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REVIEW

Advances in Diagnosis of Schistosomiasis: Focus on Challenges and Future Approaches

Bayissa Chala

Department of Applied Biology, School of Applied Natural Science, Adama Science and Technology University, Adama, Ethiopia

Correspondence: Bayissa Chala, Email baychal07@gmail.com; bayissa.chala@astu.edu.et

Abstract: Schistosomiasis is the second most devastating parasite prevalent in the tropical region of the world, posing significant public health impacts in endemic areas. Presently, several disease mitigation measures have shown a decline in transmission of the infection rate in risk localities using mass drug administration (MDA) of school-based or community-wide treatments. Despite all the endeavors made, the decline in transmission of infection rate has not been attained in the entire medicated segment of the population. Perhaps the current challenges of control of the disease appear to be strongly associated with a lack of appropriate diagnostic tools. It's well known that the current diagnosis of schistosomiasis greatly relies on conventional methods. On the other hand, minor symptoms of schistosomiasis and low sensitivity and specificity of diagnostic methods are still unresolved diagnostic challenges to clinicians. Numerous scholars have reviewed various diagnostic methods of schistosomiasis and attempted to identify their strengths and weaknesses, currently on function. As a result of the known limitations of the existing diagnostic tools, the need to develop new and feasible diagnostic methods and diagnostic markers is unquestionable for more precise detection of the infection. Hence, advances in diagnostic methods have been considered part of the solution for the control and eventual elimination strategy of the disease in endemic areas. As of today, easy, cheap, and accurate diagnostics for schistosomiasis are difficult to get, and this limits the concerted efforts towards full control of schistosomiasis. While looking for new diagnostic methods and markers, it is important to simultaneously work on improving the existing diagnostic methods for better results. This review tries to give new insights to the status of the existing diagnostic methods of schistosomiasis from conventional to modern via summarizing the strengths and limitations of the methods. It also tries to recommend new, sensitive and feasible diagnostic methods for future approaches.

Keywords: diagnostic challenges, new diagnostic methods, schistosomiasis, detection methods

Introduction

Schistosomiasis is the second most important parasitic infection after malaria in terms of public health impact.[1] It is a disease of poverty often associated with other helminth parasites and food insecurity.[2] Schistosomiasis remains one of the major neglected tropical diseases (NTDs) causing chronic human illness with serious consequences for socio-economic development in tropical countries. It is widespread in 74 nations around the world, more common in Asian, African and South American continents.[3] According to WHO reports close to a quarter of a million people in 78 nations are infected and the number of people at risk may be projected to 800 million.[4]

As of today, the diagnostic standard for active schistosomiasis is detection of viable ova in urine (*S. haematobium*) or faeces (*S. japonicum, S. mansoni*).[5] Nevertheless, the presence of a schistosomes infection cannot be ruled out definitively owing to the low sensitivity of standard urine and faecal tests. Despite the drawbacks, the WHO recommends microscopic examination of polycarbonate filters for ova in the urine, urine dipstick assays for heme, or the Kato-Katz faecal examination for schistosome mapping and field-based control of schistosomiasis.[6]

During the past few decades, control strategies of schistosomiasis relied on treatment of patient targeting to decrease morbidity, largely in sub-Saharan Africa and other high-prevalence areas. The use of targeted mass drug administration (MDA) has managed to reduce local prevalence among the victimized population after repeated treatment programs in school children and communities.[7,8] To date, mass drug administration has been the pivotal arm of schistosomiasis

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control along with supplemental snail control. Such control strategies are practiced primarily through the use of preventive chemotherapy with 40 mg/kg praziquantel (PZQ) (the drug of choice for schistosomiasis), as advocated by the World Health Assembly in 2001 through resolution 54.19. Even though it has strong efficacy against adult worms, PZQ has no protection against reinfection.[9]

It has been reported that several schistosomiasis interventions have managed to reduce local transmission in high disease burden areas largely with the use of targeted MDA but the global disease burden is still quite high despite extensive efforts and immense integrated control measures practiced in the past years.[10] In particular, the use of targeted MDA be it school-based or community-based, the infection rate decline has not been successful in all treated communities.[11,12] Such persistence of the disease despite all attempts can be partly associated with the absence of precise analytical devices for case identification and community screening where endemicity prevails. Recently, extensive research trials scrutinized the drawbacks of both forms of treatment regimens.[11,12]

Given that schistosomiasis has significant public health importance, new intervention tools are necessary for the precise diagnosis of the infected cases to prevent morbidity. Methods that enable infections to be properly diagnosed are a prerequisite for successful disease control. The achievement of new diagnostic tests may be possible by the consistent and continuous trial on identification of specific biomarkers. In this regard, several studies reviewed common diagnostic methods of schistosomiasis along with their strengths and weaknesses from different parts of the world.[11–14]

In this paper the existing diagnostic methods and the recent diagnostic developments in schistosomiasis are reviewed. It also attempts to address the strengths and limitations of the available methods and the challenges and possible future approaches pertaining to diagnosis of schistosomiasis.

Present Status of Schistosomiasis Diagnoses and Their Limitations

The diagnosis of schistosomiasis has been transformed from clinical and conventional diagnostic approaches to the most sensitive methods. The usual diagnosis involves medical history, physical examination, laboratory investigations and radiological techniques as key indicators. The clinical, subclinical, or biochemical morbidity markers are the indirect diagnostic methods with non-specific presentations.[15,16]

Schistosomiasis can be diagnosed by direct or indirect laboratory methods. Direct parasitological examinations aim to detect parasite ova in fecal samples, urine samples or in the tissues while direct immunological approaches target the detection of the schistosome-derived antigens in the circulation and excreta. On the other hand, indirect immunological tests detect the specific antibodies induced against the different stages of the parasite in blood.[17]

Present Diagnostic Methods of Schistosomiasis

Conventional Parasitological Diagnosis

Currently, the available diagnostic methods for schistosomiasis are those that rely on stool and urine microscopy for parasite detection. These include Kato-Katz (KK) and urine microscopy, serum antibodies, antigen detection, and the detection of DNA of the parasite. The conventional parasitological methods are the classical diagnostic approaches to be discovered. These involve direct microscopic finding of ova in fecal smears for intestinal schistosomiasis, or urine for urogenital schistosomiasis and miracidium hatching.[18] Moreover, cercarial shedding and microscopic examination of snails are part of conventional diagnostic methods. It is evident that microscopic examination of stool and urine remains the gold standard test for diagnosis of schistosomiasis with some limitations.[10] *Schistosoma haematobium* ova are released in urine and are detected by microscopy in a urine sample concentrated by sedimentation, centrifugation, or filtration and forced over a paper or nitrocellulose filter.[19] Concerning intestinal schistosomes like *S. mansoni* and *S. japonicum*, the Kato-Katz fecal smear technique is the standard method recommended by the WHO for the quantitative assessment of infection intensity.[20] The parasitological method Kato-Katz thick stool smear method was first introduced in 1972.[21]

The Kato-Katz technique has been reported to show a high level of specificity, is simple to use, inexpensive, less laborious and suitable under field conditions as compared to other similar procedures. On the other hand, sensitivity of the technique for detection of schistosomiasis varies by the species, intensity of infection and number of stool samples examined.[22] For instance, when the magnitude of infection is considerable, with a huge worm burden in the host, KK

exhibits high sensitivity owing to considerable egg output. On the contrary, the sensitivity of the test is challenged by samples using low-magnitude of infections in low infection rate areas.[23,24] Similar reports have shown that the daily difference in schistosome ova release and the over dispersal of egg output can result in significant variability in KK test outcomes from day to day, particularly under light infection scenarios or after PZQ medication.[25–28] It is also suggested that clumping together of ova in stool specimens can result in considerable variation in egg counts which may affect precise reporting of infection rate and/or intensity on the ground.

Additional parasitological techniques, namely: formol-ether sedimentation, salt flotation and centrifugation, and the interaction of magnetic microspheres with ova, have been available for the betterment of the microscopic analysis of fecal specimens.[29] The miracidium hatching test which depends on the positive phototrophic behavior of schistosome miracidia, has also demonstrated a valuable role in the detection process[30] where improvement of sensitivity of the test prevails.[31] On the other hand, the general shortcomings linked with the direct observation of ova by microscopy is normally inevitable. It is also remarkable that such adjustments are tedious and are often not selected for routine or extensive screening.

It's well established that urine microscopy is regarded as the gold standard for *S. haematobium* ova detection in endemic areas. Urine filtration and concentration of the ova constitute the major parasitological techniques employed in the diagnosis of urogenital schistosomiasis.[32] Perhaps in comparison with urine concentration, syringe filtration is chosen in public and school assessments for its ease of handling and the need for less equipment. Similar to stool sample testing for ova, syringe filtration too suffers from low sensitivity.[33]

In general, conventional parasitological diagnostic techniques using microscopy are inexpensive and do not need wide-ranging training and sophisticated facilities. Nevertheless, in addition to their low sensitivity for detection of light infections, conventional parasitological methods are labor-intensive and time-consuming. On top of these, centrifugation or filtration steps can be needed for the concentration of eggs which elongates the step of diagnosis. Since oviposition in intestinal and urogenital schistosomes starts at about 4 to 6 weeks and 90 days after cercarial infestation, respectively, early diagnosis of the disease prior to the parasite becoming patent is not possible using the conventional direct egg detection methods.[34]

Indirect methods for diagnosing schistosome infection using clinical, subclinical, or biochemical morbidity markers have been practically used. For instance, variations in urinalysis give vital evidence for the detection of urogenital schistosomiasis. The presence of blood and protein in urine, respectively were reported to have obviously been linked with urogenital schistosomiasis. Such evidence, including macro- and microhematuria, were employed suitably in public surveys through rapid-detection reagent strips coupled with demographic data.[35] Dipstick diagnosis of microscopic haematuria has been valuable in detecting low-level infection. Likewise, urine heme dipsticks have been shown to be practical in evaluating the outcome of PZQ in mass treatment programs on *S. haematobium* in Africa.[36] Though they are non-specific markers as haematuria can be caused by diseases other than urogenital schistosomiasis.[37] Other indirect methods include the use of clinical assessment of the patient along with ultrasound, liver biopsy, and subsequent histological examination and the measurement of biochemical markers.[19]

Malacological Survey

A malacological survey is the assessment of infection in snail intermediate hosts. The finding of infected snails and the identification of miracidia in aquatic samples are the two vital components of malacological surveys.[38] Little attention has been given to malacological approaches as one of the controls of schistosomiasis.

Morbidity reduction and ultimate elimination via integrated control actions have been the targets of the existing schistosomiasis control approaches. It has been suggested that precise identification of the infections in humans and snails, the definitive and intermediate hosts, respectively, is of paramount use in achieving these goals. The vast majority of researches on schistosomiasis have been given major emphasis on disease prevalence and intensity of infection among human populations. Although previous studies reported that snail infections are supposed to indicate infection rate and magnitude of human schistosomiasis, efforts to identify and target the intermediate snail hosts in endemic areas are apparently overlooked. But integrating snail distribution with human infection data is quite useful for the ongoing control program. Recent studies showed that vibrant modeling of snail vectors is vital for a precise forecast of spread in the state

of unclear transmission status.[39] The PCR of snail pools has been shown to be practical for large-scale monitoring of *S. haematobium* transmission, however there is a need to improve the cost-effectiveness of the test by development of a method that is cheaper and more field friendly.[40] On the other hand, very limited studies have applied molecular techniques to identify and map potential areas for transmission of schistosomiasis using intermediate host snails.[41,42]

There are two commonly used methods for detecting schistosome infections in snail intermediate hosts. These are smashing of snails between glass slides and examination for sporocysts and cercariae and shedding of cercariae by isolating snails in small containers under constant light and temperature. The technique of cercariae shedding has been reported to be the popular method to assess snail infection status.[43,44] However, the cercariae shedding technique suffers from some limitations in detecting the parasite under low parasite prevalence and during prepatent infections. Moreover, screening for cercarial shedding in snails with mixed infections may only identify mature infections thereby underestimating the true prevalence.[45] In addition to detection limitations with the techniques, the costs and labor-intensive nature of these procedures poses major hindrances in their application. Actually, interaction of snail-parasites is a dynamic process influencing the development of the larval stages of the parasites.

Immunological Diagnosis

As of today, we have constantly been introduced with various methods developed to measure the host's immune response using crude or purified egg and adult worm antigens to detect antibodies. Immunological assays target antischistosomal immunoglobulins or schistosomal antigens circulating in body fluids (plasma, serum, urine, or sputum). Intradermal test (ID), indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), dipstick dye immuno-assay (DDIA), circunoval precipitin test (COPT), dot immunogold filtration assay (DIGFA), indirect immunofluorescence test (IFT), etc. are among the most familiar of such tests.[46]

Immunological methods complement the conventional methods mainly when the latter show negative results for those with low infections.[47] The advance of serological tests has created a convenient scenario for smart and speedy tests that are practical both in public and facility-based settings in endemic localities. By the decline in infection rate and magnitude after mass treatment, conventional parasitological methods were not able to isolate cases precisely leading to false-negative results. Under such situations, indirect immunodiagnostic assays are chosen as a result of their simplicity and high sensitivity. The assay is particularly vital in surveillance of the disease and preliminary inspection where low infection rates prevail. Nonetheless, the targeted serum antibodies are likely to stay for an extended period even after clearance of the infectious agents.[48,49]

ELISA is considered as the major serological assay exhibiting top sensitivity and specificity in diagnosing schistosomiasis so far. The process follows binding of soluble proteins (antigens or antibodies) on a surface of multiple-well plates. It offers the option of detecting different antibody classes using a wide range of antigens. Through an affinity of binding between antigen and antibody, a qualitative or quantitative result is generated.[50] The detection of *Schistosoma* antigens by ELISA was initially based on the use of crude soluble egg antigens (SEA) and soluble adult worms proteins (SWAP) and slowly followed by purified antigen, commonly called excretory/secretory antigens. The detection of SEA and SWAP in serum and excreta provides a lot of diagnostic potential as the antigen levels correlate well with parasitic load so that early treatment would be initiated.[51,52] Assays for the detection of circulating schistosome adult worm antigens provided an alternative method for the diagnosis of schistosomiasis. The main advantages of those circulating antigens include the high specificity, positive correlation with worm burden, and the possibility for estimation of infection intensity. Moreover, circulating *Schistosoma* antigens disappear rapidly after treatment and can therefore be used for assessment of cure.[53]

As compared to other diagnostic methods, immunological testing (both antigens and antibodies) are relatively simple. They are good options for detecting early infections with relatively high specificity. Other than the worm or egg antigens, antigens from schistosomula or cercariae have been used for ideal immunodiagnostic assay to detect early infections;[54] though extracting antigens from the schistosomula or cercariae is not an easy task.

On the other hand, a couple of drawbacks have been linked with immunodiagnostic methods. Previous studies have revealed that the reactivity of antibodies against crude worm antigens remains low until the infections become patent in the experimental hosts. Similarly, since antischistosomal antibodies tend to remain longer, it is not easy to distinguish

active infection from earlier exposure to an infection that has already been cleared.[48] However, previous studies reported that a 200-kDa tegument protein (Sm200) of *S. mansoni* was employed in an ELISA diagnosis for *S. mansoni* infection in a mouse model, revealing very high sensitivity and specificity. The use of such tegument protein may be a potential for diagnosis of intestinal schistosomiasis.[55]

As a result of the shortcomings linked with antibody tests, misdiagnosis of active disease is usually a problem,[56] and the curative response following PZQ treatment has not been precisely evaluated. In several antibody detection assays, the antigen prepared is a crude parasite extract of varied components which may result in high rates of cross-reactivity where related trematodes and soil-transmitted helminths (STH) occur, leading to lower test reliability and reduced specificity.[23,57] Antibody testing methods are limited not only by their inability to distinguish active infections from previous ones but also their inability to distinguish between *Schistosoma* species.[56]

Strengths and Limitations of the Present Schistosomiasis Diagnostic Methods

Current diagnostic tools for schistosomiasis have realistic shortcomings regardless of their technical advancement. For instance, the implementation of new methods both in field and facility-based can be possible limitation if not the techniques are of reasonable price and convenient for field condition. Normally, schistosomiasis is endemic in low income nations where road and health facilities are limited.

Surveillance and diagnosis play key roles in schistosomiasis control; however, current direct parasitological techniques for surveillance and diagnosis of the disease have limitations. Although these methods are the gold standard for detecting schistosome infections, they are labor intensive and time consuming and not suitable for large-scale disease surveillance. The sensitivity of the parasitological diagnostic techniques depends on the rate of egg excretion and a major disadvantage is that the techniques have low sensitivity in low-prevalence endemic areas, resulting in high false-negative rates.[58,59] Hence, this method of diagnosis may prevent the benefits of early detection and the treatment with praziquantel.[60] It is a general recommendation that testing for schistosomiasis be repeated by follow up with two consecutive visits for increased accuracy, since none of the current diagnostic tests is absolutely accurate.[61]

The Kato-Katz technique is still the gold standard in schistosomiasis diagnosis.[21] Due to the ease of its use in the field and relatively low cost, the Kato-Katz technique is recommended by the World Health Organization (WHO) for surveillance and epidemiological field survey of STH infections and schistosomiasis.[62] Although this technique is cheap, convenient, and qualitative.[21] It is limited in the diagnosis of low-grade and prepatent infections, as well as in evaluating drug therapeutic effects.

Although immunological methods have comparable sensitivity and specificity as that of the PCR technique, the former cannot distinguish between current infections and past infections[63] which may lead to inappropriate treatment intervention. Serologic assays have proven useful clinically[64] for diagnosis by the detection of antibodies against schistosomal antigens. This approach, with an extremely wide variety of reported immunodiagnostic assays, is particularly useful for symptomatic travelers or for serosurveys. But, the level of antibodies persisted after treatment could not indicate whether the patients were cured or not.[65,66]

In general, molecular techniques for schistosome DNA detection in faecal, urine or blood specimens have high sensitivity, but are expensive and still suffer somewhat from sampling limitations.[67,68] Obviously, nucleic acid-based detection has been shown to be superior in identifying different schistosomes in cases of low-grade infections. The rate of positivity is dramatically higher than that when using the Kato-Katz method and miracidium hatching.[69,70] PCR has been reported to suffer from the same limitations as microscopy and does not provide a significant clinical benefit because only a small volume of the sample can be processed for DNA extraction. Hence, it is dependent on chance whether the processed sample contains ova or not. In most low resource settings, the limited resources and the requirement of expensive technology, cold chain logistics, uninterrupted power supply, and highly skilled manpower has limited the use and application of molecular screening methods like PCR.[67,71] Ibronke et al[71] added that, since DNA is extracted from a small volume of sample success depends on whether the sample processed contains ova or not. Other limitations in the field application of DNA based diagnostics is total DNA sample preparation and the purification from urine.[72]

Moreover, DNA-based assays remarkably reduce the false-negative rate and effectively monitor potential exposure to schistosomiasis. PCR can not only precisely evaluate drug therapeutic effects,[73] but can also identify early infections[74]

and assess infection density in hosts. However, the dependence on expensive apparatus and on specialized training in molecular biology limits their extensive applications for field conditions. As an alternative, the loop-mediated isothermal amplification (LAMP) may play a role for diagnosis of schistosomiasis. The LAMP assay was first reported by Notomi et al[75], has become accepted rapidly for detection of various pathogens, including human schistosomes. This technique does not require sophisticated equipment for DNA amplification or for amplicon detection, which is of great value for field study. The method employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. An inner primer containing sequences of the sense and antisense strands of the target DNA initiates LAMP.

New Diagnostic Methods of Schistosomiasis

Proteins as Diagnostic Markers

Current developments in proteomics and transcriptomics have revealed vital progresses. They resulted in the detection of a range of schistosome molecules released at various stages in the life cycle, like proteins and other components, with promising diagnostic candidates. For instance, study reports from transcriptomic and proteomic analysis showed a strong genetic relationship between *S. japonicum* and *S. mansoni*. Moreover, the reports further identified specific tegument protein (SjTs4) and an eggshell protein (MF3) expressed in different life cycle stages of *S. japonicum*,[76] which may prove valuable alternative diagnostic targets.

Extracellular vesicles protein and micro-RNAs have been recent diagnostic targets of schistosomiasis. Likewise, *Schistosoma* tegument proteins (adult tegument), are considered as valuable vaccine candidates and markers for diagnosis. The identification of certain tegument proteins as potential diagnostic markers can contribute in improving diagnostic sensitivity of *Schistosoma* infection when used instead of a crude extract from an adult. However, tests used for detection of schistosomal miRNA are still expensive and need more validation at field level. Recent reports showed that two tegument proteins (TG proteins SjPGM and SjRAD23) of adult *Schistosoma japonicum* were identified as potential diagnostic markers for the disease. The two recombinant proteins might have the potential to evaluate the effectiveness of drug treatments and for distinguishing between current and past infections.[77] It can be suggested that these recombinant proteins may solve the major drawbacks of the indirect immunological approaches.

Nucleic Acid Tests

As a result of several ongoing intervention programs currently underway, the landscape of schistosomiasis is changing across the world in general and sub-Saharan Africa in particular. Despite some variations, the progress seems to gear towards achieving the goal of ultimate elimination as reported by TchuemTchuenté et al.[78] It's to be noted that differences in prevalence of schistosomiasis may be due to various factors including methods of diagnosis of the disease. The methods of diagnosis ranging from conventional with low sensitivity and specificity to modern ones with better sensitivity and specificity contributed a lot to the current achievements yet to succeed in the progress further employment of new diagnostic tools with higher sensitivity and specificity is unquestionable.

Owing to the limitations of the existing conventional diagnostic methods development of novel detection methods with higher rapidity, reliability, and convenience should be at our disposal. Nowadays, the application of nanotechnology for detection of the nucleic acid of Schistosoma has been given major emphasis. In recent studies, nanoparticle-based detection for control of schistosomiasis has been targeted by scholars.[79]

Among the existing diagnostic tools, nucleic acid tests have become top priorities in diagnosis of parasite infections in general and schistosomiasis in particular. The gene chip test employs a particular glass or silicon chip as a carrier and bears several nucleic acid probes on it. The chip gives important information on gene sequences by fluorescence or from sample reaction with the nucleic acid probe.[80] The major strong sides of the gene chip test include easy convenience, high sensitivity and specificity.[81] Similarly, some diagnostic successes in previous studies have been reported by using smaller volumes of body fluids such as blood, saliva, and urine, nonetheless its practicability is to be evaluated.[82]

Polymerase Chain Reaction and Multiplex Polymerase Chain Reaction

Conventional polymerase chain reaction (PCR) is a process whereby schistosome DNA is isolated from a stool, urine or blood samples of a patient, Followed by DNA extraction, gel electrophoresis will be run to detect the size of a particular nucleic acid fragment band of a schistosome. The detection of a specific sized DNA band is a confirmatory mark for the presence or absence of an infection.

Specific and highly sensitive PCR based assays which combine PCR-ELISA have been developed for the identification of schistosome DNA in faeces or sera and plasma.[83] This approach has the potential to provide a test for detection of schistosomes in all stages of clinical disease. Such molecular techniques have also been proved to be extended for diagnosis of the intermediate snail hosts. On the other hand, multiplex polymerase chain reaction amplifies multiple nucleic acid fragments of a pathogen using multiple (two or more) pairs of primers in every reaction concurrently. It demonstrates superior efficiency over the corresponding conventional PCR.[84] In spite of the fact that new molecular techniques in the snail test are sensitive and sharp in accuracy, former conventional methods are being extensively used in practice perhaps due to their ease of handling, field applicability, and accessibility in resource-limited areas.[38]

Real-Time Quantitative Polymerase Chain Reaction

Real-time quantitative polymerase chain reaction (qPCR) is a method used to quantify the amount of PCR products often via addition of fluorophores to the reaction scheme. After the preparation of the reaction the qPCR, the generated fluorescence signal will be measured in each amplification cycle. As there is no need for electrophoresis to observe the bands, the procedure is less labor-intensive. It also has additional advantages over conventional PCR in that lower concentrations of target DNA can be detected.

Loop-Mediated Isothermal Amplification

Loop-mediated isothermal amplification is a new technique of nucleic acid amplification at constant temperature. It was developed recently as a fairly reasonable cost and practicable option to classical PCR for the identification of DNA of schistosomes in stool, urine and serum specimens. LAMP is by and large a very specific and sensitive test as it uses specific primers from both inner and outer parts,[75] designed to target a particular gene for amplification at steady state temperature (60–65°C) and 15 to 60 min.[85] LAMP skips thermal denaturation of template, temperature cycling and electrophoresis, all processes being core elements in conventional PCR-based assays. Moreover, it has also the advantages of being easy, quick, and with considerable efficiency of amplification.[86] In addition, the results can be examined via observation, so the method has extensive possibility of practical use in field settings. The protocol is also suitable for low-income countries and hence, quite cost-effective.

Conversely, one major drawback is that the DNA amplification method involved in the LAMP method may suffer from contamination, ending-up with invalid outcomes.[87] Other drawbacks include complexity in optimization and the drawbacks of multiplexing connected with the use of multiple numbers of LAMP primers (often six).[88]

Recombinase Polymerase Amplification

Recombinase polymerase amplification (RPA) is an isothermal DNA amplification technology that can be performed in the field due to its low resource requirements. Recent reports showed that the RPA assay has been tested in detecting low levels of *S. haematobium* and *S. japonicum*.[89,90] Moreover, the assay is rapid, requiring low temperatures, materials that are needed for the assay can be preserved at room temperature and positive reactions are interpreted using lateral flow strips, suggesting good potential for field application. This assay works through the amplification of the Dra1 DNA region of *S. haematobium*, and it was demonstrated to provide sensitivity as low as 100 fg even when crude urine was spiked in the preparation, suggesting tolerance to inhibitors.[88]

On the other hand, the major limitations in the field application of DNA based diagnostics are the costs, the technical and time consuming nature of total DNA sample preparation and the purification from urine in resource limited settings.[88] This RPA assay also has other shortcomings that may hinder its use for field diagnosis. The first is the difficulty to distinguish the minute degree of fluorescence for positive or negative reactions by non-experts and the second is the heavy isothermal devices are not portable in the field.[91] Actually, the use of molecular screening

methods have not been widely common due to the limited resources and the requirement of expensive technology, cold chain logistics, uninterrupted power supply, and highly skilled manpower.[91]

Challenges and Future Approaches in the Diagnosis of Schistosomiasis

It is clear that the landscape of schistosomiasis is changing across SSA owing to the many ongoing intervention progresses currently underway. Although the progress may vary from country to country, there are real prospects to transition from control into interruption of transmission and, ultimately, elimination. Successful interruption and/or elimination could undoubtedly be influenced by the accuracy of the currently available diagnostic tools.

Recently, extensive reviews of the available diagnostic methods and their challenges has been widely reported and efforts towards designing new, cheaper, ultrasensitive, and specific diagnostic tools that allow highly accurate determination of the infection status in low transmission areas should be intensified.[92,93]

It is obvious that diagnostic tests play a crucial role in disease management of schistosomiasis and for successful control and elimination programmes of the disease. Despite the significant efforts made in the areas of diagnostic test development, several barriers and obstacles exist that may impede the widespread use of new diagnostic assays in clinical practice. Problems associated with diagnostic methods with considerably high sensitivity and specificity are among the constraints challenging the control of schistosomiasis. It's now well understood that the current preventive chemotherapy based global strategy for schistosomiasis control has been practiced without prior diagnosis.[94] On top of this, the scientific advance of new diagnostic tools has showed slow progress partly due to the apparent shortage of financial benefits to the diagnostic companies[95] implying compound challenges on more precise, standardized and sensitive diagnostic methods for diagnosis of schistosomiasis.

Scientific advancement, including development of new diagnostic tools, costs finance. The development of new diagnostic tests for schistosomiasis has passed through similar challenges and difficulties as other neglected tropical diseases (NTDs) that may include shortage of financial support and limited commercial interest by private companies to invest in the development of tests targeting poor and endemic countries.[96] The main components of cost of diagnosis include the supply of kits and reagents, labor, the nature of the test (whether multi-step or not) and logistic supplies for field-based diagnosis.[97] Actually, the expense of performing a laboratory experiment differs significantly on the basis of various factors such as the frequency of replicate tests required, the expenses of particular reagents and equipment.[11] On the contrary, affordability of diagnostics coupled with high sensitivity is a tough challenge for stakeholders and partners to overcome. This will be a big problem for endemic countries in the developing world unless there are collaborations from the international community.

Molecular diagnostic techniques are preferred over the rest of conventional and serological diagnostic methods mainly due to their sensitivity. However, those methods including PCR and qPCR have not been practical in endemic field settings due to some limitations such as expense, time consuming, and requiring a significant laboratory infrastructure and training.[72,98] Hence, the best analytical examinations need to be cost-effective with regard to manual labor, devices, and reagents.

Despite the fact that the drive towards schistosomiasis elimination calls for developing novel diagnostic tools with a high sensitivity and high specificity,[99] the validity of the tests is posing another problem for the diagnostic methods. False positive and false negative issues have been among the major challenges facing the diagnosis of pathogens in general and schistosomiasis in particular. In addition, differential diagnosis is also required in most cases which incurs additional expense.

Other than, the commonly urogenital schistosomiasis, female genital schistosomiasis (FGS) demands accurate and precise diagnostic techniques. In this instance, FGS is currently diagnosed by visual inspection of the characteristic genital sandy patches or lesions caused by the ova of the *S. haematobium* on the cervix and vaginal wall.[100] Microscopy of urine and hematuria are nonspecific and insensitive predictors of FGS. Recent reports showed that home-based self-sampling may represent a scalable alternative method for FGS community-based diagnosis in endemic resource limited settings.[101] Generally, a summary of the different schistosomiasis diagnostic methods is presented in Table 1.

Table 1 Comparison of Strengths and Weaknesses of Schistosomiasis Diagnostic Methods

Assay Type		Strengths	Weaknesses	Remarks
Conventional microscopic methods	Parasitological methods	• Cost effective and practical gold standard for routine activities	• Low sensitivity and specificity • Labor intensive	
	Malacological methods	• Alternative method	• Detection limitations • Labor intensive procedure	
Serological and antigen detecting methods	Antibody detecting methods	• More sensitive than conventional methods	• Unable to differentiate active infection from past infection	
	Antigen detecting methods	• Differentiate active infection from past infection	• Possible antigen cross-reactivity	
Molecular methods	cPCR	• Relatively low cost compared to qPCR	• Relatively time consuming	
	nPCR	• Use of two sets of primers the specificity	• Time consuming relative to cPCR	Needs to complete two rounds of cPCR
	qPCR	• Quantify the amount of amplicon • Higher sensitivity and specificity than cPCR	• More expensive than other PCR methods.	
	LAMP	• Less equipment and reagents are required • Field applicability	• Prone to carryover contamination	

Abbreviations: cPCR, conventional PCR; nPCR, nested PCR; qPCR, quantitative PCR; LAMP, loop-mediated isothermal amplification.

Concluding Remarks

The idea of using a single magic bullet has not been a successful control approach for most infectious diseases including schistosomiasis. Challenges for monitoring the efficiency of the current control measures by themselves can be implicit for lagging behind from proposed elimination of the parasite by 2020. The major, if not the sole, challenge that contributed to the failure of successful elimination of the disease appears to be lack of appropriate diagnostic tools. To be frank, elimination can be achieved not solely by highly sensitive and specific diagnostic techniques rather via implementing all inclusive integrated control approaches. Apparently, despite the presence of an integrated control program, the present diagnosis of schistosomiasis in most developing countries still relies heavily on conventional parasitological methods unsuitable for low prevalence areas. As a result, advances in diagnostic methods have been considered part of the solution for the control and eventual elimination strategy of the disease in endemic areas. The development of new diagnostic methods and improving the existing ones should be the vital element for the target of disease control and eventual elimination. The developments of newly ultrasensitive diagnostic tools that allow highly accurate determination of the infection status should be promoted to play a significant role for the intended program. Despite the need of good and feasible diagnostic tools as integral parts of the strategy of choice for the elimination of the disease, scholars suggest that the fate of diagnostics remain neglected perhaps until the cost of diagnosis outweighs that of treatment (eg MDA without test), another competitive challenge from an economic point of view.

Disclosure

The author reports no conflicts of interest in this work.

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