Diagnosing schistosomiasis: where are we?

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ABSTRACT
In light of the World Health Organization’s initiative to extend schistosomiasis morbidity and mortality control programs by including a disease elimination strategy in low endemic settings, this paper reviews diagnostic tools described during the last decades and provide an overview of ongoing efforts in making an efficient diagnostic tool available worldwide. A literature search on PubMed using the search criteria schistosomiasis and diagnosis within the period from 1978 to 2013 was carried out. Articles with abstract in English and that used laboratory techniques specifically developed for the detection of schistosomiasis in humans were included. Publications were categorized according to the methodology applied (parasitological, immunological, or molecular) and stage of development (in house development, limited field, or large scale field testing). The initial research generated 4,535 publications, of which only 643 met the inclusion criteria. The vast majority (537) of the publications focused on immunological techniques; 81 focused on parasitological diagnosis, and 25 focused on molecular diagnostic methods. Regarding the stage of development, 307 papers referred to in-house development, 202 referred to limited field tests, and 134 referred to large scale field testing. The data obtained show that promising new diagnostic tools, especially for Schistosoma antigen and deoxyribonucleic acid (DNA) detection, which are characterized by high sensitivity and specificity, are being developed. In combination with international funding initiatives these tools may result in a significant step forward in successful disease elimination and surveillance, which is to make efficient tests accessible and its large use self-sustainable for control programs in endemic countries.

Keywords: Human schistosomiasis. Diagnosis. Review. Parasitological, immunological and molecular methods.

INTRODUCTION
During many decades, the schistosomiasis control strategies were based on chemotherapy and aimed to reduce morbidity, mainly in sub-Saharan Africa and other high-burden areas. With the passing time, where significant progress in the control of schistosomiasis and other diseases has been achieved by national control programs (in countries such as Saudi Arabia, the Philippines, Tunisia, Algeria, Japan, Morocco, China, the Caribbean, Venezuela, Egypt, Mauritius, the Islamic Republic of Iran and Brazil) integrated actions in resource capabilities, health educational activities and environmental sanitation were identified as crucial targets for the setting of disease elimination.

In 2008, the World Health Organization (WHO) Department of Control of Neglected Tropical Diseases convened more than 30 international experts and representatives of countries where schistosomiasis has been controlled at varying levels to discuss tools and strategies for monitoring schistosomiasis in low-transmission areas as well as criteria for determining and validating disease elimination[1]. International approval of a definition of schistosomiasis elimination, guidelines as to whether or not elimination has been achieved, and a confirmation/verification process to recognize this status has not yet been established and is urgently required[2].

In this scenario, there is a need for more accurate, standardized and sensitive diagnostic techniques for Schistosoma infection diagnosis. Unfortunately, the technological development of new assays has evolved slowly, partially because of the current global strategy for schistosomiasis control that is built around preventive chemotherapy, which is the regular administration of drugs to at-risk populations without prior diagnosis[3], and partially due to the perceived lack of financial return to the diagnostic companies that contributes to making the investments insufficient and discontinuous[4].

Nonetheless, as result of public, mostly academic investments, a number of diagnostic tests have been developed during the last hundred years. A few of these methods survived the critical non-industrial tactical approach generated by the classic academia-industry disconnect. Consequently, a number of great opportunities may have been buried or left put aside by universities and research institutes. In the same way, hard-won resources may have been spent on attempts to develop less robust assays.

The purpose of this review was to compile information on the laboratorial methods for schistosomiasis diagnosis, considering the steps for test development and focusing on tests that achieved validation steps by large evaluations in field, critically looking at what is available and what has become useful.
REVIEW METHODOLOGY

This review was structured around a literature search of the PubMed database using the keywords schistosomiasis and diagnosis. All papers that spanned the period between 1978 and 2013 (October), had an abstract in English and pertained to humans were included. This search generated 4,535 papers, which were screened by title to select only those referring to laboratory diagnosis of Schistosoma mansoni, Schistosoma japonicum and Schistosoma haematobium via parasitological, immunological or molecular methods. In total, 643 papers remained for detailed analysis. Additionally, a standard form to extract data on development step: proof of principle (in house development), specimen banks/limited study groups (limited field study) and population study (large field study) and type of method (parasitological, immunological and molecular tests) was used. The data were entered in a spreadsheet, and Table 1 presents the results. A formal evaluation of test performance and their step of development were considered to identify relevant papers for the review.

FINDINGS

Egg detection: it is not surprising that the urine filtration and centrifugation methods are effective tools in the diagnosis of S. haematobium infections in high prevalence settings. Urine is easily collected but ideally should be collected between 10am and 2pm due to the circadian pattern of egg excretion. In the case of S. mansoni and S. japonicum detection by stool examination, methods such as the Kato-Katz fecal smear technique also provide good sensitivity in highly endemic areas. These parasitological methods (urine filtration or Kato-Katz), which cost approximately US$ 2, have been widely studied and their usefulness with respect to morbidity control is well established.

The scientific contributions to and progress in schistosomiasis diagnosis in stool samples over the last decades have not resulted in the full availability of new methods; rather, they have resulted mainly in a better understanding of the advantages, limitations and utility of available methods. Overall, these studies indicate that an increased sample size or number of examined slides increases a technique’s sensitivity, which is of paramount importance in low transmission areas. Unfortunately, this approach limits the operational advantages of these methods, as large-scale fieldwork is required. Nevertheless, the simplicity and the laboratory infrastructure independence of these techniques remain a considerable advantage given that the highest infection and morbidity rates for the disease occur in the poorest and least developed regions.

The development of other coproscopical techniques based on sedimentation, centrifugation, fluctuation and miracidium hatching, may constitute alternatives to the Kato Katz technique. In general, these methods provide better sensitivity but are more laborious, being more useful for research, schistosomiasis diagnosis in travelers and as additional diagnostic tools for in the depth analysis of infection rates before and after treatment.

Antibody detection: Several immunological methods have been proposed for schistosomiasis diagnosis, but few have reached the large field and validation steps of development. The most commonly used techniques that were validated in large-scale field trials include different formats of enzyme-linked immunosorbent assays (ELISA), the circumoval precipitin test (COPT), the indirect hemagglutination test (IHAT), the indirect immunofluorescence test (IFT) and the skin reaction test.

The immediate response to the Schistosoma adult worm skin test antigen was largely used in China for schistosomiasis investigations in the mid 1950s and for various epidemiological surveys conducted in Puerto Rico in 1963, 1969 and 1976. However, the observation that it remains positive for many years after effective chemotherapy, with documented follow-up to ensure cure, limits its usefulness.

Using IFT with adult worm sections, Kanamura et al. reported that gut-associated fluorescence may occur 44 days after infection and is consistently associated with acute schistosomiasis mansoni infection. A sample is defined as immunoglobulin G-indirect immunofluorescence test (IgG-IFT) positive (frozen sections) when fluorescence is present in the parenchyma and/or gut of the worms and as immunoglobulin M-indirect immunofluorescence test (IgM-IFT) positive (paraffin sections) when fluorescence is only present in the gut of the worms. The IFT has been applied as diagnostic tool in epidemiologic studies of Schistosoma mansoni infection in 1998 in a low endemic area, in Brazil. The prevalence rate was 1.6% according to the parasitological Kato-Katz method and 33.2% and 33.5% according to the IgG-IFT and IgM-IFT methods, respectively. This discrepancy was explained by the low diagnostic sensitivity of the parasitological methods for a single stool sample.

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<th>Phase</th>
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<td>parasitological</td>
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<td>In-house development</td>
<td>28</td>
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<td>Limited field testing</td>
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<td>Large scale field testing</td>
<td>33</td>
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<td>Total</td>
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Table 1 - Classification of the 643 papers that remained in the study for detailed evaluation.
The need for an expensive and complex microscope, experienced personnel and delicate reagents limits the use of this technique in endemic areas.

The IHA technique uses lyophilized red blood cells coated with antigen, most commonly crude soluble egg antigen (SEA), for the detection of positive patient sera through agglutination. Due to its simplicity, this technique can be employed in field conditions and requires only basic laboratory equipment. IHA was applied in Chinese population studies as an alternative test to detect human infection with *S. japonicum*. Although this technique shows good levels of sensitivity (71%-97%) and specificity (96%-100%) [31], this method was applied in a modified form as a commercial kit in China and requires only basic laboratory equipment. IHA was tested in combination with other coproscopic and serologic tests in a modified form as a commercial kit in China and Venezuela to evaluate low transmission areas [31]. In the case of infection with *S. mansoni*, a study carried out by Sorgho et al. [36] obtained similar results, reflecting the limitations listed above.

The circumoval precipitin test (COPT) is based on patient serum precipitation with lyophilized eggs or purified live eggs identified under microscope. This method is useful for the diagnosis of *S. mansoni* and *S. japonicum* due to its high sensitivity (92%-100%) and specificity (96%-100%) [31]. This method was applied in combination with other coproscopic and serologic tests in a modified form as a commercial kit in China and Venezuela to evaluate low transmission areas [31]. The limitations of this test are that it is time consuming (48 hrs), is complicated to execute and has a varying period of seroconversion after treatment, according to the experiences reported for Venezuela (negative rate of 64% of patients after 12 months of praziquantel treatment) and China (negative rate of 80%-83% of patients after 4 years of praziquantel treatment).

ELISA is the most commonly used test for the serological detection of schistosomiasis, offering the option of detecting different antibody classes as well as using a wide range of antigens. The detection of *Schistosoma* antigens was initially based on the use of crude soluble egg antigens (SEA) and soluble adult worms proteins (SWAP). Early studies revealed low specificity, which resulted in the search for purified antigen preparations such as cationic fraction 6 (CEF6) [32]; adult microsomal antigens for *S. mansoni* (MAMA), *S. japonicum* (JAMA) and *S. haematobium* (HAMA) [33]; gut associated antigen 31/32 kilodalton adult antigens [34-36] and keyhole limpet hemocyanin (KLH) [37]. The latter has not been evaluated under field conditions and is therefore not included into this review.

CEF6 is a purified antigen from *Schistosoma* eggs, described by Dunne et al. [38]. In the Mott and Dixon’s comparative study [39], this antigen in an ELISA platform showed 91.7% of sensitivity and 90% of specificity and the highest correlation with egg output compared to other antigens. Since that report was published, this antigen was criteriously evaluated in three field studies in *S. mansoni* endemic areas in Kenya [40], Saudi Arabia [40] and Burkina Faso [40]. The sensitivity in all three assessments was confirmed to be high (97%, 90% and 97%, respectively); however, the specificity remained low (59%, 55% and 17.2%, respectively). In all of these studies, the question of the adequacy of the reference tests arose, and the low specificity was attributed to the low sensitivity of the Kato-Katz method, which was used as the gold-standard.

Adult worm microsomal antigens were tested in combination with the Falcon assay screening test-enzyme-linked immunosorbent assay (FAST-ELISA) and confirmed by Western Immunoblot. A study in Egypt using HAMA and MAMA in a *S. haematobium* endemic area as the screening test revealed sensitivity of 83.6% for MAMA FAST-ELISA and sensitivity of 98.5% for HAMA FAST-ELISA. By adding the Western blot technique to HAMA FAST-ELISA for additional analysis, all egg-passing individuals were identified, revealing 100% of sensitivity and 59.4% of specificity, compared to egg detection in urine [41]. A systematic serosurvey conducted in 1995 in almost all municipalities in Puerto Rico evaluated 2,955 healthy donor plasma samples using MAMA FAST-ELISA, followed by enzyme-linked immunoelectrotransfer blot (EITB) confirmation. The data showed that 15.4% of samples were FAST-ELISA positive, and 10.6% were confirmed by EITB. The highest seroprevalence rates (21.1-38.5%) were concentrated in 17 municipalities, which accounted for 48% of all seropositive samples and 18% of the total population of Puerto Rico [42]. Variations in the results obtained from these purified preparations were observed in different settings and thus limit conclusions about test accuracy and performance at this stage. A recent study by Abdel-Fattah et al. [43] using a purified a HAMA antigen that is highly specific for *S. haematobium* and a new standard curve suggests that FAST-HAMA can be used as a diagnostic assay for *S. haematobium* infection, as it showed a positivity rate of 95%. Nevertheless, additional studies are necessary to establish its specificity and its performance in field conditions.

In the case of 31/32 kilodalton adult *Schistosoma* proteins, population studies on *S. mansoni* in Sudan and *S. japonicum* in China revealed high sensitivity but low specificity [44]. This low specificity was justified by various factors, such as cross-reaction, infection with non-human *Schistosoma* species, and lack of sensitivity of the parasitological reference test. Unfortunately, further evaluations of this methodology’s accuracy are not available, and this issue remains unresolved.

Rapid tests for antibody detection in new assay formats have been reported for *S. japonicum*, including the dot immunogold filtration assay [44], the silver-enhanced colloidal gold metalloimmunoassay [45] and the colloidal dye immunofiltration assay [46]. A western blot assay has also been evaluated for anti-*S. mansoni* antibody detection [47] and an immunoblot assay of *S. mansoni*, *S. intercalatum* and *S haematobium* membrane antigens against *Schistosoma* infected patient sera has been tested [48]. These new formats use crude antigens and still need to be validated in large-scale field trials.
Recently, a novel rapid dipstick with latex immunochromatographic assay (DLIA) using *S. japonicum* SEA as antigen was developed to detect anti-*S. japonicum* antibodies in human serum. In a Schistosoma-endemic area in China\(^6\) the test was evaluated on 102 serum samples that had tested positive for schistosomiasis by the Kato-Katz method (epg: 8–1352) and 275 serum samples from healthy individuals (negative for schistosomiasis on the Kato-Katz method), showing 95.1% (97/102) of sensitivity and 94.9% (261/275) of specificity. Fifteen clonorchiosis, 11 intestinal nematode and 8 Angiostrongylus cantonensis samples were tested with DLIA and showed no signs of cross-reactivity. When 19 paragonimiasis samples were tested by DLIA, the cross-reaction rate was 42.1% (8/19). Additionally, serum samples (333 in total) from a schistosomiasis endemic area in China were tested by the DLIA and Kato-Katz methods. The positive rate was 21.2% (72/333) by DLIA and 3.9% (13/333) by Kato-Katz. DLIA showed 92.3% of sensitivity and 81.3% of specificity. In the same study, an ELISA-SEA test was performed, and there were no significant differences in positive and negative detection rates between the two serological methods. ELISA reagents require cold-chain logistics, and the test takes several hours to complete. One important advantage of DLIA is cost, as the latex microspheres produced in China are considerably less expensive than enzyme conjugates or colloidal gold. In addition, the control band on each dipstick contributes to the quality control of the assay. A dipstick dye immunoassay (DDIA) that has recently become commercially available in China, was evaluated in a cross-sectional survey that included 6,285 individuals aged 6–65 years from seven villages with low schistosomiasis endemicity. Stool samples were collected and examined by the Kato-Katz method and the miracidium hatching technique. Using the stool examination as the gold reference, DDIA exhibited a high overall sensitivity of 91.3% and a high negative predictive value (mean, 99.3%), but 53.1% of specificity. The authors conclude that the moderate DDIA specificity was mainly caused by the stool examination low sensitivity and by the high frequency of previous exposure to schistosomes. As a rapid, simple, apparatus-free and sensitive assay, DDIA is feasible and of practical use as a screening tool in areas of low *S. japonicum*\(^9\) endemicity. As for other antibody-based tests, specificity remains to be clarified.

Recombinant antigens and those derived from proteomic studies constitute a new promising line of research in the diagnosis of schistosomiasis, but they are still in the experimental phase, awaiting further improvement and validation. One example is the rS26-Si32 fusion protein, which is used mainly with a magnetic bead-based immunoassay\(^9\) that was developed for chronic schistosomiasis japonica immunodiagnosis in China.

**Antigen detection:** Similarly, a number of assays to detect circulating antigen have been described. The detection of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) in serum and urine, using the ELISA\(^2,35\) or dipstick platforms\(^44\), and antigen capture with monoclonal antibodies\(^55\) have been the most widely studied methods. Their main advantages are high specificity, positive correlation with worm burden, and the possibility for estimation of infection intensity\(^56-58\). Moreover, circulating *Schistosoma* antigens disappear rapidly after treatment and can therefore be used for assessment of cure\(^59\).

Overall, the sensitivity of antigen detection is low in low endemic areas, similar to the sensitivity obtained with stool examination and urine filtration. Therefore, this technique can be used as a substitute for parasitological examinations in settings with high infection rates or as a complementary method in settings with low infection rates\(^60\).

To date, currently available CCA tests have been shown to have moderate to good reliability in detecting *S. mansoni*\(^64,65\) but poor performance in detecting *S. haematobium*\(^66-69\). Recently, the Bill & Melinda Gates Foundation sponsored evaluation studies on the accuracy of the urine CCA tests for determining schistosomiasis prevalence in young children in African countries. The main results were obtained by evaluating the CCA urine cassette assay, a commercial kit produced in South Africa, performed at ambient temperature according to the manufacturer’s instructions. In Côte d’Ivoire, the sensitivity of triplicate Kato-Katz was 47.9%, 73.9% and 94.2% for the settings A, B (both endemic for *S. mansoni*) and C (where *S. haematobium* co-exists) in comparison with one CCA test, that performed at sensitivity levels of 56.3, 69.6 and 89.6%, respectively. The specificity of CCA test was moderate (76.9-84.2%)\(^70\). In the Lake Victoria region of western Kenya, where *S. mansoni* infection is prevalent and *S. haematobium* is not, CCA test was compared to Kato-Katz in 484 children, with sensitivity of 94.2% and specificity of 59.4%. The authors also utilized latent class analysis (LCA) incorporating the CCA, Kato-Katz and schistosome-specific antibody results to determine their sensitivities and specificities. By LCA, CCA test had 96.3% of sensitivity and 74.7%\(^71\) of specificity. In the same line of research, an independent study conducted in southern Sudan showed a moderate performance (sensitivity of 89.1% and specificity of 74.2%) for the CCA test in an evaluation of 373 children. For the authors, this may be a slight underestimation of the true CCA accuracy, as only one single stool and urine samples were examined by microscope\(^72\).

**DNA detection:** During the last decade, polymerase chain reaction (PCR)-based assays have become more accessible and have been applied in the diagnosis of infectious and parasitic diseases. According to Reithinger & Dujardin\(^73\) PCR assays can be divided in three distinct formats, namely the **mid tech** approach, represented by PCR, the **high tech**, represented by real-time PCR and the **low tech**, represented by the loop mediated isothermal amplification (LAMP).

In the specific case of human schistosomiasis, the first application of PCR as a diagnostic tool in an endemic area was a PCR assay for *S. mansoni* DNA detection in stool samples\(^74,75\). The target DNA is a tandem repeat 121 base pair (bp) sequence\(^76\). Comparing the PCR assay to stool examinations by the Kato-Katz technique, the PCR assay showed sensitivity of 96.7% and specificity of 88% when applied among 194 inhabitants from a Brazilian endemic area. The assay showed the detection limit (analytical sensitivity) of 1 fg of *S. mansoni* genomic DNA and no...
cross-reaction (analytical specificity) with DNA from four other helminthes: *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Taenia solium* and *Trichuris trichiura*. The authors emphasize that this technique might prove to be useful in low transmission settings due to its high sensitivity (96.7%) compared to the 79% of sensitivity obtained by stool examination, when the criteria for positivity was determined as positivity by any of the direct methods. The sensitivity of this technique was reevaluated in other studies in which it was applied to preselected individuals with low worm burden and schistosomiasis positive patients after treatment. Under these specific conditions, both studies confirmed the high sensitivity of the PCR assay.

Furthermore, this assay using a tandem repeat 121 base pair (bp) sequence was improved by the simplification of PCR product visualization with the polymerase chain reaction–enzyme-linked immunosorbent assay (PCR-ELISA) and with oligochromatography-polymerase chain reaction (OC-PCR) and a modified DNA extraction procedure without losing its high sensitivity. Recently, the same DNA sequence was used successfully in the detection of *Schistosoma* DNA in plasma and in urine samples by Enk et al. and Ichimone et al.

PCR was also applied for species-specific DNA amplification. In a study reported by Sandoval et al., this technique was used for genus- and species-specific amplification of the main four *Schistosoma* species that cause disease in humans and *S. bovis*. Using easy-to-handle urine samples from 50 healthy donors, 18 patients with schistosomiasis (6 with *S. mansoni* infection, 10 with *S. haematobium* infection and 2 with mixed infection) and 29 with other parasite infections, 94.4% of sensitivity and 99.9% of specificity were found for a genus specific primer and a *S. mansoni* species-specific PCR revealed sensitivity of 100% and specificity of 98.9%. Recently, a PCR assay to detect *S. mansoni* infection (species-specific DNA) from filtered urine samples in Zambia proved to be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and control programs of schistosomiasis.

The high tech approach of real-time PCR systems has recently been applied to parasitology, as they are powerful alternative tools for quantifying parasitic load. These assays are performed with a single setup and dye detection within a closed tube, which decreases the risk of DNA contamination. The first method described for schistosomiasis diagnosis used SYBER Green dye for the detection of *S. mansoni*, targeting a small 96bp fragment on the small subunit-ribosomal ribonucleic acid (SSU rRNA) gene. DNA extracts from adult *S. mansoni* worms were used in the system evaluation, showing a parasite DNA detection limit of 10fg. The authors emphasize the potential of this method to detect *S. mansoni* DNA in different biological samples, such as human feces, snails and water. In addition, it can be useful to quantify parasite burden in human and snail infection.

Another real-time PCR approach was described for *S. japonicum* diagnosis in stool samples. Similar to the previous method, SYBER Green dye was used as an intercalating dye into double-strand DNA to measure the change in fluorescence after each PCR cycle. In artificially spiked stool samples, this method demonstrated high sensitivity, even in samples containing a single egg. The same research group used this test in a comparative study in China among 1,727 participants from an endemic area. In China, results obtained with the commonly used algorithm for schistosomiasis control, consisting of an initial antibody screening and subsequent confirmation with Kato-Katz stool examination, were compared to results obtained by substituting the confirming coproscopical test by PCR. The results revealed an increase from 22 to 50 positives when PCR replaced the Kato Kutz technique. In conclusion, considering the decrease in prevalence and infection intensity, the authors emphasize that the gains in sensitivity must be weighed against additional costs and operational implications of both choices.

In Ghana, a real time PCR system using TaqMan chemistry and primers, as well as a probe based on the internal-transcribed-spacer-2 (ITS-2) for *S. haematobium* detection in urine samples of 153 children, was studied by Obeng et al. A total of 66 positive participants were detected by PCR, and 74 were detected by urine microscopy, indicating sensitivity of 89.2% and specificity of 100%. It is worth noting that all 8 cases that were missed by PCR had less than 50 eggs/10 ml urine in the microscopic examination, a finding that can be explained by the very small amount (200µl) of urine used for PCR. This same real time PCR system was recently applied in epidemiological survey of schistosomiasis in Ghana and proved to be a powerful tool, providing more precise and sensitive results than microscopy.

Recently, Crop et al. developed a similar assay using TaqMan chemistry and the 28S ribosomal RNA gene as target to detect different *Schistosoma* species for diagnosis in international travelers and migrants. After showing an analytical sensitivity of 0.2 eggs per gram of feces, the assay’s performance was evaluated in feces, urine, and serum samples from patients presenting at the outpatient clinic of the Institute of Tropical Medicine in Antwerp (Belgium). *Schistosoma* DNA was detected in 76 fecal (50%) and five urine (15.6%) samples, of which nine and one, respectively, were not detected by standard microscopy. Only two of the 38 serum samples from patients with confirmed schistosomiasis were PCR positive. The same group developed a real-time PCR targeting the Dra1 sequence for *S. haematobium*-specific detection in urine, feces, and particularly serum of international travelers and migrants. The assay revealed a positive result in 7/7 urine samples, 11/11 stool samples and 1/1 biopsy containing *S. haematobium* eggs as demonstrated by microscopy and in 22/23 serum samples from patients with parasitological confirmed *S. haematobium* infection. *Schistosoma haematobium* DNA was additionally detected by PCR in 7 urine, 3 stool and 5 serum samples of patients suspected of having schistosomiasis without egg excretion in urine and feces.

The multiplex format of real time PCR was tested in fecal samples by Ten Hove et al. in 88 participants from a northern Senegalese village that is endemic for both *S. mansoni* and *S. haematobium*. The system uses TaqMan probes as the detection
chemistry and primers, and probes were designed to target the cytochrome c oxidase subunit 1 (cox1) gene in the mitochondrial genome because this DNA sequence shows sufficient divergence between separate Schistosoma species. The analytical specificity was evaluated using 150 DNA controls derived from a wide range of intestinal microorganisms, and amplification was not detected in any of these samples. Although an in-depth analysis of this multiplex real time PCR system regarding its sensitivity and specificity in comparison to microscopic stool and urine analysis was not provided by the authors, the overall test performance revealed that 70% (79.5%) of the 88 subjects were positive by microscopy, whereas 74 (84.1%) were found to be PCR positive.

The low tech PCR approach, loop mediated isothermal amplification (LAMP) developed by Notomi et al. was recently used for the detection of S. japonicum DNA in rabbit fecal and serum samples, as well as in 50 human serum samples. The analytical test evaluation revealed high analytical sensitivity, detecting 0.08 fg of DNA, and no cross-reaction was found with S. mansoni and Clonorchis sinensis. The evaluation of the 50 human samples showed sensitivity of 96.7% and specificity of 100%. This initial result indicates that LAMP might become a useful tool for routine diagnosis and therapeutic evaluation due to the practicality of using a simple water bath for amplification and a dye for visualization.

The role of PCR-based assays in schistosomiasis diagnosis outside of research settings remains to be fully defined, despite their significant potential in low transmission settings and in situations where high sensitivity and specificity are required. Additionally, as cost efficiency improves through increased demand for molecular diagnosis of other infectious diseases such as human immunodeficiency virus (HIV) and tuberculosis, PCR assays are expected to become more affordable and may constitute a new available tool for schistosomiasis diagnosis.

Futures perspectives of novel funding schemes and product development partnerships: there are now more funding agencies in the diagnostic research and development fields for neglected tropical diseases, notably the Bill & Melinda Gates Foundation (BMGF). The initiatives are mainly concentrated on the development of point-of-care (POC) diagnostics that can rapidly and accurately identify infections in patients, promoting the use of standardized and validated commercial kits and applying molecular high-throughput multiplexing assays for simultaneous diagnosis of helminths and intestinal protozoan infections, at least in return travelers at specialized laboratories in the industrialized world, but hopefully not limited to these scenarios.

Importantly, the BMGF, through a five-year grant to the University of Georgia Research Foundation, sponsored the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) (http://score.uga.edu/). The Consortium was established in December 2008 to answer strategic questions about schistosomiasis control and elimination. In 2010, SCORE funded a 5-country (Cameroon, Ethiopia, Cote d’Ivoire, Kenya, and Uganda) evaluation of the CCA urine strip test for the diagnosis of S. mansoni in low and moderate endemic areas, as well as in mixed S. mansoni-S. haematobium foci. Based on the preliminary study results (see the Antigen Detection section), SCORE suggested that a single urine examination using a commercially available POC/CCA cassette-based test instead of a single stool examination by the Kato-Katz method could be used to assess S. mansoni prevalence in school age children. Should these validations prove successful, efforts should be made to further reduce the cost per test strip (ideally below US$2.00) to make this technique useful as a POC diagnostic approach in resource-constrained settings.

Also in 2003, the BMGF offered a 5-year start-up grant, which helped to set up the Foundation for Innovative New Diagnostics (FIN Diagnostics, which has gradually expanded its portfolio of activities with a growing emphasis on developing and validating novel diagnostics for neglected tropical diseases.

Most importantly, technological advancement is only one aspect of this issue. A solid governmental strategy must integrate diagnosis and treatment into the primary healthcare system. In China, the benefit of this integration was clearly demonstrated by Guo et al., who reported that the cost of a strategy based on passive chemotherapy and health education was approximately half the cost of mass drug distribution in two villages with 11% and 12.3% prevalence of S. mansoni infection, with similar treatment coverage.

Even when a test with acceptable performance is available, laboratory capacity and local autonomy are essential to carrying out and maintaining realistic, collaborative and self-sustained control programs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES


60. 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; 77:69-80.


