

Diagnosis of Human Schistosomiasis: What Technique to Use and When

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INTRODUCTION

Human schistosomiasis is one of the most important Neglected Tropical Diseases (NTDs) [1], which is caused by an intravascular blood trematode. Schistosome species which infect humans include *S.haematobium*, *S.mansoni*, *S.japonicum*, *S.mekongi* and *S.intercalatum*, among which the first three species are the most prevalent. Schistosomiasis is endemic in 77 countries with an estimated total of 237 million people are infected worldwide, and 732 million people are at risk of being infected (Figure 1) [2-4].

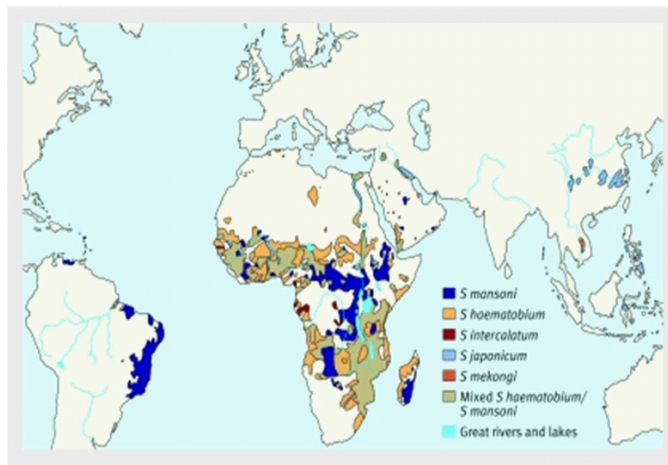


Figure 1: Global distribution of schistosomiasis. Adapted from Gryseels, et al. 2006 [3].

The life cycle of the parasite is characterized by alternation of generation where asexual reproduction occurs in the snail intermediate host and sexual reproduction occurs in humans (Figure 2) [5]. The disease is characterized by residual tissue damage due to egg-mediated immune response presented as granuloma formation followed by fibrosis and leading to obstructive manifestations in the Gastrointestinal Tract (GIT) in case of intestinal schistosomiasis and in the urinary tract in case of urinary schistosomiasis [6-8]. Nevertheless, eggs can be disseminated to other organs including the brain, the lungs, the spinal cord and genital organs. Squamous cell carcinoma of the urinary bladder is one of the serious sequelae of *S. haematobium* in Egypt and North Africa [9].

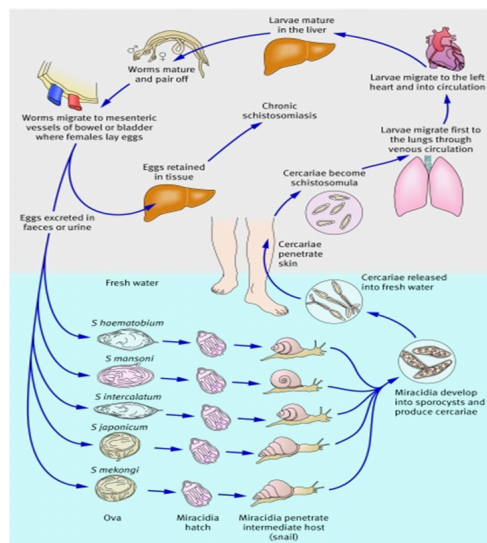


Figure 2: Schistosome life cycle showing alternation of generation in the five *Schistosoma* species affecting human, Gray, et al. 2011[5].

SCOPE OF DIAGNOSTICS IN HUMAN SCHISTOSOMIASIS

Accurate diagnosis of schistosomiasis is paramount for both individual patient management as well as for community based studies, such as drug efficacy trials, surveillance of schistosomiasis, control and elimination programs. In line with the WHO guidelines [10] infection prevalence must be determined prior to the implementation of a control program [11,12]. Accordingly the extent of chemotherapy coverage will be determined based on the level of disease transmission in the community. For example, repeated Mass Drug Administration (MDA) at regular intervals depending on the population prevalence has been recommended by the WHO [12-14]. In addition, evaluation of chemotherapy and control measures build on the results of diagnostic tests. Therefore, standardized diagnostic assays are essential for valid comparison of intervention strategies across various study settings.

Because of the fundamental role of diagnostic tools, rigorous efforts are still going on searching for sensitive and specific diagnostic assays for schistosomiasis. The available diagnostic tools include; Microscopic Examination (ME) known as conventional parasitological techniques, immunological assays detecting stage specific antigens or antibodies and molecular techniques detecting the DNA in serum, blood or excreta.

Despite the availability of this wide range of diagnostic assays ranging from simple to sophisticated techniques, ME of urine/stool samples to detect the eggs during the stage of patent infection remains the proxy of definitive diagnosis where most of community based studies rely on these techniques to map schistosome infection and to evaluate control programs. On the other hand, acute stage or Katayama syndrome which is seen among people newly exposed to infection as in case of travelers to endemic areas, the diagnosis is mainly dependent on immunological techniques, since manifestations are mediated by immune complex [5].

MICROSCOPIC EXAMINATION

For the diagnosis of patent or mature schistosomiasis, microscopic detection of excreted eggs in stool in case of intestinal schistosomiasis or in urine in case of urinary schistosomiasis remains the gold standard (Figure 3). Varieties of techniques are available such as; direct concentration, sedimentation concentration, concentration floatation and filtration assays.

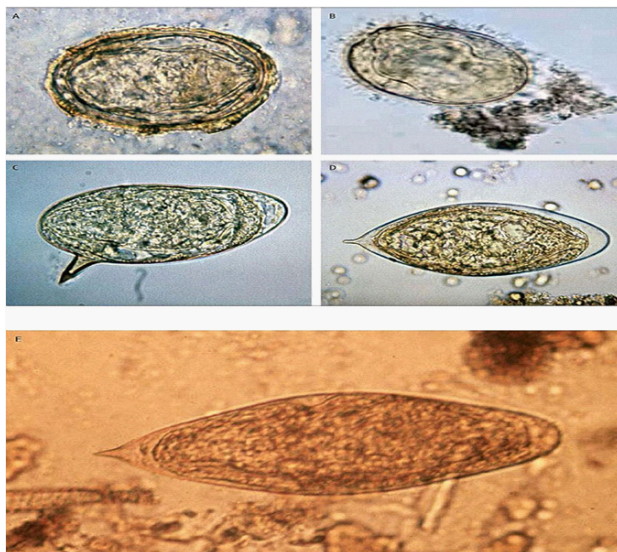


Figure 3: Schistosome eggs representing the 5 species affecting human: (A) *S.japonicum*, (B) *S.mekongi*, (C) *S.mansoni*, (D) *S.haematobium*, (E) *S.intercalatum*, Gray, et al. 2011 [5].

COPROSCOPIC TECHNIQUES

Examination of faecal material by Kato Katz (KK) technique is the most widely used assay for the diagnosis of intestinal schistosomiasis. It is a direct concentration quantitative technique with high specificity reaching 100% [15]. The technique is particularly preferable in epidemiological studies since it is rapid, non-expensive and easy in processing. In addition, quality control of Kato slides is operationally feasible because slides can be kept without marked alteration in egg integrity and egg counts. When recounts were performed 1-18 months post preparation, only 6.38% of the counts and recounts disagreed on the presence or absence of eggs, but less than a third of these negatives were found positive on recount. When duplicate counts were performed, false negatives dropped to 1.3%, and with triplicate counts, false negative rate dropped further to < 1% [16].

However, one of the major shortcomings of KK technique is its low sensitivity in individuals with low intensity of infection or in low endemic areas. Although increasing the number of stool samples or increasing the number of slides from a single stool sample can improve sensitivity, yet this might be time consuming and expensive. In a high *S. mansoni* endemic area of Uganda, baseline diagnosis by two KK was highly sensitive (98.6%). However, one year later, a minimum of three KKs two slides each, and two years later, five KKs two slides each were required for accurate diagnosis and drug-efficacy evaluation (sensitivity 90%). Accordingly, in high transmission areas, the authors recommended two KKs for pre-treatment infection mapping, and as Mass Drug Administration (MDA) continues, five KKs were required for diagnosis of children after three to ten PZQ treatments [17]. These data refuted the claims of da Frota, et al. 2011; who reported that, in low intensity regions the additional benefit of more than six Kato smears from repeated stools was negligible [18].

The consequences of relying on insensitive techniques as KK in low transmission areas are failure to portray the actual force of infection and underestimation of the true prevalence. Hence using this technique in disease mapping and monitoring schistosomiasis control program is questionable [18-20]. Of note that the KK assay has documented day-to-day and intra-stool variability, especially in moderate and low risk communities. Day-to-day variation is due to biological factors [21], whereas intra-stool variation is due to sampling error based on the amount of stool evaluated. Missing genuine infection and underestimation of intensity of infection will result in false interpretation of high cure rates and drug efficacy which would increase the risk of missing drug resistance.

The FLOTAC technique is a recently developed stool floatation procedure which is gaining interest in the diagnosis of helminths and protozoa among humans and animals. The method's principle is based on the centrifugation of stool samples in a Floatation Solution (FS) with a given specific gravity and the subsequent microscopic examination of the upper part of the faecal suspension containing the helminth eggs and intestinal protozoa. The technique is quantitative with a sensitivity exceeding that of KK [22, 23]. This might be attributed to the amount of stool processed for each technique, while 41.7 mg of stool is used per a single Kato smear, one gram of stool is used for the FLOTAC technique [24].

In the diagnosis of *S. mansoni*, single FLOTAC from stool samples preserved in sodium acetate-acetic acid-formalin showed a higher sensitivity (91.4%) than the Formol Ether Sedimentation Concentration Method (FECT) on stool samples preserved in formalin (85.0%) or triplicate KK using fresh stool samples (77.4%). However, faecal egg counts are consistently lower when compared to the KK [25]. In a previous study, similar results were also reported for the FECT where the geometric mean egg count was 94 eggs per gram (epg) of stool by two KKs and only 43 epg by the sedimentation technique. This means that more than 50% of eggs were missed when using the sedimentation technique, a fact that should be taken into consideration when relating infection level with morbidity [26]. This difference might be due to loss of eggs during the multiple operational steps of FLOTAC and the sedimentation concentration techniques.

It is observed that increasing the number of KK smears improved the diagnostic capacity of the test to diagnose more cases. However, intensity of infection is inversely related to the number of KK smears. In a comprehensive study which encompassed 1265 individuals as the Total Population (TP), prevalence and infection intensity were established by examining two KK smears. A subset of 305 individuals, representing the Experimental Group (EG), was defined to assess the comparative advantage of an increased sampling effort using the KK. In addition stools of the participants of the EG were examined by FECT. The overall infection intensity calculated as GMEC, was 83 epg for the TP based on one KK slide, 78 epg for the EG based on one KK slide and 28 epg based on 10 KK slides of three stool samples. The same study demonstrated that prevalence among TP based on two KK slides was 12.5%, whereas prevalence among the EG varied from 13.8%, based on one KK slide, over 27.2% based on 10 KK slides of three stool samples to 35.4% when both KK and FECT served as the gold standard to estimate prevalence of schistosomiasis [27].

Performance of KK and FECT was evaluated in high risk versus moderate risk communities in the Nile Delta Egypt for the diagnosis of schistosomiasis mansoni. A single stool specimen was collected from 915 individuals representing a high prevalence community (63.3%) and from 471 individuals representing a moderate risk community (40%). The overall sensitivity of a single KK was 70.8%, and increased with each additional slide to reach 91.7% on examining four smears. However, the sensitivity was 83.3% when using FECT [26]. These data clearly show that KK is more efficacious in high and moderate risk areas than in low risk areas.

Percoll is another sedimentation technique which was found more efficient than KK in low transmission areas [28, 29]. Amongst Kato negative cases Percoll detected 11% positive samples. In addition, KK overestimated cure rate, being 97% compared to 90% by Percoll technique [28]. The amount of processed stool in Percoll technique (250 mg) is about three fold that examined in the routinely recommended two Kato slides (83.7 mg), added to this, the two steps of stool sieving and the malachite green staining of the eggs in Percoll technique explain its superior performance.

MICROSCOPIC EXAMINATION IN UROGENITAL SCHISTOSOMIASIS

Regarding urogenital schistosomiasis, microscopic detection of excreted ova in urine samples remains as the gold standard to reflect active infection using either sedimentation/centrifugation or filtration methods [3,30]. These conventional methods are of particular use under field conditions since they are cheap, easy to perform and rapid.

Urine filtration is recommended by the WHO for *S. haematobium* [31]. A standard 10-ml of the urine to be tested is forced through the device with a syringe. If eggs are present, they are unable to pass through a Millipore filter and can be observed and counted under the microscope. The cost of the filtration equipment is considerably high (\$2 for each device) which might limit its use for mass diagnosis. A new, inexpensive filtration device (\$ 0.080) for the diagnosis of urinary schistosomiasis demonstrated comparable sensitivity to the millipore filter and hence can be used for community based studies [32].

In urinary schistosomiasis, the limitations of ME of urine samples to detect *S. haematobium* eggs parallel those of coproscopic assays used to diagnose intestinal schistosomiasis. Several studies documented that the parasitological approach underestimated infection prevalence in low intensity of infection which makes these techniques inaccurate in young children and in older individuals as well as in low risk areas for example in communities subjected to repeated PZQ treatments [33]. In addition, variability of egg counts was evident when using these assays [34,35]. Furthermore, egg detection assays do not detect single sex or pre-patent infections.

For the three common Schistosome species affecting human, ME has been applied extensively to monitor control programs. For example PZQ efficacy is measured by Cure Rate (CR) and Egg Reduction Rate (ERR); this is calculated as the percent reduction in the number of eggs in the stool/urine after treatment. The mean number of eggs is calculated using either Geometric (GM) or Arithmetic Means (AM) to represent intensity of infection as eggs per gram of stool (epg) for

intestinal schistosomiasis or eggs/10 ml of urine for genitourinary schistosomiasis. To answer the question whether to use the GM or the AM, data from 24 studies conducted in Africa, Asia, and Latin America enrolling 4,740 individuals infected with *S. mansoni*, *S. haematobium*, or *S. japonicum* and treated with PZQ were reanalyzed to validate the sensitivity of the GM versus the AM in monitoring drug efficacy through computing ERRGM and ERRAM. Results revealed that group-based AM and GM can be used interchangeably to express Egg Reduction Rates (ERR) only if treatment efficacy is above 95%. Whereas, when treatment efficacy is lower than that, AM calculations are more sensitive to capture drops in drug efficacy. According to WHO guidelines, the authors recommended using AM to better identify problems related to PZQ reduced susceptibility [36]. However, individual responses in egg excretion allow better identification of inadequate treatment response. Finally, monitoring drug outcomes must be standardized and tailored according to specific objectives as epidemiological surveys, morbidity control, elimination and clinical trials [36,37].

Regarding the optimal time to assess CR and ERR, PZQ efficacy against urogenital schistosomiasis should be measured at the earliest from week three post-treatment to assess ERR. While the optimal time point for CR assessment is the sixth week post-treatment. In settings where *S. haematobium* and *S. mansoni* co-exist, evaluation of drug efficacy, could be done at a single point, 4-5 weeks post-treatment [38].

VALIDITY OF HAEMATURIA IN UROGENITAL SCHISTOSOMIASIS

Urine dipsticks for detection of micro haematuria in urogenital schistosomiasis are considered a rapid and inexpensive means for estimating infection prevalence [39-41]. Identifying blood in the urine micro or macrohaematuria has been widely and successfully used as a good indicator of *S. haematobium* infection, mainly in high prevalence communities. According to WHO guidelines for preventive chemotherapy, prevalence of haematuria, in addition to egg count-based criteria, are recognized as effective measures for identifying communities with high, moderate, or low risk for schistosomiasis [42]. However, haematuria is a nonspecific symptom of urogenital schistosomiasis in areas of low endemicity and can be incorrectly estimated depending on the infection prevalence [5,43]. Of note that, dipstick performance after mass treatment and in communities with low indices of infection, has remained uncertain [44,45].

A Meta-analysis study of haematuria including 95 surveys to evaluate dipstick sensitivity and specificity according to factors potentially affecting test performance as age, sex and treatment status revealed that, independent of prevalence, accuracy was greater in surveys of school-aged children than in community wide surveys. This finding probably reflects lower test performance among individuals with low intensity of infection because more eggs are retained in the tissues of older individuals resulting in low level of haematuria. In addition, performance was less efficient in North Africa, as compared to other regions, indicating that *Schistosoma*-associated morbidity is not unified among different regions [46,47]. Considering the parasitological techniques as the gold

standard to diagnose urinary schistosomiasis, the overall dipstick sensitivity and specificity were estimated at 81% and 89% respectively. For low prevalence areas the estimated sensitivity for detection of active *S. haematobium* infection was higher for dipsticks (79%) than for egg detection (51%), while specificity was almost the same (98-99%). Moreover, evaluation following PZQ treatment revealed that, sensitivity for dipsticks was 79%, compared to 58% for egg detection. This suggests that reagent dipsticks may be the preferred community-level diagnostic screening tool as programs reduce parasite prevalence and move towards elimination [43].

Challenges due to false positive urine dipstick results assuming that low-intensity infection are non-morbid, has been refuted recently [48]. It appears that many haeme-positive egg-negative subjects in endemic areas are, in fact, *S. haematobium*-infected [41,49,50]. In a low prevalence community in Nigeria parasitological examination revealed that only 9.5% of examined samples were egg positive compared to 52% haeme-positive urine samples. However, infection prevalence determined by *Schistosoma*-specific PCR was estimated to be 93–98% [51]. Because of the delayed disappearance of micro haematuria which lasts for 4-6 months following PZQ treatment, urine dipstick might not be a valid indicator to monitor drug efficacy as measured by CR [38].

IMAGING TECHNIQUES

Imaging modalities as Ultrasound (US), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and X-rays are mainly employed to investigate schistosome-induced pathology and to assess evolution of pathological lesions after treatment. While intensity of infection is considered as an indirect key of disease morbidity, imaging techniques provides direct visualization of disease-induced morbidity.

Ultrasound is a non-invasive procedure applied to scan the urinary tract, liver and spleen. Pathology associated with schistosomiasis include; Periportal Fibrosis (PPF), parenchymal fibrosis having a fish scale-like structure specific for *S. japonicum*, increased blood vessel diameter, changes in organ size, bladder wall thickening and hydronephrosis of the kidney [52]. Despite being a non-invasive technique, yet it is less practical in the majority of field settings owing to the requirement of specialized equipment and trained personnel. In addition, WHO protocol for ultrasonography recommended that measurements of organ size and vein diameters should be height-adjusted, using standard reference measurements for healthy individuals of the same population [53]. As for genital schistosomiasis, trans-rectal and trans-vaginal ultrasonography is also used to investigate disease-associated morbidities [54].

The introduction of portable good quality ultrasound makes it feasible to scale up its use to involve large population in community based studies. Point of care ultrasound was employed in a large comprehensive survey in one of the Nile Delta Governorate in Egypt which encompassed 44 villages and 18168 individuals in total. While the overall prevalence was $39.3 \pm 3.3\%$, morbidity data based on sonographic examination demonstrated splenomegaly in 55 % and PPF in 47.25 %, both of them increased with age. Splenomegaly was more strongly associated with infection

than hepatomegaly and PPF, while the latter increased with history of bathing in canal water [55]. The progressive nature of schistosomiasis morbidity staying for decades explains the difference between prevalence based on ME and the proportion of cases presented with PPF and splenomegaly [56]. Additionally, damage to the portal system may be irreversible when the intensity and duration of the infection are progressive [57,58]. Therefore, morbidity data are neither sensitive nor specific indicators to assess control measures shortly after intervention [59]. On the other hand imaging data might provide additional clue to indicate the success of elimination programs. In an area of high *S. japonicum* transmission in China, US revealed that prevalence peaked at 10-14 years old patients while pathological changes peaked after the age of 30. This was explained by the progressive nature of the disease-induced pathological changes [60].

Recently, Confocal Laser Scanning Microscopy (CLSM) has been introduced as a diagnostic tool to image schistosome ova in experimental animal studies [61]. The technique was successfully applied to detect *S. haematobium* ova in the mucosal tissue of the bladder of a patient infected with *S. haematobium* [62].

MRI

MRI has been applied to diagnose neurological complications of schistosomiasis resulting from dissemination of eggs into the CNS. It has been successfully used to diagnose 16 cases of Schistosomal Myelo Radiculopathy (SMR). Cases were followed-up two and six months post treatment using the same technique [63]. Similarly, in a retrospective study which included 23 cases, MRI was implemented to diagnose SMR. Patients were from a schistosomiasis endemic area and presented clinically by lower limb weakness associated with lumbar and/or lower limb pain, urinary retention and intestinal dysfunction [64].

Regarding CT, it has been reported in limited numbers of case studies to diagnose complications of schistosomiasis. For example, chest helical computed tomography demonstrated pulmonary micro nodules and lung consolidation in the left upper lobe in a case diagnosed with acute schistosomiasis *mansoni* [65]. In Colorectal Carcinoma (CRC) associated with *S. japonicum*, the important CT features include; irregular thickening of the intestinal wall, soft tissue masses, and multiple *S. japonicum* ova calcifications inside the tumour with obscured margins and multiple intestinal masses. These lesions were detected in only some cases of those diagnosed with CRC [66].

IMMUNODIAGNOSTIC TECHNIQUES

The steep decrease in sensitivity of parasitological assays in Low-Endemicity Areas (LEAs) makes them less reliable in estimating true infection indices. Under these conditions, serology remains as a very valuable diagnostic tool, since it gives a closer approximation to the true prevalence.

Despite the restricted utilization of immunodiagnostic and molecular techniques, recent improvements and advances have been contributing to change this scenario, especially in LEAs. Nonetheless, the main issue in a new era of diagnosis overcomes technical advances per se and relates to the loss of 'gold standards' in schistosomiasis diagnosis in LEAs [67]. Here, we review and discuss the current role of immunodiagnostic and molecular methods in the diagnosis of human schistosomiasis.

ANTIBODY DETECTION TESTS

Over the last decades, various serological methods have been developed to detect antibodies against *Schistosoma* antigens. Different techniques have been applied, including Indirect Immuno fluorescent- Antibody Tests (IFATs), Indirect Haemagglutination Assays (IHAs), and Enzyme-Linked Immunosorbent Assays (ELISAs) using different antigens, such as crude or purified adult worm antigen (AWA), Soluble Egg Antigen (SEA), and Cercarial Antigen (CA) preparations [6,44,68-81].

The performance of eight serological assays, including four commercial kits, in the diagnosis of schistosomiasis in individuals from nonendemic areas, including six enzyme-linked immunosorbent assays using three different antigens, an indirect haemagglutination assay, and an indirect immunofluorescent-antibody test was evaluated. The sensitivity values ranged from 41% to 78% and were higher for *S. mansoni* than for *S. haematobium* infections. Specificity values ranged from 76% to 100%; false positive results were most frequent for samples from patients with cestode infections. By combining two or more tests, sensitivity improved markedly and specificity decreased only moderately. Serological tests proved to be useful instruments where the disease is not endemic, but due to limitations in test sensitivities, it is recommended using two or more assays in parallel [82].

Studies have shown that an indirect immune fluorescence reaction, using paraffin-embedded sections of adult worms, allows for the detection of immunoglobulin M, A and G (IgM, IgA, IgG) against antigens of the parasite's digestive tract, making it a highly sensitive method for the diagnosis of acute and chronic schistosomal infections. The specificity of this reaction has also proved to be adequate [83,84].

Recently, a study comparing the indirect immunofluorescence assay (IFA-IgM), the Circumoval Precipitin Test (COPT) and KK technique showed that positivity rates were highest using IFA-IgM. The positivity rates were 15.8%, 5.1%, and 0.9% respectively [85]. The authors suggested that, confirmation of IFA-IgM-positive cases with COPT would be beneficial, as it is a more sensitive and specific technique in patent infection [86].

ELISA is preferred for mass diagnosis. This assay fulfils the requirements of low cost, reproducibility, objectivity and rapid results [87]. The *S. mansoni* Soluble Egg Antigen (SmSEA) in the ELISA format has been shown to hold promise as a diagnostic method for the monitoring of schistosomiasis in endemic areas [88,89]. SEA-ELISA tests are the frequent method of choice

in travelers' medicine clinics [90] and there is also a commercially-produced SmSEA-ELISA kit available for use in the field [91].

Alkaline Phosphatase Immune Assay (APIA) is an antibody detection technique which permits the diagnosis of schistosomiasis using a butanolic extract preparation from adult worms [92]. APIA has demonstrated high sensitivity and specificity but it was not able to confirm active infections [93].

However, ELISA with crude antigens of *S.mansoni* was reported to overestimate the prevalence of schistosomiasis infection since it does not discriminate between active and past infections and because false positives are present due to cross-reactivity with other parasites as hookworms [94]. To overcome this situation, a modification was introduced to reduce false positives by treating the antigen with sodium metaperiodate. Specificity of ELISA-SEA-SMP improved from 73 to 97%, and maintained a sensitivity of 99% [95]. In a later study comparing ELISA-SEA-SMP, APIA, COPT and KK [96] it was suggested that, for field work, the whole population can be screened though ELISA-SEA-SMP or APIA. Then, KK and COPT would be applied only to the positives as proposed earlier [86]. In this sense, COPT, ELISA-SEA-SMP, and APIA joined to coprology allow the identification of patent infection [96].

The performance of *S.mansoni* cercarial Transformation Fluid (SmCTF) was compared with SmSEA in ELISA detecting anti- *S.mansoni*, anti- *S.haematobium* and anti- *S.japonicum* antibodies. The SmCTF antigen appeared to perform equivalently to SmSEA. Moreover, SmCTF is more easily and cheaply produced than SmSEA, with the added advantage of a reduced number of laboratory animals required for antigen production [97].

A Rapid Diagnostic Test (RDT) incorporating SmCTF has recently been developed [98] following promising preliminary results on this antigen's ability to detect anti-schistosome antibodies in an (ELISA) format [71,97]. The SmCTF-RDT meets the "assured" criteria for diagnostic tests [99]. Using a finger-prick blood sample, the test performed well for the diagnosis of both *S. mansoni* and *S. haematobium* infections in preschoolchildren. The test was at least as sensitive as duplicate KK and a single urine filtration for detection of *S. mansoni* and *S. haematobium*, respectively [98]. This has been further confirmed in Uganda. The sensitivity of the test was however reduced in children aged below three years [11]. In Zimbabwe, the SmCTF-RDT was found to be as sensitive as traditional parasitological methods (100%), and as specific as other routine antibody-detection methods (39.5 %) for the diagnosis of both *S. haematobium* and *S. mansoni* [87].

SEROLOGICAL DIAGNOSIS OF *S.JAPONICUM*

Among the techniques that have been successfully applied in the field is the ELISA incorporating *S. japonicum* Soluble Egg Antigens (SjSEA) for detection of the anti-schistosome antibodies. The assay has high sensitivity and good specificity [100-102]. However, the same problems as those with the SmSEA-ELISA are encountered: SjSEA is expensive to produce, there is the problem of requiring sera for ELISA and the ELISA format is generally not suitable for use outside of a designated laboratory [97].

Xu, et al. 2014; predicted putative secreted proteins of *S. japonicum* (SjSPs) and expressed them as Glutathione S-Transferase (GST)-fusion proteins. The fusion proteins were arrayed on glutathione (GSH)-immobilized microplates and screened with serum samples from patients with schistosomiasis diagnosed by the KK method. They further assessed an identified protein marker for sensitivity and specificity. The reactivity of SjSP-13 to 476 serum samples showed 90.4% sensitivity and 98.9% specificity. Of 1371 residents enrolled in a field study, only 74 individuals were identified as being egg-positive, whereas 465 were diagnosed as positive by the SjSP-13-based ELISA kit (rSP13-ELISA). Of the 394 individuals found egg-negative but rSP13-ELISA-positive, 363 (92.4%) were confirmed to be positive for schistosome infection by PCR detection of *S. japonicum* SjR2 retro transposon [103].

A schistosome protein microarray was used in screening of samples from individuals living in an area of *S. japonicum* endemicity. The test has demonstrated significant differences in the antibody profiles for sera from individuals in the acute, advanced, chronic, and exposed but Stool Negative (SN) groups. A set of 25 antigens, most of which are surface-located proteins, including several *S. mansoni* and *S. japonicum* tetraspanins, as well as previously described vaccine candidates, were identified. Subjects in the acute and advanced disease had a high total anti-parasite antibody response but a reduced antibody response against protein microarray antigens, compared with subjects in the chronic and SN groups. This suggests that subjects with mild pathology use a broad and strong antibody response, particularly against surface-exposed proteins, to control pathology and/or infection. Specific antigens identified could discriminate between *S. japonicum*-exposed groups with different pathologies [104].

Another study strongly suggested that SjSP-216, a highly expressed gene in the young worm stage, could serve as a potential biomarker for the early immunodiagnosis of *S. japonicum* infections in vertebrate hosts. The authors recorded 100 % diagnostic sensitivity and specificity of SjSP-216-based ELISA in both infected mice and rabbits, three weeks after infection [105].

LIMITATIONS OF ANTIBODY DETECTION TECHNIQUES

Although antibody detection techniques are very efficient, they do not constitute part of the diagnostic routine for schistosomiasis, and also they are not commonly used in epidemiologic studies. Antibody detection methods often cannot distinguish between current and past infection. [106], and are often criticized for their lack of specificity [87]. However, possible alternative explanations for the lack of specificity are that antibody-positive, egg-negative patients may in fact have infections that are missed by insensitive parasitological methods that failed to detect eggs in subjects who are lightly-infected [107] or who have been treated with sub-curative drug doses [108]. There is of course also the possibility that antibody false-positives are due to cross-reactivity with antigens of other helminths [44,67]. Slow reduction of specific antibody levels after treatment is another disadvantage [109]. Moreover, blood collection is not easily applicable under field conditions in endemic areas [3].

Despite their failings, antibody-detection is for some time likely to remain the best available method for diagnosis in areas of low intensity of schistosome infection [110,111]. Antibody tests may also have a role in checking for maintained exposure to schistosomiasis in areas that are moving towards elimination [112].

Antigen Detection Tests

These tests are based on detection of schistosome antigens in the serum and urine of infected individuals [3,113,114]. The main circulating antigens are; adult worm gut-associated circulating antigens, Circulating Anodic Antigen (CAA) and Circulating Cathodic Antigen (CCA). The tests can detect current infections and are potentially more sensitive for diagnosis of cases in low transmission areas [115-117].

Assays detecting Circulating Antigens (CAA) and (CCA) have been evaluated in replacements for microscopy in the diagnosis of infection due to *S.haematobium* or *S.mansoni*. These tests can differentiate between active and past infections, as the circulating antigens are probably present only when there is active infection [44]. As circulating antigens are released from living worms, antigen levels may correlate directly with parasite load, while microscopy does not. Hence, these tests seem very promising, as serum levels of circulating antigens are related to actual worm burden and rapidly decrease following drug treatment [67,112,118]. This monoclonal antibody-based ELISA determines serum CAA levels for all human *Schistosoma* species with virtually 100% specificity [119]. However, the sensitivity has been shown to vary with prevalence of disease and intensity of infection [120-124].

A Lateral Flow (LF) assay was developed to test CAA in serum for individual diagnosis of imported active schistosome infections. Application of fluorescent submicron-sized Up-Converting Phosphor Technology (UPT) reporter particles increased analytical sensitivity compared to that of the standard ELISA method. The UPT-LF assay identified 36 CAA-positive samples, compared to 15 detected by CAA-ELISA. The UPT-LF assay proved to be a low-complexity test with higher sensitivity than the CAA-ELISA, well suited for laboratory diagnosis of individual active *Schistosoma* infections [125].

A test that is affordable, rapid, easy-to-use and interpret and works on whole blood will obviate the need for a laboratory or even electricity and allow diagnosis at the Point-Of-Care (POC). POC tests may have comparable or higher sensitivity to microscopy [126]. POC diagnosis is beneficial not only for individual patients, but it will also allow for more rapid mapping of disease prevalence [127], and monitoring of schistosomiasis control programs, factors which are important in efforts to meet the 2020 goals [128].

A rapid immuno chromatography strip test for the detection of *Schistosoma* CCA in urine has been introduced. This simple-to-use field test has shown its value in epidemiological surveys, particularly in remote areas of *S. mansoni* endemicity [123,129].

The urine POC- CCA test is a lateral flow immuno-chromatographic urine dipstick assay that uses a nitrocellulose strip with a monoclonal antibody coated test line to detect the presence of Schistosoma-specific CCA antigen in urine. When urine from an infected individual flows through the strip, the antigen will bind to the test line, which becomes visible with the binding of added labelled monoclonal antibodies (Figure 4).

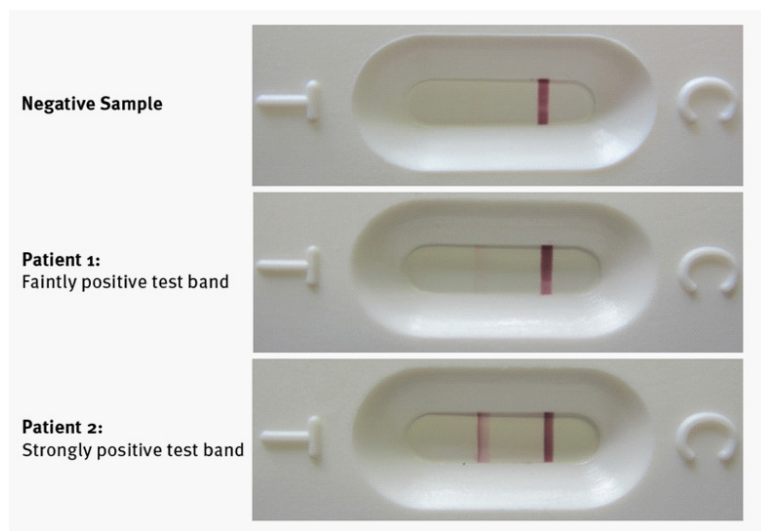


Figure 4: Point of care rapid test for the detection of CCA (POC-CCA) in urine of two positive cases infected with schistosomiasis mansoni, Becker, et al. 2015 [167].

Evaluation of the diagnostic performance of the urine strip test CCA and single-day double KK measurements over three consecutive days for the diagnosis of *S. mansoni* revealed that, CCA is an appropriate tool for mapping surveys of *S. mansoni* infection. Its diagnostic performance remained constant after PZQ treatment and the test was overall more sensitive (approximately 90%) but less specific than single-day double KK faecal slides [129,130].

The performance of (POC/CCA) cassette test was evaluated by several recent studies. They reported better performance of a single urine-CCA cassette test for diagnosis of *S. mansoni* infection compared to single or more KK thick smears [34,120,131-134].

When evaluated in *S. mansoni*-infected preschoolers, the sensitivity of one POC- CCA was much higher than a single KK for diagnosis before (69.7% versus 28.3%) and after treatment (80.0% versus 4.0%) [98].

In low endemic areas, it has been shown that most (if not all) KK- negative/POC-CCA positive individuals are likely to harbor low level infections with *S.mansoni* [135]. Moreover, in areas with a moderate to high prevalence of infection, the POC-CCA test for *S.mansoni* could also detect a very large proportion of microscopy negatives. This is possibly because the test is potentially more sensitive than microscopy [136]. While not a perfect test (as seen in the day-to-day variability)

the POC-CCA assay appears to be highly suitable for initial prevalence mapping of *S.mansoni* infection, and for subsequent post-treatment programmatic decision making [135].

When compared to ELISA testing for host antibodies (IgG/M) to Soluble Egg Antigens (SEA), CCA was best to capture and identify infections in early stages showing very promising diagnostic performance and robust field performance with high sensitivity. In addition, prevalence based on the CCA, when considering trace as positive, was very close to that of ELISA [34].

In spite of being sensitive for *S.mansoni* infections [120,131-134,137], the POC-CCA test is less sensitive for *S.haematobium* [91,123]. In addition, its widespread use may be limited to some extent by its cost which is around US\$1.75/test [111,120].

Regarding the urine CCA ELISA, it was found to have the best diagnostic performance, followed by the serum CAA assay for *S.mansoni* [118,138,139].

The urine-based CCA and CAA assays for *S.mekongi* and *S.japonicum* diagnosis was evaluated by examining banked urine samples by the Up Converted Phosphor Lateral Flow (UCP-LF) CAA assay and the POC-CCA urine assay. Based on 250µl - urine samples, UCP-LF CAA sensitivity outcomes surpassed a single stool examination by the KK technique. The sensitivity of a single urine POC-CCA was in the same order as that of a single KK thick smear examination, while the sensitivity approached that of triplicate KK when a combination of both CAA and CCA assays was used [140].

MOLUCAR TECHNIQUES

Polymerase Chain Reaction (PCR) methods have been used to improve the direct detection of *Schistosoma*. They are less labour intensive when many samples are to be examined, and also have the advantage of being less influenced by observer variation/bias as compared to microscopy-based methods. PCR is done on stool, urine, serum or organ biopsy samples [141,142]. The source of DNA is the adult worm, which sheds tegument at regular intervals and detection of such parasite-specific DNA proves the presence of the parasite [143].

Detection of Parasite-Specific DNA In Faecal Samples

Pontes, et al. 2002; reported the first use of conventional PCR for the diagnosis of *S.mansoni* DNA in faecal samples. This work standardized and evaluated the technique in endemic areas and it detected 1 fg of *S.mansoni* DNA. The specificity of this method was demonstrated by the absence of the DNA of other helminths (*Ascaris lumbricoides*, *Ancylostomoduodenale*, *Necator americanus*, *Taenia solium* and *Trichuris trichiura*), which commonly infect people in endemic areas [109]. In addition, PCR can be an important tool for detecting *S. mansoni* infection in individuals excreting few eggs, and it can be used to confirm the results of seropositives [144]. However, this technique requires several steps after DNA amplification, including gel electrophoresis, which limits the number of samples that can be conveniently analyzed. In spite of its high sensitivity and specificity, PCR gave negative results among few cases considered sure positives by microscopy.

It was reported that these missed cases were misdiagnosed by the DNA amplification assay due to many factors such as: inhibition of the amplification reaction by faecal compounds and / or DNA degradation during transportation from the field, variation in egg output and uneven distribution in faeces [141]. Similar results were also obtained by many authors [28,145-147].

A multiplex real-time PCR assay for the detection and quantification of schistosomal DNA in faecal samples was developed and evaluated. It proved to be sensitive and 100% specific. The simple faecal sample collection procedure and the high throughput potential provide a powerful diagnostic tool for epidemiological studies on schistosomiasis in remote areas. Moreover, it demonstrated high sensitivity, even in samples containing a single *Schistosoma* egg in stool [148,149].

A highly specific multiplex real-time PCR targeting the *Schistosoma* Internal Transcriber-Spacer-2 Sequence (ITS2) was developed. ITS2 PCR on one stool sample detected more infections, especially in people with light-intensity infections and in children from low-risk area compared to stool microscopy. Consequently, it may be very useful for the detection of *S. mansoni* in areas with low levels of infection [150].

Espirito-Santo, et al. 2014; evaluated the performance of a TaqMan® Real-Time PCR system; a quantitative Polymerase Chain Reaction (qPCR) technique in serum and faeces DNA samples. The positivity rate presented by the qPCR approach was far higher than that obtained by parasitological techniques. The qPCR-faeces presented the highest positivity [151]. A SYBR Green (a fluorescent based technology) and Taq Man probe real time PCR assays were developed for the detection and quantification of schistosomal DNA and proved to be useful for quantification of parasite burden in human infection [145,148,152].

Gomes, et al. 2010, developed the PCR-ELISA system for the detection of *Schistosoma* DNA in human faeces as an alternative approach to diagnose light infections. The system permits the enzymatic amplification of a specific region of the DNA from minute amounts of parasite material. Then DNA is detected using an ELISA platform, which measures colorimetric intensity in a microplate reader. It can be considered a semi-quantitative method for diagnosis of low intensity of schistosomiasis, as the mean absorbance readings corresponded to the intensity of infection. The high sensitivity of the *Schistosoma* PCR-ELISA system was evidenced by the high number of positive samples. The advantage of PCR-ELISA as compared to PCR-electrophoresis is that it makes use of standard equipment widely used for the processing of ELISAs, and the reagents used are easy to obtain commercially. Therefore, PCR-ELISA allows for the use of PCR-based DNA diagnosis for routine purposes in laboratories in less developed countries with fewer resources [153].

PCR-ELISA was further evaluated by Siqueira, et al. 2015; and showed a positivity rate of 23.4% (47/201). When patients were followed up 30, 90 and 180 days after treatment to establish the cure rate, PCR-ELISA revealed cure rates of 98.5%, 95.5% and 96.5%, respectively.

They concluded that, PCR-ELISA is very useful to confirm suspicious cases that were not detected using conventional parasitological methods and to evaluate cure after chemotherapy [154].

Detection of Parasite-Specific DNA in Urine Samples

For *S. haematobium*, it was demonstrated that detection of a species-specific DNA fragment from urine sediment obtained by filtration is the best estimator of true infection. It was also concluded that this approach is closer to the actual “gold standard” for schistosome diagnosis than presence of eggs, antigen capture or serology. Furthermore, *S. haematobium* DNA becomes undetected two weeks after drug treatment [143,155]. Concerning *S. mansoni*, species-specific DNA detection from urine sediment obtained by filtration proved to be more sensitive and specific than KK [156].

Amplification of species-specific DNA by PCR showed much higher sensitivity (99%-100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30% respectively) for both schistosome species. The test was able to differentiate between single and mixed infections of *S. mansoni* and *S. haematobium* in a single urine specimen. Hence, this test can be an important tool for rapid identification of multi Schistosome species- infected individuals before and after chemotherapy [157].

Sandoval, et al. 2006; developed a diagnostic approach based on the detection of parasite DNA by PCR in urine, which could detect parasite-derived DNA seven days p.i. in a murine model. They concluded that this PCR technique was potentially useful for the detection of acute *S. mansoni* infection in human and can be used on non-invasive samples such as urine, but had less reliable results when performed in other samples such as stool. In addition, this technique was more sensitive than the two classical methods-KK and indirect ELISA-for the diagnosis of schistosomiasis [158].

An Internal Transcribed Spacer (ITS) -based real-time PCR showed excellent sensitivity of 100% and 85.2% in urines with > 50 eggs/10 mL urine and ≤ 50 eggs/10 mL urine respectively. Additionally, *Schistosoma*-specific DNA was amplified in 102 of 673 samples in which *Schistosoma* eggs could not be detected with microscopy. This technique proved to be a powerful tool in epidemiological surveys of schistosomiasis providing more precise and sensitive results than microscopy [159].

Obeng, et al. 2008; using a real-time PCR achieved 100% specificity and sensitivities of 100% and 87% in urines with > 50 eggs/10 mL urine and ≤ 50 eggs/10 mL of urine, respectively [122]. A higher sensitivity of the PCR in samples with low egg counts might be achievable by using a larger volume of the urine sample by applying filter-based DNA isolation methods [143,155]. It is worth mentioning that real time PCR demonstrated less day to-day variation and higher sensitivity compared to microscopy. It was proved also to be an accurate and reproducible tool for monitoring treatment efficacy two and 18 months post treatment [160].

A real-time PCR targeting the *Dra*I sequence for *S. haematobium*-specific detection in urine, faeces and serum was reported to be a promising tool to confirm the diagnosis, also during the acute phase of urogenital schistosomiasis. A novel method of collecting the urine specimens in the field and filtering them through heavy Whatman No. 3 paper was introduced. It was concluded that the urine filter paper method was sufficiently sensitive to detect low and cryptic infections, that DNA detection was more sensitive than egg detection, and that the filtration method facilitated specimen collection and transport from the field [161].

Detection of Parasite-Specific Dna in Serum Samples

Pontes, et al. 2002; reported for the first time the high sensitivity of PCR in the detection of free circulating *S. mansoni* DNA in serum of two infected patients with active intestinal schistosomiasis [109]. Using the same primers Sadek, et al. 2008; found that PCR for the detection of circulating DNA of *S. mansoni* proved to be of high significant value in the diagnosis of active intestinal schistosomiasis with a sensitivity of 97.2% and a specificity of 100% [162]. When PCR based on the same primers was applied to mice sera, detection of the free circulating DNA of *S. mansoni* started on the third day p.i., with increase in the strength of the specific band as the infection progressed [163]. Furthermore, a specific PCR assay detected *S. japonicum* DNA in the sera of experimentally infected rabbits at the first week p.i. [164]. Hence, detection of circulating DNA could be a novel diagnostic tool for the diagnosis of schistosomiasis in the early prepatent period allowing early treatment to avoid the occurrence of irreversible pathological reactions caused by the deposited eggs [163].

A real-time PCR, targeting a highly repeated 121-bp sequence of *S. mansoni* (named Sm1-7) was developed to detect cell-free schistosome DNA in serum and proved to be successful in diagnosing acute and chronic *S. mansoni* infection [165].

PCR may currently be the most sensitive and specific method available for the diagnosis of schistosomiasis in low-endemic areas. However, it is unlikely to become useful for mapping purposes in the near future due to the costs and difficulties associated with performing PCR in the field [87]. Table 1 summarizes diagnostic assays available for human schistosomiasis.

In conclusion, comparing parasitological (Kato-Katz), immunological (ELISA) and molecular (real time PCR) techniques, in the diagnosis and cure assessment after treatment with PZQ, it is suggested that the methods should be used in low endemic areas as follows: serology should be used in the initial diagnosis in a population with potential positive cases; subsequently, coproscopy should be used in IgG positive cases to confirm the current infection; and qPCR should be used to evaluate the infection cure after treatment and is also a very valuable tool when there are cases showing positive IgG and negative coproscopy [166].

Table 1: Diagnostic assays available for human schistosomiasis.

Diagnostic assay	<i>S. mansoni</i>	<i>S. haematobium</i>	<i>S. japonicum</i>
Parasitological diagnosis	Stool Kato Katz	Urine filtration	Stool Kato Katz
Antibody detection	✓	✓	✓
Antigen detection	✓	+/-	+/-
PCR	✓	✓	✓
Assessment of infection intensity	(EPG/g of stool) Kato Katz	Egg10/ml of urine Filtration	(EPG/g of stool) Kato Katz
Assessment of drug efficacy	CR, ERR	CR, ERR	CR, ERR
Mapping/elimination	Seroepidemiology	Seroepidemiology	Seroepidemiology

CR: Cure rate, ERR: Egg Reduction Rate

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