer, B.H., N'Goran, E.K., 2009. Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 136, 1859–1874.

Utzinger, J., N'Goran, E.K., Caffrey, C.R., Keiser, J., 2011. From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis. Acta Trop. 120 (Suppl. 1), S121–S137.

van Dam, G.J., de Dood, C.J., Lewis, M., Deelder, A.M., van Lieshout, L., Tanke, H.J., van Rooyen, L.H., Corstjens, P.L., 2013. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of *Schistosoma* circulating anodic antigen. Exp. Parasitol. 135, 274–282.

van Dam, G.J., Xu, J., Bergquist, R., de Dood, C.J., Utzinger, J., Qin, Z.Q., Guan, W., Feng, T., Yu, X.L., Zhou, J., Zheng, M., Zhou, X.N., Corstjens, P.L.A.M., 2015. An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of *Schistosoma japonicum* in a low-endemic area, People's Republic of China. Acta Trop. 141, 190–197.

Van Lieshout, L., Panday, U.G., De Jonge, N., Krijger, F.W., Oostburg, B.F., Polderman, A.M., Deelder, A.M., 1995. Immunodiagnosis of schistosomiasis mansoni in a low endemic area in Surinam by determination of the circulating antigens CAA and CCA. Acta Trop. 59, 19–29.

van Lieshout, L., Polderman, A.M., Deelder, A.M., 2000. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop. 77, 69–80.

Wang, C., Chen, L., Yin, X., Hua, W., Hou, M., Ji, M., Yu, C., Wu, G., 2011. Application of DNA-based diagnostics in detection of schistosomal DNA in early infection and after drug treatment. Parasit. Vectors 4, 164.

Webster, M., Libranda-Ramirez, B.D., Aligui, G.D., Olveda, R.M., Ouma, J.H., Kariuki, H.C., Kimani, G., Olds, G.R., Fulford, A.J., Butterworth, A.E., Dunne, D.W., 1997. The influence of sex and age on antibody isotype responses to *Schistosoma mansoni* and *Schistosoma japonicum* in human populations in Kenya and the Philippines. Parasitology 114, 383–393.

Xu, J., Xu, J.F., Li, S.Z., Zhang, L.J., Wang, Q., Zhu, H.H., Zhou, X.N., 2015. Integrated control programmes for schistosomiasis and other helminth infections in P.R. China. Acta Trop. 141, 332–341.

Zhou, X.N., Bergquist, R., Leonardo, L., Yang, G.J., Yang, K., Sudomo, M., Olveda, R., 2010. Schistosomiasis japonica: control and research needs. Adv. Parasitol. 72, 145–178.