Comparison of novel and standard diagnostic tools for the detection of Schistosoma mekongi infection in Lao People’s Democratic Republic and Cambodia

Youthanavanh Vonghachack 1,2,3, Somphou Sayasone 4, Virak Khieu 5, Robert Bergquist 6, Govert J. van Dam 7, Pytsje T. Hoekstra 7, Paul L. A. M. Corstjens 8, Beatrice Nickel 2,3, Hanspeter Marti 2,3, Jürg Utzinger 2,3, Sinuon Muth 5 and Peter Odermatt 2,3*

Abstract

Background: Given the restricted distribution of Schistosoma mekongi in one province in Lao People’s Democratic Republic (Lao PDR) and two provinces in Cambodia, together with progress of the national control programmes aimed at reducing morbidity and infection prevalence, the elimination of schistosomiasis mekongi seems feasible. However, sensitive diagnostic tools will be required to determine whether elimination has been achieved. We compared several standard and novel diagnostic tools in S. mekongi-endemic areas.

Methods: The prevalence and infection intensity of S. mekongi were evaluated in 377 study participants from four villages in the endemic areas in Lao PDR and Cambodia using Kato-Katz stool examination, antibody detection based on an enzyme-linked immunosorbent assay (ELISA) and schistosome circulating antigen detection by lateral-flow tests. Two highly sensitive test systems for the detection of cathodic and anodic circulating antigens (CCA, CAA) in urine and serum were utilized.

Results: Stool microscopy revealed an overall prevalence of S. mekongi of 6.4% (one case in Cambodia and 23 cases in Lao PDR), while that of Opisthorchis viverrini, hookworm, Trichuris trichiura, Ascaris lumbricoides and Taenia spp. were 50.4%, 28.1%, 3.5%, 0.3% and 1.9%, respectively. In the urine samples, the tests for CCA and CAA detected S. mekongi infections in 21.0% and 38.7% of the study participants, respectively. In the serum samples, the CAA assay revealed a prevalence of 32.4%, while a combination of the CAA assay in serum and in urine revealed a prevalence of 43.2%. There was a difference between the two study locations with a higher prevalence reached in the samples from Lao PDR.

(Continued on next page)
Conclusions: The CCA, CAA and ELISA results showed substantially higher prevalence estimates for S. mekongi compared to Kato-Katz thick smears. Active schistosomiasis mekongi in Lao PDR and Cambodia might thus have been considerably underestimated previously. Hence, sustained control efforts are still needed to break transmission of S. mekongi. The pivotal role of highly sensitive diagnostic assays in areas targeting elimination cannot be overemphasised.

Keywords: Cambodia, Food-borne trematodes, Kato-Katz, Lao People’s Democratic Republic, Point-of-care circulating cathodic antigen, Schistosoma mekongi, Serology, Soil-transmitted helminths, Up-converting phosphor-lateral-flow circulating anodic antigen

Multilingual abstracts
Please see Additional file 1 for translations of the abstract into the five of the six, official working languages of the United Nations.

Background
Human schistosomiasis is caused by any of six species of blood flukes, namely Schistosoma mansoni, S. japonicum, S. haematobium, S. mekongi, S. intercalatum and S. guineensis [1]. The latter three species are not only in a clear minority but are also geographically restricted. S. intercalatum is endemic along part of Congo River and S. guineensis is found in lower Guinea on the African continent, while S. mekongi exists in limited areas near the border between Lao People’s Democratic Republic (Lao PDR) and Cambodia. Transmission of S. mekongi is highly focal [2, 3] with the overall distribution delineated by environmental variables suitable for the intermediate host snail Neotricula aperta [4]. The at-risk population is estimated at around 50,000 households comprising an estimated 150,000 people [2] (Fig. 1). Infection and re-infection in the endemic areas sustain the severe, chronic consequences of schistosome infection with its various complications [5]. Due to their high level of water contact, children are at the highest risk, which might result in retardation of growth and cognitive development.

The World Health Organization (WHO) roadmap for elimination of neglected tropical diseases (NTDs) [6] and the Regional Action Plan for NTDs in the Western Pacific Region for 2012–2016 issued by WHO’s Western Pacific Region Office (WPRO) [7] recommend targeting schistosomiasis mekongi for elimination. Delineation of infection occurrence based on valid documentation is a necessary step to reach this goal and success depends crucially on the availability of highly sensitive diagnostic techniques providing non-equivocal prevalence values in remaining endemic pockets. In mass deworming campaigns, schistosomiasis is treated with oral single-dose praziquantel (40 mg/kg body weight) since the early 1980s when this drug was introduced [8]. The current approach in communities affected by S. mekongi consists of preventive chemotherapy targeting at-risk populations (e.g. the entire population of villages along the Mekong) without prior diagnosis, complemented with the distribution of information, education and communication (IEC) packages and improvement of water, sanitation and hygiene (WASH) whenever resources allow [9]. The next stage now being considered is the elimination of this infection as a public health problem. Given its restricted distribution, eradication of S. mekongi might be envisaged. However, the preventive chemotherapy programmes implemented in endemic areas in Cambodia and Lao PDR make individuals harbouring mainly light-intensity infections likely to be missed by the standard and widely used Kato-Katz thick smear technique [10], resulting in imprecise assessment of the impact of preventive chemotherapy and other interventions. The solution lies in modifying the methodology applied according to the prevailing diagnostic need [11], which obliges assays to be more sensitive and specific when priorities shift from control of morbidity to interruption of transmission followed by surveillance [11, 12].

Apart from egg deposition, schistosome worms excrete (regurgitate) a number of different antigens into the host’s blood. The circulating anodic antigen (CAA) and its cathodic counterpart (CCA), described by Deelder and colleagues as early as in 1976 [13], are the most well-studied ones. Their detection in serum and urine has been followed up with continuously improving techniques, e.g. by de Jonge et al. [14], van Lieshout et al. [15], van Dam et al. [16] and Corstjens et al. [17]. Importantly, detection of the antigens in either blood or urine is evidence for an ongoing active infection as both antigens are subject to rapid renal clearance from the human circulation [13]. On the other hand, stool examination is marred by the problem that schistosome eggs can be detected up to several weeks after cure [14].

Diagnostic assays should preferably be applied in the field providing results at the point-of-care (POC) to allow appropriate test-and-treat approaches. Diagnostics utilizing the user-friendly, rapid-test platform based on lateral-flow (LF) immunochromatography are well suited for this type of test protocols [18]. As an alternative to...
egg detection in stool samples, a rapid POC assay for CCA detection in urine (POC-CCA) was developed for *S. mansoni* infection [16]. The POC-CCA assay is a visually read field assay, which takes about 20 min to perform and which does not require any equipment except the disposables provided with the kit. The colour intensity of the test line on the LF strip has a correlation to the number of eggs in the stool sample investigated [19, 20] and the read-out is at least as sensitive as duplicate Kato-Katz thick smears and considerably less laborious [21–23]. Although it was specifically developed for detection of *S. mansoni* infections, it has been shown to be suitable for other intestinal schistosomiasis-causing species such as *S. mekongi* and *S. japonicum* [24]. In order to increase sensitivity and wider applicability to other schistosome species, another LF-based test that detects CAA specifically and is based on the luminescent up-converting phosphor (UCP) reporter technology has been developed [17]. This test is referred to as UCP-LF CAA and includes different formats, depending on the matrix and sample volume used for testing [25]. The UCP-LF CAA test provides an assay applicable for all known *Schistosoma* species (including veterinarian ones) and is assumed to allow detection down to the level of a single worm pair while maintaining 100% specificity [25]. The CAA concentration is considered a good proxy for the number of worms present in the host [17]. This approach has been shown to work for *S. mansoni* [26] and *S. haematobium* infections [27, 28] as well as for infections by *S. japonicum* [29] and *S. mekongi* [24]. In the People’s Republic of China, the UCP-LF CAA assay demonstrated a *S. japonicum* prevalence of about 10 times higher than that estimated by triplicate Kato-Katz thick smears [29]. However, unlike the POC-CCA, the current UCP-LF CAA assay format is still a laboratory-based assay due to the need of centrifugation steps, hence not yet convenient for POC test-and-treat approaches.

Realizing that verification of transmission interruption requires a high level of sensitivity, we aimed to evaluate the new diagnostic techniques and to compare their results to the standard tools (e.g. Kato-Katz). We used
the UCP-LF CAA assay formats to validate the POC-CCA test results. The POC-CCA test was expected to have higher sensitivity than the stool examination. As an extra control for sensitivity, Schistosoma serology based on an enzyme-linked immunosorbent assay (ELISA) was included as all active infections indicated by the POC-CCA assay should test positive with this approach unless the infection was only acquired very recently. It has to be noted that a positive test for specific antibodies is not only assured during active infections but indicate also former infections as antibody titres normally persist for a long time. This study compared a set of available assays to get a handle on the real prevalence and intensity of S. mekongi infections in the endemic enclaves in Cambodia and Lao PDR as the results should indicate a negotiable way forward with regard to elimination of the disease.

Methods

Study design, area and population
A cross-sectional study was conducted between February and April 2016 in S. mekongi-endemic villages in Lao PDR and Cambodia. Repeated stool examinations for intestinal helminth infections were conducted with particular emphasis on S. mekongi infection. Furthermore, urine and serum samples were obtained from each study participant to be tested for Schistosoma infection by the POC-CCA, UCP-LF CAA and ELISA assays.

Four villages, two in each of the endemic districts of Lao PDR and Cambodia, respectively, were selected. The villages Som VenOok and Ban Yai VeunSom in Khong district, Champasack province in southern Lao PDR were selected together with the villages Kbal Chuor and Sre Khoeurn in Kratié province in northern Cambodia (Fig. 1). The main occupation of the villagers was farming and fishing. All household members older than 6 years were enrolled. They were invited to fill in a questionnaire pertaining to demographic details and risk factors for infection, information on hygiene, disease knowledge and anthelminthic drugs taken during the last 6 months.

In Lao PDR, about 200 individuals living in Som VenOok and Ban Yai VeunSom, situated on islands in the Mekong River, were approached about the study. The study households were randomly selected from a list of households of the two villages. In Cambodia, according to the 2008 census, the total population was 2339 people (1602 in Kbal Chuor and 737 in Sre Khoeurn). Between 120 and 130 individuals were randomly selected from 30 to 35 households in each village.

Sample collection and handling

Stool samples
Three stool samples were obtained from each participant during five consecutive days. Stool samples were subjected to examination by duplicate Kato-Katz thick smears (41.7 mg stool per smear) examined under a light microscope [30] by an experienced technician within 1 h after preparation on site in the study villages. Prior to microscopy, the thick smears were allowed to clear for 30 min after set-up. Eggs of all intestinal helminth species were counted and recorded for each species separately. The Kato-Katz thick smear examinations were performed directly in a convenient place in the study village (i.e. the village temple in Lao PDR; the village chief’s house in Cambodia).

Serum and urine samples
Blood samples were obtained from each participant, i.e. 5 ml venous blood (taken with vacutainers without anticoagulant) for serodiagnosis of S. mekongi infection and for the UCP-LF CAA assay. Urine samples (i.e. 10 ml urine) were obtained for CCA/CAA examination. Blood and urine samples were stored in cool-boxes at around 4 °C. In Cambodia, blood samples were centrifuged at Kratié Provincial Hospital a few hours after collection. Coagulated blood samples were centrifuged at 3000 rpm for 5 min and the upper part (serum) transferred to fresh tubes that were frozen and kept at −20 °C immediately after spinning, while the urine samples were directly frozen at −20 °C in the 15 ml-tubes they were collected in [29].

All samples were transferred frozen to a central national laboratory in Cambodia or Lao PDR and eventually shipped on dry ice to speciality laboratories at Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland and Leiden University Medical Center (LUMC) in Leiden, The Netherlands.

Laboratory procedures

Detection of S. mekongi antibodies
Schistosoma serology was performed by ELISA at Swiss TPH using S. mansoni adult worm extract (AWE) and S. mansoni soluble egg antigen (SEA). Both S. mansoni antigens show cross-reactivity with antibodies elicited by other Schistosoma spp. (S. haematobium, S. mekongi or S. japonicum). The combination of both serological tests exhibits a sensitivity of 94.5% for S. mekongi infections and a specificity of 96% and 92% for AWE and SEA, respectively [31, 32].

AWE was prepared as described previously [31]. In brief, adult S. mansoni worms were homogenized in phosphate-buffered saline (PBS) of pH 7.2 containing 2-mM phenylmethylsulfonyl fluoride (PMSE). The extract was centrifuged at 80,000 g for 3 h at 4 °C and the pellet further extracted with PBS containing 1% Nonidet P40. After overnight incubation at 4 °C, the suspension was centrifuged again in the same way. After the supernatant had been concentrated and centrifuged at 15,300 g
for 5 min at 4 °C, it was stored in aliquots at −80 °C until use. SEA was made from frozen S. mansoni eggs homogenized in PBS of pH 7.2 on ice and subsequently extracted for 3 h at 4 °C. The extract was centrifuged at 100,000 g for 2 h at 4 °C and the supernatant was stored in aliquots at −80 °C until use.

ELISA testing was carried out using Immulon 2HB plates (Thermo Labsystems; Beverly, MA, USA) coated with S. mansoni antigens in 0.05 M sodium carbonate buffer (pH 9.6) for 48 h at 4 °C. After washing with tap water containing 0.05% Tween 20, diluted sera (1:160 in PBS, pH 7.2, 0.05% Tween 20) were added to the plates that were incubated for 15 min at 37 °C. After additional washing steps, horseradish peroxidase conjugated goat-anti-human-IgG from Kirkegaard & Perry Laboratories (KPL) was added. Plates were incubated for 15 min at 37 °C, subsequently washed and o-phenylenediamine dihydrochloride (OPD) from Sigma (http://www.sigmaaldrich.com), diluted in 0.6-M sodium phosphate buffer of pH 5.0 supplemented with 0.03% H2O2, was added. The reaction was stopped with 8-M H2SO4 and the absorption read with a Thermo Scientific Multiscan FC reader (http://corporate.thermofisher.com) at 492 nm. The results of the ELISA tests were interpreted according to the cut-offs previously determined by receiver operating characteristic (ROC) analysis with sera from healthy Swiss blood donors, sera from S. mansoni infected patients and sera from patients with other parasitic infections, as described before [31, 32].

Detection of circulating schistosome antigens

This part of the study was carried out at LUMC. The POC-CCA test devices were obtained from Rapid Medical Diagnostics (Pretoria, South Africa) and tests were performed according to the manufacturer’s description. The amount of urine analysed per strip was 30 µl applied by pipette. Test results were visually interpreted, including distinction of trace-signals (weak colouration of the test line).

The UCP-LF CAA assay for urine was performed with 2 ml urine (the UCAA2000 assay format) as described earlier [25]. In short, 2 ml urine was extracted with 2-ml 4% (w/v) trichloroacetic acid (TCA). An Amicon centrifugal filtration device was used to concentrate the resulting clear supernatant (approximately 4 ml) to a final volume of 20–30 µl, of which 20 µl was analysed on UCP-LF CAA test strips using the wet-reagents format [25]. CAA concentrations were determined from standard series spiked in a negative urine sample and treated similarly to the clinical urine samples. The quality control (QC) cut-off threshold for singlet testing using the UCAA2000 wet-assay is 0.1 pg CAA per ml urine and the lower limit of detection = 0.05 pg/ml for testing performed in triplicate. Samples generating test results with a concentration between 0.05 and 0.1 pg per ml were counted as indecisive; samples with test results below 0.05 pg were considered CAA-negative [25].

The UCP-LF CAA assay for serum was performed with 0.5 ml serum (SCAA500) as described earlier [25]. The procedure was the same as described above with the difference that 0.5 ml TCA serum supernatant was concentrated to a final volume of 20 µl and the QC cut-off threshold was 1 pg CAA per ml serum with the lower limit of detection = 0.5 pg/ml. Samples generating test results with a concentration between 0.5 and 1 pg per ml were counted as indecisive; samples with test results below 0.5 pg were considered CAA-negative [25].

Note that the ultrasensitive assay format, specifically the SCAA500 test, is considered to allow identification of the majority of all active infections (including single-worm ones) [25]. In order to achieve the highest specificity, results were analysed considering the POC-CCA trace scores as well as the urine- and serum-CAA indecisive scores as negative. As this is a preliminary analysis, we decided to follow a conservative approach [25]. Generally, samples generating test results in the indecisive category would ideally require retesting with a larger sample volume to verify the true infection status.

Statistical analysis

Demographic details of participants and their exposure to infection were obtained by questionnaire. Data were digitally collected using electronic tablets. The questionnaires and forms were developed in Commcare (http://www.commcarehq.org) format using the open data kit (ODK) programme (version 2.8) that was installed on the tablets for field data collection. Statistical analyses were performed in STATA version 13.1 (Stata Corp.; College Station, TX, USA). Only results from participants who had completed their questionnaires and stool examination were included in the final analysis.

The intensity of infection, expressed as eggs per gram of stool (EPG) obtained from Kato-Katz thick smear examinations were classified as light, moderate or heavy [33, 34]. The χ2-test was used to examine the association of categorical variables. The Spearman rank correlation test was used to correlate the results of the different diagnostic tests with each other. Spearman r- and p-values were reported. A p-value below 5% was considered statistically significant.

The Schistosoma ELISA assay (a marker of former or active infection) was composed of two separate assays, one based on AWE and the other based on SEA. For this study both these ELISAs were combined and an overall interpretation of both test results was applied. A result was interpreted as positive if at least one of the two ELISA tests was positive. A result was interpreted as
inconclusive if both ELISA tests generated an inconclusive result. A result was considered negative if both ELISA were negative.

**Combined reference**

We compared the diagnostic performance of all our stool, urine- and serum-based diagnostic tests to a combined reference. This consisted of a combination of test results of the Kato-Katz test, the urine-CAA test and the CAA-serum assay, all assays with a very high specificity, in particular as we followed a conservative approach accepting a relatively high cut-off threshold for the CAA tests. The sensitivity, specificity, positive and negative predictive values were calculated for all diagnostic tests based on this composite measure. For these calculations, traces and indecisive tests results of CAA and ELISA were taken as negative results. The urine- and serum-CAA tests were also combined into a total CAA outcome, which was deemed as positive when at least one of the two tests produced a positive outcome. This approach of comparing assays to a combined reference is a widely recognized method for assessment of diagnostic tests in the absence of a highly sensitive and specific ‘gold’ standard method and has been recommended by the WHO/TDR Diagnostics Evaluation Expert Panel [35].

**Results**

**Study population**

Data records could be completed for a total of 377 persons and they were included in the analysis carried out as shown schematically in Fig. 2. Of these, 196 (52.0%) were from Cambodia and 181 (48.0%) from Lao PDR. The age of the participants ranged from 6 to 79 years with a median of 25 years; slightly more females than males were enrolled (52.3% versus 47.8%). About half of the participants had finished primary school (53.3%); most of them were subsistence rice farmers and fishermen (61.3%). The social and demographic characteristics of study participants are summarised in Table 1.

**Egg detection**

The status of the participants according to helminth infection intensity categories is shown in Table 2. Overall, *S. mekongi* infection prevalence was 6.4% (24/377) with a much higher prevalence of 12.7% (23 positives) in Lao PDR, compared to 0.5% (one positive) in Cambodia. The overall results for prevalence of other helminth infections, such as *O. viverrini*, hookworm, *Trichuris trichiura*, *Ascaris lumbricoides* and *Taenia* spp. were 50.4%, 28.1%, 3.5%, 0.3% and 1.9%, respectively. Significantly higher prevalence rates were found for *O. viverrini* (90.1%), hookworm (50.8%) and *Taenia* (3.3%) in Lao PDR. Multiparasitism was observed in both countries with much higher frequency in Lao PDR than in Cambodia. Table 3 shows infection intensity categories recorded as EPGs. All infections were found to be light in Cambodia, while a large number of the *O. viverrini* infections were of moderate intensity in Lao PDR; some heavy infections (4 out of 181) were also identified there.

**Antigen detection**

In total, 377 urine and serum samples were tested for *S. mekongi* infection (Table 4). In the urine samples, the CCA- and CAA-based test formats detected *S. mekongi* infections in 21.0% and 38.7% of all subjects, respectively. Compared to Cambodia, both urine tests diagnosed a higher *S. mekongi* prevalence in Lao PDR: 23.8% versus 18.4% with respect to CCA, and 42.5% versus 35.2% with respect to CAA. In serum, the latter test format detected a 32.4% overall prevalence with a similar difference between the two countries as found with the urine samples, 26.0% for Cambodia versus 39.2% for Lao PDR.

**Detection of *S. mekongi* antibodies**

The combined results of the two ELISA tests were positive in 34.5% of study participants, with a more
### Table 1  Demographic characteristics of the study participants

| Parameter                  | Overall       | Cambodia     | Lao PDR      | $\chi^2b$ | $P$-value$^c$ |
|----------------------------|---------------|--------------|--------------|-----------|---------------|
| Number of subjects         | 377 (100)     | 196 (52.0)   | 181 (48.0)   |           |               |
| Age (years)                |               |              |              |           |               |
| Median (IQR)$^a$           | 25 (12–44)    | 14 (11–35)   | 35 (15–47)   | NA        | NA            |
| Sex                        |               |              |              |           |               |
| Male                       | 180 (47.8)    | 101 (51.5)   | 79 (43.7)    |           |               |
| Female                     | 197 (52.3)    | 95 (48.5)    | 102 (56.4)   | 2.3       | 0.126         |
| Age group (years)          |               |              |              |           |               |
| $\le$ 9                    | 40 (10.6)     | 25 (12.8)    | 15 (8.3)     |           |               |
| 10–16                      | 116 (30.8)    | 82 (41.8)    | 34 (18.8)    |           |               |
| 17–36                      | 92 (24.4)     | 43 (21.9)    | 49 (27.1)    |           |               |
| 37–50                      | 68 (18.0)     | 22 (11.2)    | 46 (25.4)    |           |               |
| $\ge$ 51                   | 61 (16.2)     | 24 (12.2)    | 37 (20.4)    | 33.5      | <0.001        |
| Educational level          |               |              |              |           |               |
| Illiterate                 | 11 (2.9)      | 0            | 11 (6.1)     |           |               |
| Primary school             | 201 (53.3)    | 101 (51.5)   | 100 (55.3)   |           |               |
| Secondary school           | 134 (35.5)    | 95 (48.5)    | 39 (21.6)    |           |               |
| High school                | 23 (6.1)      | 0            | 23 (12.7)    |           |               |
| Technical school or higher | 8 (2.1)       | 0            | 8 (4.4)      | 64.9      | <0.001        |
| Occupation                 |               |              |              |           |               |
| Farmer/fisherman           | 231 (61.3)    | 101 (51.5)   | 130 (71.8)   |           |               |
| Student                    | 146 (38.7)    | 95 (48.5)    | 51 (28.2)    | 16.3      | <0.001        |

$IQR^a$ Inter quantile range; $^b$comparison between countries; $^{NA}$, not applicable.

### Table 2  Prevalence of S. mekongi, O. viverrini and other helminth infections among all study participants according to Kato-Katz examination

| Helminth species            | Overall (%) | Cambodia (%) | Lao PDR (%) | $\chi^2a$ | $P$-value$^a$ |
|----------------------------|-------------|--------------|-------------|-----------|---------------|
| Number of subjects         | 377 (100)   | 196 (52.0)   | 181 (48.0)  |           |               |
| Trematode                  |             |              |             |           |               |
| Schistosoma mekongi        | 24 (6.4)    | 1 (0.5)      | 23 (12.7)   | 23.5      | <0.001        |
| Opisthorchis viverrini     | 190 (50.4)  | 27 (13.8)    | 163 (91.1)  | 219.0     | <0.001        |
| Nematode                   |             |              |             |           |               |
| Hookworm                   | 106 (28.1)  | 14 (7.1)     | 92 (50.8)   | 88.9      | <0.001        |
| Ascaris lumbricoides       | 1 (0.3)     | 0            | 1 (0.6)     | 1.1       | 0.297         |
| Trichuris trichiura        | 13 (3.5)    | 7 (3.6)      | 6 (3.3)     | 0.02      | 0.892         |
| Cestode                    |             |              |             |           |               |
| Taenia spp.                | 7 (1.9)     | 1 (0.5)      | 6 (3.3)     | 4.1       | 0.044         |
| Multiparasitism            |             |              |             |           |               |
| No infection               | 157 (41.6)  | 150 (76.5)   | 7 (3.9)     |           |               |
| Single infection           | 115 (30.5)  | 42 (21.4)    | 73 (40.3)   |           |               |
| Double infection           | 90 (23.9)   | 4 (2.0)      | 86 (47.5)   |           |               |
| Triple infection           | 14 (3.7)    | 0            | 14 (7.7)    |           |               |
| Quadruple infection        | 1 (0.3)     | 0            | 1 (0.6)     | 228.1     | <0.001        |

$^a$Comparison between countries
than 16% higher rate in Lao PDR than in Cambodia (43.1% versus 26.5%). For all the diagnostic tests performed, the positivity rates were statistically significantly higher in Lao PDR compared to Cambodia (Table 4).

Table 3 Intensity of helminth infections among the infected study participants according to Kato-Katz examination

| Species/Type of infection | Overall (%) | Cambodia (%) | Lao PDR (%) |
|---------------------------|-------------|--------------|-------------|
| Number of subjects        | 377         | 196          | 181         |
| Schistosoma mekongi       |             |              |             |
| Light infection           | 24 (100)    | 1 (100)      | 23 (100)    |
| Moderate infection        | 70 (36.8)   | 0            | 70 (42.9)   |
| Heavy infection           | 4 (2.1)     | 0            | 4 (2.5)     |
| Opisthorchis viverrini    |             |              |             |
| Light infection           | 116 (61.1)  | 27 (100)     | 89 (54.6)   |
| Moderate infection        | 70 (36.8)   | 0            | 70 (42.9)   |
| Hookworm                  |             |              |             |
| Light infection           | 104 (98.1)  | 14 (100)     | 90 (97.8)   |
| Moderate infection        | 2 (1.9)     | 0            | 2 (2.2)     |
| Ascaris lumbricoides      |             |              |             |
| Light infection           | 1 (100)     | 0            | 1 (100)     |
| Trichuris trichiura       |             |              |             |
| Light infection           | 12 (100)    | 6 (100)      | 6 (100)     |

Table 4 Diagnosis of S. mekongi infection using serum and urine samples (n = 377)

| Type of sample/method | Overall (%) | Cambodia (%) | Lao PDR (%) | $\chi^2$ | P-value |
|-----------------------|-------------|--------------|-------------|---------|---------|
| Urine                 |             |              |             |         |         |
| POC-CCA               |             |              |             |         |         |
| Negative              | 174 (46.2)  | 97 (49.5)    | 77 (42.5)   | 7.4     | 0.0068  |
| Trace                 | 124 (32.9)  | 63 (32.1)    | 61 (33.7)   |         |         |
| Positive              | 79 (21.0)   | 36 (18.4)    | 43 (23.8)   | 2.4     | 0.308   |
| UCAA                  |             |              |             |         |         |
| Negative              | 206 (54.6)  | 110 (56.1)   | 96 (53.0)   |         |         |
| Indecisive range      | 25 (6.6)    | 17 (8.7)     | 8 (4.4)     |         |         |
| Positive              | 146 (38.7)  | 69 (35.2)    | 77 (42.5)   | 4.0     | 0.133   |
| Serum                 |             |              |             |         |         |
| SCAA                  |             |              |             |         |         |
| Negative              | 240 (63.7)  | 133 (67.9)   | 107 (59.1)  |         |         |
| Indecisive range      | 15 (4.0)    | 12 (6.1)     | 3 (1.7)     |         |         |
| Positive              | 122 (32.4)  | 51 (26.0)    | 71 (39.2)   | 10.9    | 0.004   |
| ELISA combined$^a$    |             |              |             |         |         |
| Negative              | 115 (30.5)  | 76 (38.8)    | 39 (21.6)   |         |         |
| Equivocal             | 132 (35.0)  | 68 (34.7)    | 64 (35.4)   |         |         |
| Positive              | 130 (34.5)  | 52 (26.5)    | 78 (43.1)   | 16.7    | <0.001  |
| Combined Reference$^b$|             |              |             |         |         |
| Negative              | 203 (53.8)  | 121 (61.7)   | 82 (45.3)   |         |         |
| Positive              | 174 (46.2)  | 75 (38.3)    | 99 (54.7)   | 10.2    | 0.001   |

AWE, adult worm antigen; SEA, soluble egg antigen; $^a$either AWE or SEA positive; $^b$at least one of the three tests (UCAA, SCAA, Kato-Katz) positive

Analysis of tests using the combined reference

We defined an active infection as an individual found positive for CAA (in urine or serum) or with a positive Kato-Katz thick smear. Table 5 shows the calculated sensitivity and specificity and the predictive values of the urine and serum tests in relation to this composite measure. The combined CAA tests had the highest calculated sensitivity (93.7%), followed by the urine- (83.9%) and serum-CAA (70.1%) test. The combined ELISA tests had a calculated sensitivity of 52.9% and a specificity of 81.3% against this combined reference. Triplicate Kato-Katz and single POC-CCA had a comparatively low sensitivity of 13.8% and 24.1%, respectively, and a negative predictive value of 57.5% and 55.7%, respectively (Table 5).

Analysis with respect to age and sex

Table 6 shows the positivity rate of the different diagnostic tests in relation to sex and age-groups. In general, all tests showed a higher positivity rate in males. A peak of positivity can be observed for the CAA tests in the age group 10–16 years. The ELISA results did not decrease with age to the same extent which could be explained by persistence of antibody titres for long time even after cured infections.
**Table 5** Diagnostic characteristics of the various tests to diagnose *S. mekongi* infection using a combined reference

| Method          | ELISA<sup>b</sup> | Kato-Katz | POC-CCA | CAA | CAA | CAA |
|-----------------|-------------------|-----------|---------|-----|-----|-----|
| Target          | Antibodies        | Parasite eggs | Circulating antigens | Urine | Urine | Serum | Serum + urine |
| Sample          | Serum             | Faeces    | (%)     | (%) | (%) | (%) |
| Sensitivity     | 52.9              | 13.8      | 24.1    | 83.9 | 70.1 | 93.7 |
| Specificity     | 81.3              | 100       | 81.8    | 100  | 100  | 100  |
| PPV<sup>*</sup> | 70.8              | 100       | 53.2    | 100  | 100  | 100  |
| NPV<sup>**</sup>| 66.8              | 57.5      | 55.7    | 87.9 | 79.6 | 94.9 |

<sup>*</sup>Positive predictive value; <sup>**</sup>Negative predictive value; <sup>a</sup>Infection-positive by either egg- or CAA-positivity (serum and urine combined, assuming 100% specificity of the CAA result). <sup>b</sup>For the ELISA, either AWE and/or SEA positive was considered positive.

**Correlation analysis**

Correlation analysis of the different diagnostic tests showed positive and statistically significant correlations between urine- and serum-CAA (*r* = 0.64, *p* < 0.001) and combined ELISA tests with serum-CAA (*r* = 0.55, *p* < 0.001) and urine-CAA (*r* = 0.38, *p* < 0.001). Furthermore, weakly positive but statistically significant correlations were detected between the infection intensity results of Kato-Katz and ELISA (*r* = 0.14, *p* = 0.005), POC-CCA (*r* = 0.12, *p* = 0.017), and urine (*r* = 0.11, 0.005) and serum-CAA (*r* = 0.17, *p* = 0.001) (Fig. 3). The correlation of the POC-CCA test results with the other tests were all weakly positive but statistically significant for urine-CAA (*r* = 0.15, *p* = 0.003) and serum-CAA (*r* = 0.14, *p* = 0.005). The correlation between the test results of POC-CCA and ELISA were weakly positive but not statistically significant (*r* = 0.09, *p* = 0.083).

**Discussion**

The implementation of preventive chemotherapy has decreased schistosomiasis morbidity in endemic countries worldwide, including *S. mekongi* affected areas in Cambodia and Lao PDR [36, 37]. The current lower intensity of disease, however, is a compelling fact to recommend replacing stool examination using Kato-Katz with more sensitive diagnostic tools. Serology based on antibody detection is a helpful adjunct, but in order to determine cure and the level of worm burdens detection, assays based on the detection of circulating antigens are required. This approach has been successfully used for all major schistosome species showing that it is 10–20 times more sensitive than standard stool microscopy [17].

WHO recommends targeting schistosomiasis mekongi for elimination as the endemic areas are very limited and both stool examination according to Kato-Katz and antibody testing using ELISA serology indicate low intensity of disease after several rounds of preventive chemotherapy [6]. However, as has already been shown in the People’s Republic of China, highly sensitive tests for schistosome circulating antigens give considerably higher prevalence results than Kato-Katz [29]. With the proof-of-principle of testing for excreted antigens in the urine shown for *S. mekongi* [24], it was now felt that a field study in the endemic areas in southern Lao PDR and

**Table 6** Sex and age distribution of *S. mekongi* infection: results of various approaches

| Method          | ELISA | Kato-Katz | POC-CCA | CAA | CAA | CAA |
|-----------------|-------|-----------|---------|-----|-----|-----|
| Target          | Antibodies | Parasite eggs | Circulating antigens | Urine | Urine | Serum | Serum + urine |
| Sample          | Serum | Faeces    | (%)     | (%) | (%) | (%) |
| Sex             |       |           |         |     |     |     |
| Male            | 68 (37.8) | 15 (8.3)  | 39 (21.7) | 71 (39.4) | 65 (36.1) | 80 (44.4) |
| Female          | 62 (31.5) | 9 (4.6)   | 40 (20.3) | 75 (38.1) | 57 (28.9) | 83 (42.1) |
| Age group (years) |       |           |         |     |     |     |
| ≤ 9             | 3 (7.5)  | 1 (2.5)   | 6 (15.0)  | 8 (20.0)  | 6 (15.0)  | 9 (22.5)  |
| 10–16           | 44 (37.9) | 2 (1.7)   | 25 (21.6) | 56 (48.3) | 53 (45.7) | 64 (55.2) |
| 17–36           | 36 (39.1) | 10 (10.9) | 23 (25.0) | 39 (42.4) | 27 (29.4) | 42 (45.7) |
| 37–50           | 25 (36.8) | 6 (8.8)   | 9 (13.2)  | 22 (32.4) | 19 (27.9) | 24 (35.3) |
| ≥ 51            | 22 (36.1) | 5 (8.2)   | 16 (26.2) | 21 (34.4) | 17 (27.9) | 24 (39.3) |

Vonghachack et al. Infectious Diseases of Poverty (2017) 6:127
northern Cambodia would be warranted to establish this approach. In contrast to antibody detection, the Kato-Katz stool examinations along with the tests for circulating schistosome antigens (POC-CCA and UCP-LF-CAA) are all indicators of active infections. Antibody titres can persist for very long time after cure and therefore serology is not suitable for assessing treatment outcomes or as single diagnostic approach for detection of active infections.

In the field, detection of active infection and cure are all highly important, particularly when moving from control of a disease to transmission interruption and elimination. It is equally important for the individual patient. While the better sensitivity of antigen detection compared to Kato-Katz is obvious, it is also clear that CAA detection (both in serum and urine) performs much better than CCA. These results are in agreement with previous reports for *S. japonicum* and *S. mekongi* [24, 29, 38].

The advantage of the POC-CCA test is that it is a standardized urine test applicable in the field without the need for any extra equipment (fulfilling all ‘AS-SURED’ characteristics). It has been mainly and widely validated for *S. mansoni* detection, but shows limited use for the other schistosome species [23]. However specificity is limited to some extent, because CCA has epitopes common with certain human components (Lewis-X structures) that sometimes end up in the urine causing false positive reactions [39]. The UCP-LF CAA test, on the other hand, is applicable for all schistosome species and for various human liquid samples, such as urine and serum, as well as potentially saliva [25]. In contrast to the POC-CCA assay, the UCP-LF CAA test format is not yet commercially available nor is its current format applicable for POC application because of a sample preparation procedure and the use of an UCP strip-reader. While the cost of the former is US$ 1–1.5 per test, that of the latter, being a manual laboratory test, is at least 10-fold higher. However, as shown here, the UCP-LF CAA test does display a superior sensitivity by concentration of the clinical sample and may therefore detect single-worm infections [25]. Still, as our results show that the POC-CCA assay is applicable for field diagnosis of *S. mekongi*, this assay should be the approach of choice for schistosomiasis diagnosis in Lao PDR and Cambodia with the current infrastructure.

![Fig. 3 Correlation between combined ELISA (top left), POC-CCA (bottom left) and urine (top right) and serum CAA (bottom right) and infection intensity of *Schistosoma mekongi* (eggs per gram of stool)](image-url)
We found a strong correlation of the test results of the urine and serum CAA tests and ELISA, while the correlations between the two CAA tests and the Kato-Katz and POC-CCA were weaker. These observations are consistent with previous studies in the People’s Republic of China [29] and elsewhere [28, 40] and are largely a reflection of the different sensitivities of these diagnostic tests.

It should be mentioned that the results presented here are interpreted rather conservatively with respect to the cut-off threshold, leaving the POC-CCA trace scores and the UCP-LF CAA indecisive values as negatives. A more detailed comparison of the different assays using e.g., latent class analysis may shed a better insight in the actual status of trace and indecisive samples. Such additional analyses, incorporating also a quantitative analysis of the POC-CCA results using a gold strip reader, are being planned.

In agreement with previous evaluations of the various assays for circulating schistosome antigens in areas endemic for other schistosome species, we found that the POC-CCA is both more rapid and more sensitive than multiple Kato-Katz thick smears. In the present study, the number of positives identified by POC-CCA was significantly higher than those found by Kato-Katz in both counties. These results are in accordance with published results which showed that POC-CCA prevalence was between 1.5- and up to 6-fold higher than Kato-Katz prevalence estimates in areas with low infection intensity [23]. The comparable cost levels per determination for POC-CCA and Kato-Katz [41, 42] should not prevent the application of the rapid test in national schistosomiasis control programmes. Furthermore, people are more likely to provide urine samples than any other type of sample, leading to higher compliance.

While eggs continue to be excreted by the host for a few weeks after cure, both CCA- and CAA-levels drop quickly, sometimes turning negative within 1 week after treatment [40, 43], making this approach a promising tool to monitor drug efficacy. The sensitivity of CCA-based tests is not as high as what the UCP-LF CAA assay or what DNA-based detection methods can offer [44, 45], while the ultrasensitive SCAA500 format of the UCP-LF CAA test surpasses PCR in sensitivity [46, 47]. As many different diagnostic assay systems are now available, planning to assess geographic areas potentially endemic for schistosomiasis, multiple diagnostic approaches should be compared taking into account modelling and statistical methods in combination with knowledge how biological systems operate [28, 48].

Conclusion
Where low egg counts are most common, such as in areas characterised by low endemicity slated for elimination, the sensitivity and specificity of diagnostic tests must be taken into account when deciding which approach to choose. CCA-based assays are already available for use in the field, but tests targeting CAA still need the laboratory due to some of the sample preparation steps. Although the latter approach is the most sensitive antigen test, it would still be useful to apply POC-CCA testing for screening. While the results presented here will be subjected to further analysis, it would be useful to start planning for wider testing including application of geographical information systems to establish the real boundaries of the areas endemic for S. mekongi, prevalence and intensity of disease before moving on to transmission control and eventual elimination of the disease in Cambodia and Lao PDR.

Additional file

Additional file 1: Multilingual abstracts in five of the six, official working languages of the United Nations. (PDF 902 kb)

Acknowledgements
Claudia J. de Dood (Department of Molecular Cell Biology, Leiden University Medical Center) is acknowledged for performing the antigen testing and contribution to the data analysis. We thank Christina Krebs from the Diagnostic Centre, Swiss Tropical and Public Health Institute, for expert technical assistance and Mrs. Armelle Forrer for establishing the maps.

Funding
We are grateful to financial support of the Task Force for Global Health, Neglected Tropical Diseases Support Centre, the Department of Parasitology, Leiden University Medical Center and the Swiss Tropical and Public Health Institute.

Availability of data and materials
Please contact author for data requests.

Authors’ contributions
SS, VK, RB, GJvD, BN, JU, SM and PO designed the study; YV, SS, VK and SM implemented the field work; GJvD, PTH, PLAMC, BN and HM performed the diagnosis in urine and serum samples; YV, SS, VK, GJvD, PTH and PO performed the analysis; YV, RB, PO wrote the first draft and all other authors contributed to the writing; all authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the ethics committees in Lao PDR (070 NIOPH/NECHR, 4 December 2015) and Cambodia (394 NCHR, 10 November 2015). A written informed consent was obtained from all study participants. Helminth infections diagnosed during the study were treated according to the national treatment guidelines, i.e. praziquantel (single oral 40 mg/kg body weight) for S. mekongi and O. viverrini infection and albendazole (single oral dose 400 mg) or mebendazole (single oral dose 500 mg) for soil-transmitted helminth infections. All parasitic infections diagnosed were treated with the standard treatment regimens recommended by the Ministry of Health in each country [49].

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Faculty of Basic Sciences, University of Health Sciences, Ministry of Health, Vientiane, Lao People’s Democratic Republic. 2Swiss Tropical and Public Health
Received: 1 February 2017 Accepted: 12 July 2017

Published online: 10 August 2017

References

1. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383(9926):253–64.
2. Ohnmae H, Sinoum A, Kinoki M, Matsurono JT, Chigusa Y, Soчетe D, Matsuda H. Schistosomiasis mekongi from discovery to control. Parasitol Int. 2004;53(2):135–42.
3. Sinoum M, Tshuuka R, Soчетe D, Odermatt P, Ohnmae H, Matsuda H, Montresor A, Palmer K. Control of Schistosoma mekongi in Cambodia: results of eight years of control activities in the two endemic provinces. Trans R Soc Trop Med Hyg. 2007;101(1):34–9.
4. Attwood SW, Fatih FA, Campbell I, Upatham ES. The distribution of Mekong schistosomiasis, past and future: preliminary indications from an analysis of genetic variation in the intermediate host. Parasitol Int. 2008;57(3):256–70.
5. Biays S, Stich AH, Odermatt P, Long C, Yersin C, Men C, Saem C, Lormand JD. A foci of schistosomiasis mekongi re-discovered in Northeast Cambodia: clinical perception of the illness; description and clinical observation of 20 severe cases. Trop Med Hosp Int Health. 1999;4(10):662–73.
6. WHO: Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation. Webdocument, Accessed 25 Dec 2016 (http://www.emro.who.int/neglected-tropical-diseases/ntd-infocus/ntd-roadmap.html), 2012.
7. WPRO: Regional action plan for neglected tropical diseases in the Western Pacific (2012–2016). Webdocument, Accessed 25 Dec 2016 (http://www.wpro.who.int/mpv/documents/ntd_rap_2012_2016/en/).
8. Davis A, Wegner DH. Multicentre trials of praziquantel in human schistosomiasis: design and techniques. Bull World Health Organ. 1979;57(5):767–71.
9. WHO: Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme. Geneva: World Health Organizations; 2006 (http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf).
10. Zhu HX, Xu J, Zhu R, Cao CL, Bao ZP, Yu Q, Zhang Li, Xu XL, Feng G, Zhu JG. Comparison of the miracidium hatching test and modified Kato-Katz method for detecting Schistosoma japonicum in low prevalence areas of China. Southeast Asian J Trop Med Public Health. 2014;45(1):20–5.
11. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol. 2009;25(4):151–6.
12. Utzinger J, Becker S, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect. 2015;21(6):29–42.
13. Deelder AM, Klappe HT, van den Aardweg GJ, van Meerveld EK. Schistosoma mansoni demonstration of two circulating antigens in infected hamsters, Exp Parasitol. 1976;40(2):189–97.
14. de Jonge N, Kremers PG, Krijger FW, Schommer G, Fillie YE, Kornelis D, van Zeyl R, van Dam GJ, Feldmeier H, Deelder AM. Detection of the schistosoma circulating cathodic antigen by enzyme immunoassay using biotinylated monoclonal antibodies. Trans R Soc Trop Med Hyg. 1990;84(6):815–8.
15. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop. 2000;77(1):69–80.
16. van Dam GJ, Wichers JH, Ferreira TM, Ghati D, van Amerongen A, Deelder AM. Diagnosis of schistosomiasis by point strip test for detection of circulating cathodic antigen. J Clin Microbiol. 2004;42(12):5458–61.
17. Cortijos PLAM, van Lieshout L, Zuidewijn M, Kimelis D, Tanke HJ, Deelder AM, van Dam GJ. Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum. J Clin Microbiol. 2008;46(1):171–6.
18. Fenton EM, Mascarenas MR, Lopez GP, Sibbett S. Multiplex lateral-flow test strips fabricated by two-dimensional shaping. ACS Appl Mater Interfaces. 2009;1(1):124–9.
19. Standley CJ, Lamwoob NJS, Lange ON, Kariki HC, Adniko M, Stothard JR. Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria. Parasit Vectors. 2010;3:77.
20. Mwinzi PN, Kittur N, Ochola E, Cooper PJ, Campbell CH Jr, King CH, Coley DG. Additional evaluation of the point-of-contact circulating cathodic antigen assay for Schistosoma mansoni infection. Front Public Health. 2015;3:48.
21. Casacuberta M, Kinunghi S, Vennavard BJ, Olsen A. 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;12(2):105–15.
22. Coulbally JT, NGKesse YK, Knopp S, NGuessan NA, Silué KD, van Dam GJ, NGoran BK, Utzinger J. 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.
23. Kittur N, Castleman JD, Campbell CH Jr, King CH, Coley DG. 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.
24. van Dam GJ, Odermatt P, Acosta L, Bergquist R, de Dood CJ, Kornsels D, Muth S, Utzinger J, Cortijos PLAM. 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. Acta Trop. 2015;141(1Pt B):198–203.
25. Cortijos PLAM, de Dood CJ, Kornsels D, Tjon Kon Fat EM, Wilson RA, Karuki TM, Nyakundi RK, Loverde PT, Abrams WR, Tanke HJ, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. Parasitology. 2014;141(14):1841–55.
26. Cortijos PLAM, Nyakundi RK, de Dood CJ, Karuki TM, Ochola EA, Karanja DMS, Mwinzi PN, van Dam GJ. Improved sensitivity of the urine CCA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. Parasit Vectors. 2015;8:241.
27. Stothard JR, Sousa-Figueiredo JC, Standley CJ, Lwambo NJS, Lange CN, Kariuki HC, Adriko M, Stothard JR. Infection, Assessment of circulating anodic antigen in serum. Acta Trop. 2012.
28. Knopp S, Cortijos PLAM, Koukounari A, Cercamondi CI, Ame SM, Ali SM, de Dood CJ, Mohammed KA, Utzinger J, Rollinson D, et al. Specificity of a urine circulating anodic antigen test for the diagnosis of Schistosoma haematobium in low endemic settings. PLoS Negl Trop Dis. 2015;9(5):e0003752.
29. van Dam GJ, Xu X, Bergquist R, de Dood CJ, Utzinger J, Qin QZ, Guan W, Feng T, Yu XL, Zhou J, et al. 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. 2015;141(1Pt B):180–7.
30. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop São Paulo. 1972;14(6):397–40.
31. Nickel B, Sayasone S, Vonchack, Y, Odermatt P, Marti H. Schistosoma mansoni antigen detects Schistosoma mekongi infection. Acta Trop. 2015;141(1Pt B):310–4.
32. Ampah KA, Nickel B, Asare P, Ross A, De-Graft A, Kerber S, Spallek R, Singh M, Pluschke G, Yohoba-Manu D, et al. A sero-epidemiological approach to explore transmission of Mycobacterium ulcerans. PLoS Negl Trop Dis. 2016;10(1):e0004387.
33. Maleewong W, Intapan P, Wongwajana S, Sitthithaworn P, Pipitgool V, Wongkham C, Daenseegaew W. Prevalence and intensity of infection by using image analysis in school children in Mwanza region, Tanzania. Parasite Epidemiol Control. 2016;12(2):105–15.
34. WHO. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: a manual for health professionals and programme. Geneva: World Health Organizations; 2006 (http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf).
35. van Dam GJ, Xu J, Bergquist R, de Dood CJ, Utzinger J, Qin QZ, Guan W, Feng T, Yu XL, Zhou J, et al. 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. 2015;141(1Pt B):180–7.
36. Keang H, Odermatt P, Odermatt-Biays S, Cheam S, Degremont A, Hatz C. Liver morbidity due to Schistosoma mekongi in Cambodia after seven rounds of mass drug administration. Trans R Soc Trop Med Hyg. 2007;101(8):759–65.

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

38. Varit Wout AB, de Jonge N, Wood SM, van Lieshout L, Mitchell GF, Deelder AM. Serum levels of circulating anodic antigen and circulating cathodic antigen detected in mice infected with Schistosoma japonicum or S. mansoni. Parasitol Res. 1995;81(5):434–7.

39. Polman K, Diakhate MM, Engels D, Nahimana S, van Dam GI, Falcao Ferreira ST, Deelder AM, Gryseels B. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. Trop Med Int Health. 2000;5(8):534–7.

40. Lamberton PH, Kabatereine NB, Oguttu DW, Fenwick A, Webster JP. 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.

41. Sousa-Figueiredo JC, Basáñez MG, Khamis IS, Garba A, Rollinson D, Stothard JR. Measuring morbidity associated with urinary schistosomiasis: assessing levels of excreted urine albumin and urinary tract pathologies. PLoS Negl Trop Dis. 2009;3(10):e526.

42. Worrell CM, Bartoces M, Karanjia DO, Mwinzi PN, Montgomery SP, Secor WE. Cost analysis of tests for the detection of Schistosoma mansoni infection in children in western Kenya. Am J Trop Med Hyg. 2015;92(6):1233–9.

43. de Jonge N, De Caluwe P, Hilbersath GW, Krijger FW, Polderman AM, Deelder AM. Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in schistosomiasis mansoni. Trans R Soc Trop Med Hyg. 1989;83(3):368–72.

44. Obeng BB, Aryeeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, Yazdanbakhsh M, Hartges FC, Boakey DA, Verweij JJ, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Ann Trop Med Parasitol. 2008;102(7):625–33.

45. Lodh N, Mwansa JC, Mutengo MW, Shiff CJ. Diagnosis of Schistosoma mansoni without the stool: comparison of three diagnostic tests to detect Schistosoma mansoni infection from filtered urine in Zambia. Am J Trop Med Hyg. 2013;89(1):46–50.

46. Stothard JR, Stanton MC, Bustinduy AL, Sousa-Figueiredo JC, van Dam GI, Betson M, Waterhouse D, Ward S, Allan F, Hassan AA, et al. Diagnostics for schistosomiasis in Africa and Arabia: a review of present options in control and future needs for elimination. Parasitology. 2014;141(14):1947–61.

47. Wilson AR, van Dam GI, Kariuki TM, Farah IO, Deelder AM, Coulson PS. The detection limits for estimates of infection intensity in schistosomiasis mansoni established by a study in non-human primates. Int J Parasitol. 2006;36(12):1241–4.

48. Koukounari A, Donnelly CA, Moustaki I, Tukahebwa EM, Kabatereine NB, Wilson S, Webster JP, Deelder AM, Vennervald BJ, van Dam GI. A latent Markov modelling approach to the evaluation of circulating cathodic antigen strips for schistosomiasis diagnosis pre- and post-praziquantel treatment in Uganda. PLoS Comput Biol. 2013;9(12):e1003402.

49. MOH. Diagnosis and treatment at the district hospital. A diagnosis and treatment guideline for the district hospital in Lao PDR. Vientiane: Ministry of Health; 2004.