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# Current trends in the laboratory diagnosis of schistosomiasis infection

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## ABSTRACT

Schistosomiasis is a neglected tropical disease that affects about 290 million patients worldwide. Children aged between 5 and 14 years represent 45.8% of the affected patients, in addition, schistosomiasis has been reported in Schistosoma-free areas, mostly because of tourism and immigration from endemic countries. Intestinal schistosomiasis caused by *Schistosoma mansoni* is mainly diagnosed via direct stool examination for egg detection. Immunological methods are favoured for disease monitoring and preliminary checking for communities in areas with low infection rates, and for patients with light and chronic infections where parasitological tests are negative. PCR-based diagnostic techniques are more sensitive, but expensive. Tegument proteins and miRNAs are promising markers for diagnosis of schistosomiasis.

**Keywords:** Schistosomiasis, Diagnosis and current trends

## INTRODUCTION

Schistosomiasis also known as bilharziasis, snail fever and fever is a parasitic disease caused by worms of the genus *Schistosoma* [1-4]. The agents of aetiology of these diseases are waterborne “blood-thirsty” digenetic trematodes and are harboured by freshwater *Bulinus* gastropods [5]. There are six species known to cause schistosomiasis in humans: *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum*, and *Schistosoma guineensis*. Of these six species, *S. haematobium* and *S. mansoni* are the most commonly found species in endemic areas. *S. haematobium* causes urogenital schistosomiasis and *S. mansoni* causes intestinal schistosomiasis [6]. Urinary schistosomiasis, *S. haematobium* infection or *Tsargiya* in Hausa is known worldwide as an important chronic and debilitating disease mainly affecting under privilege rural communities characterized by poverty, poor sanitation, poor housing and hygiene [7]. These have been identified to be responsible for the continued persistence of intestinal parasitic infection in children leading to continued exposure to the causal parasites and thus high rates of re-infection [8]. Schistosomiasis is one of the major neglected tropical diseases (NTDs). It was estimated about 240 million people in 78 countries are infected and approximately 90% of world cases occur in sub-Saharan Africa [9]. In Nigeria, the disease is prevalent throughout the country, with an estimated 25 million people infected and 101 million at risk of infection, respectively, and about 30 million people have been deemed to be in dire need of treatment annually. Schistosomiasis is a serious public health for rural inhabitants. They are usually exposed to infection through their agricultural practices of farming rice, recreational and domestic activities, fishing, and swimming in infected ponds or rivers where the snail intermediate hosts breed [10]. Clinical signs and symptoms include dysuria, haematuria, granulomatous host response and urinary egg excretion, renal failure and bladder and other genitourinary cancers [11]. The disease has also been reported to increase the risk of HIV infection among women due to the fact that this parasite causes genital lesions and sandy patches [12-41]. School

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age children were thought to have frequent water contact that would make them more vulnerable to schistosomiasis. It is the age group associated more frequently with schistosomiasis problems [42]. Schistosome infections are usually at their peak in late childhood to early adulthood. In some parts of Africa, the onset of haematuria due to urinary schistosomiasis is very common in adolescent boys, and due to lack of knowledge, it is seen as a normal phenomenon in some communities. Morbidity associated with the infestation in children include nutritional deficiencies, growth retardation, cognitive impairment as well as decreasing physical activity, school performance, work capacity and productivity [43]. The negative impacts can be reversed with appropriate treatment. Chronic schistosomiasis may result in death, in sub-Saharan Africa, more than 200,000 deaths per year are caused by the disease [44].

### HISTORICAL BACKGROUND

Schistosomiasis is one of the most widespread of all human parasitic diseases, ranking second only to malaria in terms of its socio-economic and public health importance in tropical and subtropical areas [45]. It is caused by blood flukes Schistosome. Schistosomiasis is known as bilharzia or bilharziasis in many countries, after a German physician Theodor Bilharz, who first to describe the cause of urinary schistosomiasis in 1851 [46]. The first physician who described the entire disease cycle was Brazilian parasitologist (Pirajá da Silva) by examining 6,200 years old skeleton [47]. Most human schistosomiasis is caused by *S. haematobium*, *S. mansoni*, and *S. japonicum*. Less prevalent species, such as *S. mekongi* and *S. intercalatum*, may also cause systemic human disease. Less importantly, other Schistosomes with avian or mammalian primary hosts can cause severe dermatitis in humans (e.g., swimmer's itch secondary to Trichobilharzia acellata).

### EPIDEMIOLOGY AND DISTRIBUTION OF SCHISTOSOMIASIS

Schistosomiasis is a major Neglected Tropical Disease (NTD) with 240 million people infected globally, and about 700 million are at risk [48]. It is acquired solely through direct contact with freshwater bodies that breed the snail intermediate host (*Bulinus* species), and which has been contaminated with schistosome eggs; which undergo polyembryony in the intermediate host, to produce infective cercariae. Schistosomiasis is largely an occupational disease of farmers, fishermen, and workers of irrigation farms. Sub-Saharan Africa harbours over 90% of the estimated 240 million world cases of human schistosomiasis and records more than 200,000 schistosomiasis-associated deaths annually [49]. Schistosomiasis caused by *Schistosoma* species is an important neglected tropical disease that attracts more attention due to its chronic nature, association with water resources, and lack of a protective vaccine. It is dominant in rural poor communities among agriculture and fishing workers, moreover, contact with infected water as in car and clothes washing, as well as recreational activities increases the risk of infections. Out of the Six *Schistosoma* species affecting humans, *Schistosoma* (*S.*) *mansoni* is responsible for intestinal schistosomiasis in African countries. Nearly, 54 million of infected cases are present in sub-Saharan Africa, while 393 million are at risk [50]. The emergence of schistosomiasis in low endemic or even non-endemic areas can be facilitated by movement of population to urban areas, international tourism, increase in migration, international cooperation programs and environmental changes [51].

In Nigeria varied from 2% to 82.5% amidst analysed studies, the pooled prevalence measure was 34.7% (95% confidence interval). *Schistosoma haematobium* surveys conducted over the period of 50 years under review using different diagnostic tools revealed that Ogun State has the highest prevalence, followed by Ekiti state, while the lowest prevalence was recorded in Adamawa. No incidence in Gombe. In terms of endemicity, this review has shown that Nigeria is divided into four zones: hyperendemic, moderately endemic, low endemic, and no endemic zones. A survey of 47 (15%) of the 323 dams in Nigeria revealed that 45 out of the 47 dams are located in the hyperendemic zone, while the remaining two are located in the moderately endemic zone. Twenty (43%) of the total surveyed dams harboured *Bulinus globosus* and/or *Biomphalaria Pfeiffer*, the local intermediate hosts of schistosomes, and 18 of these are located in the hyperendemic zone, while the other two are in the moderately endemic zone [12]. In the last two decades, Nigeria has intensified campaigns against schistosomiasis and other NTDs [42]. The efforts have generated schistosomiasis morbidity reductions in a few endemic areas [52]. It is, however, difficult to achieve long term schistosomiasis control implementation programmes. There are strong indications that Egypt, China, Japan, Philippines, and Brazil are progressing towards the elimination of the disease [42]. It appears, however, that the menace of schistosomiasis will last longer in Nigeria. There are no significant differences in the prevalence of schistosomiasis in several states in Nigeria in the different eras

Despite the availability of effective drugs, the annual death rate is around 200,000 in sub-Sahara Africa alone, making the group of parasites which cause schistosomiasis the most lethal worms in the world. In 2010, approximately 238 million people were infected with schistosomiasis, 85 percent of whom live in Africa. An earlier estimate from 2006 had put the figure at 200 million people infected [53]. In many of the infected areas, schistosomiasis infects a large proportion of children under 14 years of age. An estimated 600 to 700 million people worldwide are at risk from the disease because they live in countries where the organism is common [54-55]. In 2012, 249 million people were in

need of treatment to prevent the disease [56]. Thus, the negative impacts caused by untreated infections demoralize both social and economic development on school performance among infected children in endemic areas [57].

### LIFE CYCLE

#### SNAIL HOST

The life cycle starts when human host release worm eggs through urine or faeces. the egg hatches into a free-living miracidium on reaching fresh water under favourable conditions. The miracidium then locates and infect a suitable snail host (*Bulinus* spp.) after infiltration, the miracidium removes the ciliated plates, develops into a mother sporocyst, and then produces a daughter sporocysts. Daughter produce either cercariae (cercariogenous sporocysts) or more daughter sporocysts (sporocytogenous sporocysts). Daughter sporocysts can also experience a re-differentiation into new daughter sporocysts. Snails can shed about 200 cercariae daily [58].

#### HUMAN HOST

Cercariae enter human skin and shed their forked tail, forming schistosomula. the schistosomula migrate throughout the body's tissues through blood circulation. Schistosomula grow into schistosomes and adult worms. these adult worms each have a ZZ chromosome pair in males and ZW chromosome pair in females. Adult worms in humans reside in the mesenteric venules in various locations, depending on the species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the rectal venules. The females deposit eggs in the small venules of the portal and peri-vesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*) or mesentery, where they mature in situ and lay eggs intravascularly, possibly causing both acute and chronic disease and symptoms and are eliminated with faeces or urine, respectively [58].

#### CLINICAL MANIFESTATION

Transient pruritic dermatitis or swimmer's itch may occur in response to cercarial skin penetration. However, newly infected patients are often asymptomatic. Common symptoms are urinary frequency, dysuria and end-stream haematuria. Of these symptoms, terminal haematuria may be the most feasible and is often the basis for epidemiologic diagnosis. Iron deficiency anaemia may be exacerbated by co-morbidity with other endemic tropical diseases, such as malaria and helminthiasis. A substantial amount of iron is sequestered by vitelline cells for the formation of the parasite eggshell. In addition, host iron recycling is disrupted by a pro-inflammatory synthesis of hepcidin, an acute phase reactant [52]. Although structural deficits are sometimes reversible by medical treatment, obstructive uropathy may invariably result from progressive bladder fibrosis, ureteral dilatation and hydronephrosis, particularly in older patients [59]. Ulcerations of bladder mucosa, focal bladder wall calcification and renal stone formation may result. Urological surgery is seldom required for rehabilitation. Genital disease may cause sexual dysfunction, facilitates human immunodeficiency virus transmission and promotes infertility in adolescents and young adults. The ease of access to wider geographical regions for tourism has increased the infection rate among travelers [59]. Such individuals lack any acquired immunity and within 6 weeks after infection they may experience a severe hypersensitivity reaction in response to the first bout of egg antigen release by adult worms.

#### COMPLICATIONS

Among the several complications caused by *S. haematobium*, the mortality rate due to non-functioning kidneys and haematemesis was put at 150,000 annually.<sup>3</sup> According to the 1993 WHO reports, the above figure clearly showed that urinary schistosomiasis is an important public health problem in sub-Saharan Africa next to malaria in morbidity.<sup>2</sup> Granulomatous inflammation, severe ulceration and pseudopolypoidosis of the ureteral and vesical walls are the major complications/damages caused by the eggs of *S. haematobium*. The common signs of the infection include haematuria, dysuria, and proteinuria. However, *S. haematobium* mediated renal failure deaths due to UTI scarring, ureter and bladder deformities are less common nowadays due to the emergence of novel and highly systematic anti-helminthic drugs with promising anti-schistosome activity [60-64].

#### CURRENTS LABORATORY DIAGNOSIS

Currently available diagnostic methods for schistosomiasis include those that are relying on stool and urine microscopy for parasite detection, serum antibodies, antigen detection, and the detection of DNA. Accurate, cost effective, and easy-to-use diagnostic tests are required for successful national control programmes in endemic areas. The diagnosis methods, currently being used for schistosomiasis, have several limitations, hence warrant further investigations for development of novel detection methods with higher rapidity, reliability, and convenience. Nanoparticles have widely been used in bioassays, because of their low-cost, high sensitivity and rapidity. Nano-diagnosis of schistosomiasis is a potential prospect, so this review described the application of nanotechnology for detection of antigen, antibody and the nucleic acid of *Schistosoma*. Furthermore, the techniques described in this

review included magnetic affinity, colloidal gold, nanotechnology-based screen-printed biosensors, and electrochemical genosensors [65].

### MICROSCOPY

Urogenital schistosomiasis is usually assessed by the microscopic examination of parasite eggs in urine samples. About 10 mL of urine is filtered, and the residue that is examined for parasite eggs following centrifugation. The number of eggs per 10 mL of urine is used to express infection intensity [66]. Urine microscopy is also regarded as the gold standard for *S. haematobium* eggs detection in endemic areas. However, in areas of low transmission intensity, which were recently treated with praziquantel, young children with light infections or adults with chronic infections who usually excrete few eggs, urine microscopy is not sensitive. Microscopy sensitivity can be improved by repeated screening of the children, but this brings up the total cost of diagnosis for each person, manpower needs and resources, which might be unaffordable. This inability to carry out repeated testing will result in an underestimation of the true burden of infection, necessitating a more sensitive diagnostic and cost-effective tool in such settings [66-71]. In schistosomiasis screening campaigns, haematuria has been found to be significantly associated with *S. haematobium* infection. Macro- or micro-haematuria can be assessed using dipstick. However, a large percentage of micro-haematuria in low prevalence areas may be from other causes that are unrelated to *S. haematobium* infection.

### FILTRATION METHOD

It involves the use of vacuum pump filtration machine: 10ml of well-mixed urine was dispensed into the filtration unit of the filtration chamber. The urine was then drained through a Whatman No.1 filter paper. The filter paper was then removed and stained with the saturated ninhydrine solution (few drops of iodine was added to enhance the staining). It was allowed to stay overnight at room temperature for the eggs to pick up the stain [67]. It was examined under the light microscope under  $\times 10$  and  $\times 40$  objectives. Terminal spine eggs, characteristics of *S. haematobium* was counted for each positive sample. The result was express as eggs/10ml urine. All the urine samples were treated the same way.

### KATO KATZ TECHNIQUE

The WHO recommended the Kato-Katz (KK) thick stool smear technique for diagnosis at community level. This technique is highly specific and simple; by microscopic examination of fresh stool sample (41.7 mg) cases with a minimum of 20 eggs/g can be detected. The KK is inexpensive (US\$0.1–0.3 for a KK kit) and easily used in field studies to assess the impact of control programs by measuring the changes in the prevalence and infection intensity in post-treatment surveys where mass drug administration using praziquantel is employed. However, its poor sensitivity for detecting light intensity infections causes substantial variation in results and in turn alternative techniques are being tested [68].

### POLYMERASE CHAIN REACTION (PCR)

For low-intensity level, PCR can be beneficial. PCR has sufficient sensitivity and specificity for detection of schistosome eggs in humans [69]. The detection of *Schistosoma* DNA by PCR in stools and urine appears to be a promising, highly sensitive, and specific tool that could improve and facilitate the diagnosis of schistosomiasis, particularly in non-endemic countries where the parasite burden can be lower [70]. A few previous studies demonstrated the potential interest of blood-based PCR for the diagnosis of acute schistosomiasis. As evidenced by Wichmann *et al.*, the high potential of both *S. haematobium* and *S. mansoni* PCR assays is linked to the high repetition of the multicopy targeted genes, accounting for 12–15% of the whole *Schistosoma* genome. PCR can also be used to investigate the association between schistosomiasis in women and the increased susceptibility to human immunodeficiency virus (HIV) [71-81].

### SEROLOGY

Compared with microscopy, serology provides a sensitive tool for the diagnosis of schistosomiasis, especially for infections with low intensity. In the human host, antibodies against the four lifecycle stages of *S. haematobium* and the proteins associated with these stages *e.g.* circulating proteins regurgitated by adult worms can be detected. Some serological assays have been widely evaluated in endemic regions of varying levels of endemicity and for the diagnosis of travel related schistosomiasis [82].

### ANTIGEN DETECTION

*Schistosoma* antigens are present in the serum and urine of infected persons. Two antigens, referred to as circulating anodic antigens (CAA) and circulating cathodic antigens (CCA) can be detected in the laboratory. These assays detect gut-associated glycoproteins of adult worms, but have not been extensively evaluated and the sensitivity of these tests is variable, depending largely on the intensity of infection. Urine CCA has been shown to perform relatively well for *S. mansoni* infections with sensitivities exceeding those of stool microscopy. However, urine CCA is relatively insensitive for detecting *S. haematobium* infections [83].

## ANTIBODY DETECTION

Antibody based methods include indirect immunofluorescence (IFAT), enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA), and are designed to detect antibodies in the serum of infected people. The sensitivity of IFAT and IHA in detecting Schistosoma infection compares favourably with standardized western blot analysis, with specificity of IHA depending on the application of the right cut off values. However, its application is limited as these antibodies will only appear after 4–6 weeks in response to the laying of eggs. The test is most useful for travellers from non-endemic countries who would not be expected to have prior exposure, or in patients with signs and symptoms of disease and a history of likely exposure. The presence of Immunoglobulin E (IgE) antibody is claimed to be a marker of chronic active disease, but with sensitivity limited at about 70%. Antibody tests cannot differentiate between old and new infections and have no place in monitoring the response to treatment. A negative antibody test is a useful tool to rule out infection in endemic populations, but it should be noted that a proportion of patients will not develop any antibody response in active infections. Finally, infections with non-human schistosomes, for example, avian schistosomes, may result in false positive antibody tests [84].

## MAGNETIC AFFINITY ENZYME-LINKED IMMUNOASSAY (MEIA)

Magnetic affinity enzyme-linked immunoassay (MEIA), initially used in the 1980s for quantification of haemoglobins, [85] was recently used as an immunoassay, which combined the magnetic bead separation with enzyme-linked immunoassay to improve the detection efficiency. The principle of MEIA based upon the immune response and production of specific antibody in response to a specific antigen of the worms or eggs. In MEIA, the magnetic beads contained superparamagnetic nanoparticles and functional polymer materials. Moreover, the magnetic beads were modified with specific functional groups, thus made them able to conjugate with the molecular targets [86]. A magnetic field was applied to the reaction system after binding the molecular targets on the magnetic beads. Consequently, the immune complex was collected through magnetic separation. Subsequently, an anti-IgG antibody labelled with alkaline phosphatase was added into the reaction system and incubated. The unconjugated antibody was washed away. Furthermore, phenolphthalein monophosphate was added mixed into the reaction system as a chromogenic substrate of alkaline phosphatase. After the reaction, the absorbance was measured at 550 nm to confirm the conjugation of alkaline phosphatase to the reaction system, as well as to further verify the presence or absence of specific antibodies in the serum.

## COLLOIDAL GOLD

Colloidal gold, another kind of metal nanoparticle besides superparamagnetic nanoparticles, is applied widely as an emerging immune labelling technique. Owing to its ability to combine with biological macromolecules such as immunoglobulin, colloidal gold was introduced into immunological detection field and became a practical tool in basic research and clinical detection [87-98]. Several new detection techniques based on colloidal gold have been developed so far for the detection of antibody against Schistosoma, including rapid-dot-immuno gold staining (R-Dot-IGS), dot immune gold filtration assay (DIGFA), anodic stripping voltammetry (ASV), super hydrophobic surface-based analytical platform (SSAP)-based magnetic electrochemical immunoassay, and colloidal gold immune chromatography assay (GICA), etc. The principle of antibody detection using colloidal gold techniques involves antigen-antibody reaction. An antigen-antibody anti- IgG antibody-colloidal gold complex is formed in these reactions. The assay can be done as follows. Firstly, the antigens are absorbed and coated in the vehicle, such as the walls of a polystyrene micro well or nitrocellulose (NC) membrane. Secondly, prepare the colloidal gold-labelled anti-IgG secondary antibody by adding anti- IgG secondary antibody to the colloidal gold solution at optimum pH. In the next step, the sample sera are added into the vehicle to achieve immunoreaction of the coated antigen with the special antibody in the serum. Finally, the gold-labelled anti-IgG antibodies are added to form the antigen-antibody-anti-IgG antibody-colloidal gold complex. Hence, all these assays focus on the detection of colloidal gold [87-98].

## SCREEN-PRINTED BIOSENSOR

The changes in electrical signals, including those of current or potential caused by antigen-antibody binding, could be detected by screen-printed biosensors, thus reflected the nature and substance of the sample [88]. Moreover, the screen-printed biosensors offered the advantages of low cost, convenience, rapidity, and miniaturization. The sensitivity of these assays could be increased, and the detection limit could be decreased (as low as 38 pg/mL) by introducing Nano-materials into them [89]. At first, soluble worm antigens were immobilized on the Nano carbon working area of the electrode. In addition, the redox reaction of iron ions was performed on the electrode surface. The electron transport was influenced by the formation of antigen antibody complexes on the surface of the electrode; thus, the quantity of the antibody bound to the antigen-loaded screen-printed electrode could be determined through cyclic and differential pulse voltammetry. The presence of antibody was indicated by an increase in the separation of anodic and cathodic peak potentials ( $E$ ), as well as a decline in the peak current on voltammograms. Moreover, the peak current declined with the increase in the concentrations of antibody and

exhibited a good linear relationship. In addition, the formation of an antigen-antibody complex increased the insistence and blocked the electron transfer. Thus, the nano-technology based screen-printed electrochemical immunosensors could be used for a rapid and sensitive detection of antibody [88].

#### EXTRACELLULAR VESICLES (EV) PROTEIN AND MICRO-RNAS:

Parasite EV are membrane-bound vesicles classified based on their sizes, specific proteins and RNA contents into exosomes and microvesicles. Exosomes develop within a cell by inward budding of multi-vesicular endosomes and thus contain components of the parental cell, such as RNAs or proteins [90]. Recently, *S. mansoni* exosome-like EV from adult and schistosomula have been characterized, and several proteins were tested as vaccine candidates. MicroRNAs (miRNAs) are short noncoding RNAs that regulate gene expression in eukaryotes, they are present in EV and play a role in host-parasite interplay [90]. Experimentally, elevation of miRNAs sma-miR-277, sma-miR-3479-3p, and bantam were reported in a murine model of schistosomiasis 8 weeks PI [91]. Furthermore, miR-277, miR-3479-3p and bantam were tested in serum from low and high infection sites in Zimbabwe and Uganda and miRNAs were able to detect infected individuals in these areas with high sensitivity and specificity [91]. A study has detected miRNAs within EV fractions from infected traveller [92], and highlighted the possibility of using miRNA for evaluating the efficacy of treatment, as miRNA level decreased as early as 11 weeks after treatment. However, tests used for detection of schistosomal miRNA are still expensive and need more validation at the field level [92].

#### SCHISTOSOMA TEGUMENT PROTEINS

Parasite surface proteins, particularly those expressed in the adult tegument, are considered as valuable vaccine candidates and markers for diagnosis. The identification of certain tegument proteins as potential diagnostic markers can help in improving diagnostic sensitivity of *Schistosoma* infection when used instead of crude extract from adult. Examples of those proteins are as follows: Aquaporins is an integral membrane protein involved in selective transportation of water and solutes through the plasma membrane of mammals, plants and lower organisms. Aquaporin is the most abundant transmembrane protein in *S. mansoni* tegument; expressed in all developmental stages especially intravascular stages. It plays a vital role in parasite survival through osmoregulation and nutrient transport. Dysferlin is a member of the ferlin family involved in plasma membrane repair. In *S. japonicum*, Dysferlin was reported as a tegumental protein upregulated in adult worms, particularly females and was involved in the repair process in the tegument and muscle. The rSm200 is recombinant 200-kDa tegumental protein used in ELISA for diagnosis of schistosomiasis. When used in experimental studies, there were significant differences between infected (acute and chronic) and non-infected animals, the test can also differentiate between healthy persons from non-endemic areas and infected patients, but cannot diagnose patients from low endemic areas [93].

#### CONCLUSION

Despite control programs, schistosomiasis is still a public health problem. Early and accurate diagnosis of schistosomiasis lead to early treatment, cessation of transmission cycle and prevention of chronic complications. Many diagnostic tests for schistosomiasis are available but have unsatisfactory performance. Although the application of molecular techniques to improve diagnostics for *Schistosoma* infection has resulted in some technical advances, but still with the lack of reliable diagnostic tools, underestimation of disease prevalence will lead to the misuse of protective chemotherapy and ultimately to an increase in costs and efforts. It is important to work on improving the current diagnostic methods for better results, and simultaneously look for new diagnostic markers. Surface proteins such as Aquaporin, Dysferlin and are attractive biomarkers, which could be used for development of rapid diagnostic tests and contribute to increase the accuracy of diagnosis

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