Recent Advances in the Diagnosis of Schistosomiasis

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ABSTRACT

Schistosomiasis is considered a neglected tropical disease instigated by flatworms of the genus Schistosoma, remnants a major problem in several parts of the world. A timely and accurate diagnosis is indispensable for disease management and control. Significant advances in the identification of schistosomiasis have been made in current years, improving our ability to detect and characterize infections. This chapter focuses on recent advances in schistosomiasis diagnosis, with an emphasis on new methods and approaches. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), and loop-mediated isothermal amplification (LAMP), have exhibited the ability to improve schistosomiasis findings, sensitivity, and specificity. These techniques identify Schistosoma DNA in biological specimens such as blood, urine, and stool, allowing for early detection and monitoring of infections. Furthermore, the development of multiplex PCR assays allows for the finding of multiple Schistosoma species at the same time, which aids in species-specific diagnosis and epidemiological studies. In addition to molecular approaches, serological test-based antibody detection has advanced significantly. When compared to traditional methods, novel serological assays utilizing recombinant antigens and antigen-detection methods have demonstrated improved diagnostic accuracy, offering increased sensitivity and specificity. These tests not only detect current infections but also provide information about previous exposure to Schistosoma parasites. These non-invasive methods enable assisting in treatment decisions and assessing treatment efficacy. Finally, recent advances in schistosomiasis diagnosis have greatly expanded our diagnostic capabilities, allowing for more accurate and efficient infection detection. Molecular diagnostics, serological assays, and imaging modalities have all contributed to a better understanding of the disease and better patient care. Continuous research and innovation in diagnostic methods are required to improve global schistosomiasis control and elimination efforts.

Key words: Schistosomiasis, Molecular Techniques, Serological Assays, Neglected Tropical Disease.

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1. INTRODUCTION

Infectious diseases, especially parasitic infestations, are major public health concerns in both animals and humans (Alvi et al. 2023a; Alvi et al. 2023b). Schistosomiasis is considered a neglected tropical disease instigated by flatworms of the genus *Schistosoma*, remnants a major problem in several parts of the world. A timely and accurate diagnosis is indispensable for disease management and control. Significant advances in the identification of schistosomiasis have been made in recent years, improving our ability to detect and characterize infections. This chapter focuses on recent advances in schistosomiasis diagnosis, with an emphasis on new methods and approaches. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), and loop-mediated isothermal amplification (LAMP), have exhibited the ability to improve schistosomiasis findings, sensitivity, and specificity.

These techniques identify *Schistosoma* DNA in biological specimens such as blood, urine, and stool, allowing for early detection and monitoring of infections. Furthermore, the development of multiplex PCR assays allows for the finding of multiple *Schistosoma* species at the same time, which aids in species-specific diagnosis and epidemiological studies. In addition to molecular approaches, serological test-based antibody detection has advanced significantly. When compared to traditional methods, novel serological assays utilizing recombinant antigens and antigen-detection methods have demonstrated improved diagnostic accuracy, offering increased sensitivity and specificity. These tests not only detect current infections but also provide information about previous exposure to *Schistosoma* parasites. These non-invasive methods enable assisting in treatment decisions and assessing treatment efficacy. Finally, recent advances in schistosomiasis diagnosis have greatly expanded our diagnostic capabilities, allowing for more accurate and efficient infection detection. Molecular diagnostics, serological assays, and imaging modalities have all contributed to a better understanding of the disease and better patient care. Continuous research and innovation in diagnostic methods are required to improve global schistosomiasis control and elimination efforts.

Schistosomiasis, also known as bilharzia, is a serious parasitic disorder initiated by blood flukes. It is still a persistent issue in various developing countries located in tropical regions. Schistosomiasis is ranked 2nd in terms of morbidity and mortality after malaria (Leblanc et al. 2023). Schistosomiasis is mainly caused by parasites *S. japonicum*, *S. mansoni*, *S. haematobium* (Mu et al. 2023a; Ullah et al. 2021), *Schistosoma mekongi*, *Schistosoma guineensis*, and *Schistosoma intercalatum* (Ajibola et al. 2018), in humans, and causes complicated conditions such as urinary bladder, colorectal, and liver malignancies (Qadeer et al. 2022). *Schistosoma* parasites can affect about 240 million people globally and have a serious health

problem prevalent in about 78 countries, approximately 0.7 billion individuals are in danger of contracting schistosomiasis (Ullah et al. 2022; Ullah et al. 2020a), and the estimated mortality due to this parasite is about 0.3 million yearly (Qadeer et al. 2021).

At present, there is no available vaccine to combat schistosomiasis, and the primary treatment approach involves the administration of praziquantel via chemotherapy. However, the widespread use of praziquantel has resulted in the emergence of drug resistance in the schistosomes that cause disease (Qadeer et al. 2022). Schistosomiasis has been detected using a variety of diagnostic methods. In conventional diagnostic methods, the *Schistosoma* eggs can be detected by traditional microscopic methods (such as Kato Katz, FECT, direct smear) in fecal or urine samples (Mu et al. 2023a). Several immunologic tests, including the indirect hemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and rapid diagnostic tests (RDTs), have proven to be cost-effective and have been extensively employed for infection control and transmission control purposes (Lv et al. 2022). However, they may face challenges related to lower specificity when utilizing crude extracted antigens and the difficulty of distinguishing between past and present infections (Mu et al. 2023b).

Furthermore, the use of nucleic acid-based diagnostic methods has shown higher performance in detecting various schistosome species, especially in low-level infections. When compared to traditional methods such as the Kato-Katz method and miracidium hatching (Espírito-Santo et al. 2014), these DNA-based assays have significantly higher rates of positive detection. These assays, which rely on the detection of deoxyribonucleic acid (DNA), effectively reduce the occurrence of false negative results, and provide a reliable means of monitoring potential schistosomiasis exposure (He et al. 2016).

The disease can manifest in two ways: acute and chronic presentations. The acute form also referred to as Katayama syndrome, is caused by immediate or delayed hypersensitivity reactions to the immature worm migrating in the liver and other blood vessels (Coltart and Whitty 2015), and temporary lung infiltrates are typical symptoms that appear 14 to 84 days after infection (Cimini et al. 2021). Symptoms are fever, malaise, nausea, headache, diarrhea (Oliveira et al. 2020), wheezing, and eosinophilia (Coltart and Whitty 2015). While some people may have no or only mild symptoms (Oliveira et al. 2020). The chronic form, symptom may appear several months to years after the initial infection. The release of lytic enzymes, the activation of a significant T-cell mediates immune reaction, and the occurrence of delayed typed hypersensitivity reaction all contribute to the formation of granulomas and, eventually, tissue fibrosis (Zheng et al. 2020).

2. SCHISTOSOMA LIFE CYCLE

Schistosomiasis is an infectious disease resulting from parasitic trematodes belonging to the genus *Schistosoma*, which are commonly found in endemic regions of freshwater. The parasitic life cycle includes both an intermediate and a final host. The infective stage, known as cercaria, is larvae that can actively penetrate the human skin (Cimini et al. 2021). The cercaria transforms into schistosomula once inside the host and migrates through the bloodstream, eventually reaching the heart and lungs. From where it travels to the liver's portal and venous flow, wherever they develop into fully-grown worms of different sexes (sex determination occurs in fertilized eggs). To avoid the host immune response, mature worms have developed strategies such as the production of molecules that mimic self-antigens (Anisuzzaman and Tsuji 2020). Adult male-female worm pairs migrate through the host's circulation and settle in various organs depending on the Schistosoma species. The female worm starts to deposit a significant quantity of eggs, ranging from several hundred to several thousand per day, and this egg production can last for many years, even up to 20 years. In *S. japonicum*, the worms move towards the inferior mesenteric and superior hemorrhoidal veins. On the other hand, *S. mansoni* worm migrates to the superior mesenteric vein, while adult worms of S*. haematobium* travel to the vesical plexus and veins that drain the ureters, bladder, and

other pelvic organs (Cimini et al. 2021). Female worm eggs are highly immunogenic, causing a significant inflammatory response that causes damage to surrounding tissues. Furthermore, the eggs release lytic enzymes, which contribute to tissue destruction. As a result, the eggs exit the blood vessels and are excreted in urine and feces through the bowel or bladder. When the eggs hatch in the water, they discharge miracidia, the first larval stage. These miracidia enter an intermediate host, usually an aquatic snail, and reproduce asexually for several cycles within the snail. The parasite's cercaria stage eventually forms within the snail. These cercaria are then discharged from the snail into the water, where they can infect the ultimate host once more, thus perpetuating the cycle (Fig. 1) (Lindner et al. 2020).

3. OVERVIEW OF SCHISTOSOMIASIS DIAGNOSIS

To effectively control schistosomiasis in endemic areas, accurate, affordable, and user-friendly diagnostic methods are required. Microscopy of feces and urine samples (such as Kato Katz and direct microscopy) to detect parasites, as well as testing for serum antibodies, antigen detection, and DNA detection, are currently available diagnostic techniques for schistosomiasis. Nevertheless, there is a need for enhanced diagnostic methods that are affordable and user-friendly to effectively support national control programs in regions impacted by schistosomiasis. An overview of various diagnostic methods is enlisted in Fig. 2.

3.1. CONVENTIONAL PARASITOLOGICAL DIAGNOSIS

The conventional techniques were old method which is still practiced in some country due to limited resources. The conventional method was cheap, and quick but was not convenient for early diagnosis.

3.1.1. MICROSCOPIC METHODS

The first schistosomiasis diagnostic techniques relied on parasitological methods, such as identifying eggs in stool samples for intestinal schistosomiasis or urine samples for urinary schistosomiasis. These methods, however, have limitations in terms of early disease detection because they rely on detecting eggs, which are only produced by the parasites several weeks or months after infection. Consequently, these direct egg detection tests are not efficient in identifying the disease during its initial phase before the parasites become fully established and symptoms worsen (Weerakoon et al. 2015).

3.1.1.1. DIRECT FECAL SMEAR

In this method, approximately 2 mg of fresh stool or urine is taken and put on a drop of saline, properly mixed, and examined under a microscope (Utzinger et al. 2015). The direct fecal smear is a conventional method used for finding ova in fecal smears for intestinal schistosomiasis, or urine for urinogenital schistosomiasis and miracidial hatching (Chala 2023). It is widely recognized that microscopic examination of stool and urine samples remains the preferred and most reliable Schistosomiasis diagnostic test, serving as the gold standard. These methods, however, have some limitations (Ross et al. 2013). It is considered labor-intensive, time-consuming, and unsuitable for widespread disease surveillance. The effectiveness of parasitological diagnostic techniques is dependent on the rate of egg excretion, this lowers their sensitivity in regions with low disease occurrence, leading to a significant number of false negative outcomes (Chala 2023). In the case of *Schistosoma haematobium*, for example, the eggs are excreted in the urine and are typically detected through microscopic examination of concentrated urine sample obtained through sedimentation, centrifugation, or filtration, followed by passage through a paper or nitrocellulose filter (Chala 2023).

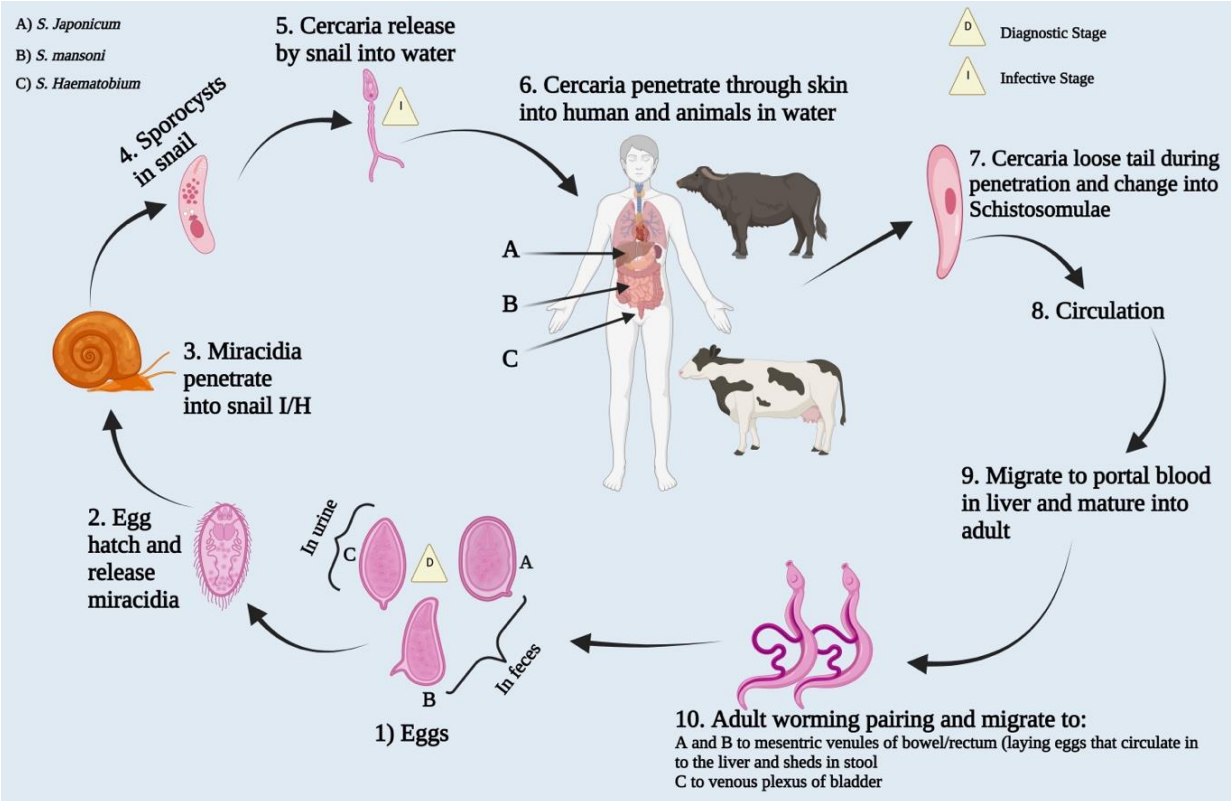

Fig. 1: Schematic life cycle of Schistosomiasis; *Schistosoma* parasites, which are trematode parasites, go through various stages of their life cycle in both bodies and water (steps 2-5) and the veins (steps 6-10) of their ultimate human/animal's hosts. The parasites rely on aquatic snails as intermediate hosts, which can be found in bodies of water. Humans/animals become infected when they encounter snail-infested waters while performing occupational or recreational activities. They then contribute to parasite transmission by contaminating local freshwater lakes and streams with urine or feces; The intermediate host snail is different for each species like *Oncomenania* species for *S. japonicum*, *Biomphalaria* species for *S. mansoni*, *Bulinus* species for *S. haemtobium* & *S. intercalatum*, *Neotricula* species for *S. mekongi*. This figure is generated by using Biorender.com.

3.1.1.2. FORMALIN ETHER CONCENTRATION TECHNIQUE (FECT)

The FECT is a laboratory technique used in high-income countries, particularly in a hospital laboratory, in conjunction with direct fecal smear analysis. Its goal is to improve the detection of parasites in stool samples (Utzinger et al. 2015).

The FECT consists of four major steps: 1) the homogenization of small amounts of stool (1-1.5gm) mixed with formalin, 2) filtration of stool through 400 µm sieve or surgical gauze to remove the debris, 3) the addition of ether to formalin-stool suspension, 4) centrifugation and examination of sediment material through a microscope. FECT generally exhibits greater sensitivity compared to direct fecal smear (Utzinger et al. 2010).

3.1.1.3. KATO-KATZ

In endemic countries, the Kato-Katz method is widely used as the standard way for detecting the occurrence and intensity of *S. japonicum*, *S. mansoni*, and other soil-transmitted helminths. There are several steps involved in the Kato-Katz procedure (Yap et al. 2012). 1) fresh stool samples are passed through a fine mesh (60 - 105 - µm) to remove large debris and to achieve a uniform consistency. 2)

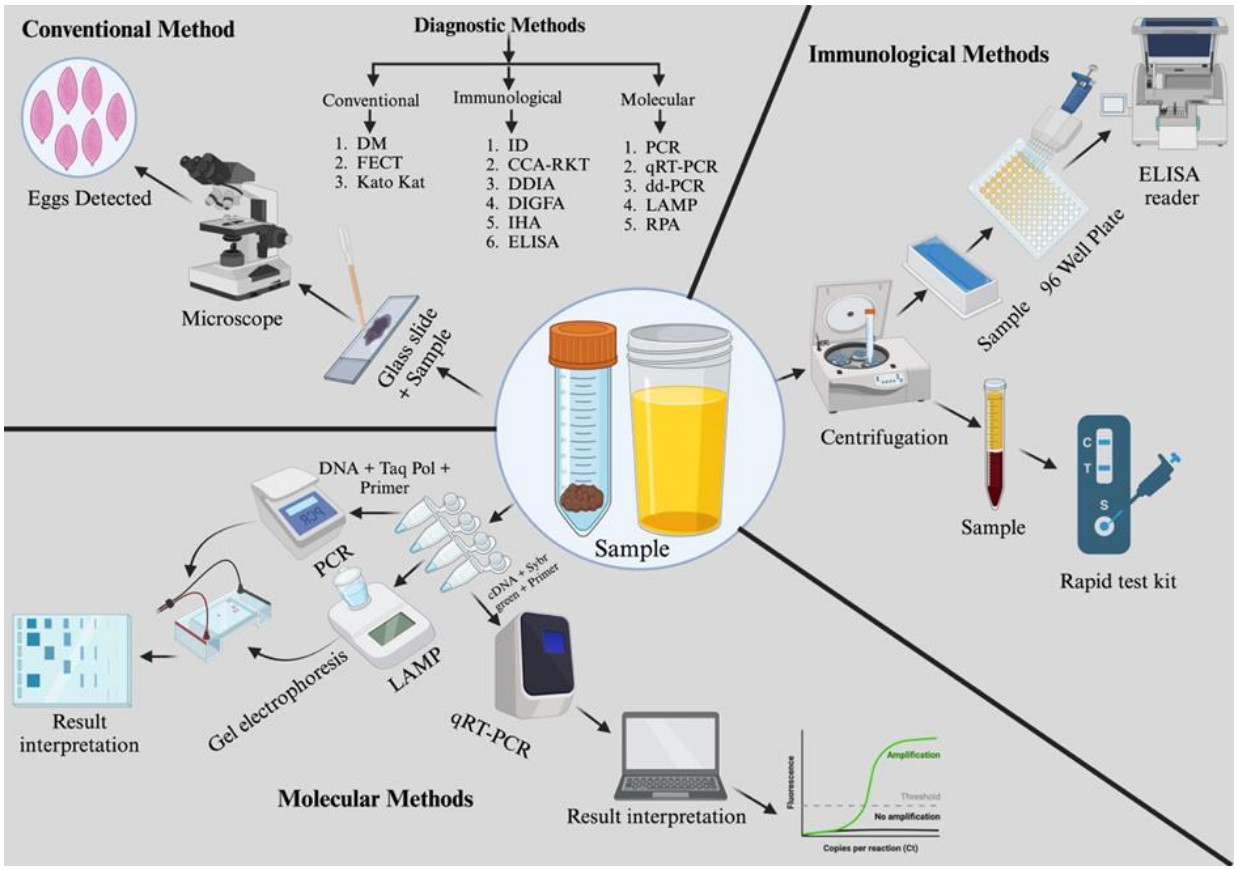

Fig. 2: General Pictorial representation of various diagnostic methods; The diagnosis of schistosomiasis can be primarily accomplished through three distinct approaches, as illustrated in the provided diagram. Each of these methods encompasses various subtypes, as depicted in the figure, with an emphasis on the commonly employed techniques.

The stool is transferred on the slide spread properly on the slide and covered with glycerol-methylene blue-soaked cellophane. The slide is then allowed to clear for at least 30 minutes, preferably 24 hours. 3) The slide is examined under a microscope after it has been cleared. The eggs seen under the microscope are counted and expressed as egg per gram of stool (EPG) by multiplying the apt factor (subject to the quantity of stool taken) (Utzinger et al. 2015). EPG values are used to determine the severity of an infection. The Kato-Katz method is a simple, inexpensive, and effective technique for detecting moderate to severe infection (Knopp et al. 2013). However, it has limitations in detecting light infections due to its comparatively low recognition threshold (around 25-50 EPG), depending on the template used. This can result in an underestimation of true prevalence, especially in low-prevalence areas, and can also complicate the confirmation of cure after treatment (Adriko et al. 2014).

3.2. NEW DIAGNOSTIC METHODS IN SCHISTOSOMIASIS

Recent advancements in proteomics and transcriptomics have led to significant breakthroughs in the study of schistosomes. These cutting-edge techniques have enabled the identification and characterization of a diverse array of molecules, including protein and other components, that are released during various stages of the schistosome life cycle. These newly discovered molecules hold great promise as a probable entrant for the development of diagnostic tools for schistosomiasis.

3.2.1. IMMUNOLOGICAL TEST:

With time various methods have been developed to assess the host's immune response by using crude or purified antigens from schistosome eggs and adult worms to detect antibodies. Immunological tests are used to detect anti-schistosomal immunoglobulins or to identify schistosomal antigens in body fluids such as plasma, serum, and urine. The following methods are commonly used for immunological detection.

3.2.1.1. INTRADERMAL TEST (ID)

It is a diagnostic test for *Schistosoma* based on immune reactions introduced by (Gan 1936), involving the use of antigens to detect IgE. These antigens are extracted from various stages of *Schistosoma*, such as fresh adult worms, frozen adult worms, eggs, and miracidia (Zhang et al. 2016). The intradermal test proved to be easily applicable, cost-effective, and highly sensitive (90 %), which led to its use in the 1950s in schistosomiasis control programmed to assess the prevalence and distribution of *S. japonica* (Maegraith 1958; Mao and Shao 1982). However, due to its low specificity, the intradermal test was eventually replaced by other diagnostic methods (Zhang et al. 2016).

3.2.1.2. CIRCULATING CATHODIC ANTIGEN (CCA) RAPID KIT TEST

This test is mostly used in humans to detect *Schistosoma* using urine samples. The CCA test, which is specifically designed for detecting *S. mansoni* is used to diagnose schistosomiasis. However, this test is less effective for the diagnosis of *S. haematobium* (Coulibaly et al. 2013). The CAA test works by binding Circulating cathodic antigen from the urine to a labeled monoclonal antibody immobilized on the sample membrane. When the solution is passed over the strip, a pink color appears if the antigen-antibody complex binds to another monoclonal antibody immobilized at the test line. The intensity of color reflects the severity of the infection (Sousa et al. 2020).

3.2.1.3. DIPSTICK DYE IMMUNOASSAY (DDIA)

It is a rapid testing kit designed to identify antibodies against *S. japonicum*. China developed the DDIA, which uses soluble egg antigen (SEA) that has been dyed colloidally. This test is particularly helpful for field screening (Zhu et al. 2005). This kit works on the principle that *S. japonicum's* soluble egg antigen (SEA) conjugates with a blue colloidal dye. This is cast-off to find antibodies in *Schistosoma*-infected patients' serum. Immunochromatography is used to immobilize the antigen-antibody complex onto a nitrocellulose membrane dipstick, employing anti-human IgG as the capturing agent (Zhu et al. 2002). In the case of S*. haematobium* infection dipstick reagent is available to detect hematuria, which is a common indicator of infection, the presence of blood can fluctuate over time (Kosinski et al. 2011).

3.2.1.4. DOT IMMUNOGOLD FILTRATION ASSAY (DIGFA)

DIGFA is a fast method used for the diagnosis of *S. japonicum*, this will detect anti-*Schistosoma japonicum* antibodies (Wen et al. 2005). The DIGFA was developed for the detection of schistosomiasis using rabbit anti-human IgG that has been tagged with colloidal gold as a probe and SEA as an antigen to make a diagnosis (Ding 1998). Tang et al. (2008) used a sheep antihuman IgM immunogold conjugate as a probe in their study to improve the precision of their assay (Tang et al. 2008). This modification produced remarkable results, with the test detecting acute schistosomiasis with 100% sensitivity and chronic schistosomiasis with 96% sensitivity (Tang et al. 2008). One of the primary benefits of this method is its

simplicity, as it does not require specialized equipment and can be performed quickly. Furthermore, when stored at 4°C, the reagent used in the test remained stable for at least 6 months (Ding 1998).

3.2.1.5. INDIRECT HEMAGGLUTINATION ASSAY (IHA)

IHA is used for the detection of *S. japonicum*. It agglutinates sheep erythrocytes with soluble antigens (Mao and Shao 1982), a process that occurs when antibodies against these antigens in patients' blood samples interact with the antigens on the cell surfaces (Zhang et al. 2016). The sensitivity of the immune-hemagglutination assay (IHA) has improved to 93-100% because of this approach, with a reduced false positive rate of 2-3% in healthy individuals from non-endemic areas (Nian-Gao et al. 2011; Yang et al. 2009). The majority of previous schistosomiasis patients have a negative test result after receiving effective, and consistent therapy for at least three years. Despite its long history of use (over 50 years), IHA is still the second most used general immunoassay in China, trailing only the COPT test (Zhou et al. 2009).

3.2.1.6. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA is regarded as the foremost serological assay, demonstrating exceptional sensitivity and specificity in the diagnosis of schistosomiasis (Chala 2023). The reactivity between antibodies found in a patient's serum and antigens extracted from various life cycle stages of schistosomes can be assessed using the enzyme-linked immunosorbent assay (ELISA) (Weerakoon et al. 2015). The procedure involves the binding of soluble proteins, such as antigens or antibodies, to the surfaces of multi-well plates. It provides the flexibility to detect various classes of antibodies using a wide array of antigens. By virtue of the affinity between antigens and antibodies, ELISA generates qualitative or quantitative results (Othman 2013). Initially, the detection of *Schistosoma* antigens by ELISA relied on the utilization of crude soluble egg antigens (SEA) and soluble adult worm proteins (SWAP). This was subsequently followed by the introduction of purified antigens, often referred to as excretory/secretory antigens. Detecting SEA and SWAP in serum and excreta has shown significant diagnostic potential, as the levels of these antigens correlate closely with the parasitic load, enabling early treatment initiation (Chala 2023). Furthermore, assays designed for the detection of circulating schistosome adult worm antigens offer an alternative method for diagnosing schistosomiasis. The primary advantages of these circulating antigens include their high specificity, positive correlation with the worm burden, and the ability to estimate the intensity of infection. Additionally, circulating *Schistosoma* antigens rapidly disappear following treatment, making them valuable for assessing the effectiveness of a cure (Chala 2023).

3.2.2. NUCLEIC ACID-BASED TECHNIQUES

Nucleic acid tests have emerged as a prominent diagnostic tool for parasitic infections such as schistosomiasis. (Chala 2023). Because of their precision and sensitivity, these methods offer significant advantages in terms of accurate and timely parasitic detection. In this section, we will deliver a summary of current advancements in each method before delving into their utility in diagnosing Schistosome infections (Ullah et al. 2020b). The diagnosis of schistosomiasis relies on microscopic techniques, but it has poor sensitivity when the parasitic burden is low, time-consuming and a trained operator is required for it (Ross et al. 2017). Due to these problems, people are shifting towards molecular techniques for the diagnosis of schistosomiasis, it`s not only improves diagnosis but also uplifts effective research in current times (Cavalcanti et al. 2019; He et al. 2016). Following are different nucleic acid techniques used as an advanced diagnostic method in schistosomiasis diagnosis.

3.2.2.1. POLYMERASE CHAIN REACTION (PCR)

The use of PCR to detect Schistosoma DNA in urine and stool samples is a highly sensitive and specific method that offers significant improvement in diagnosing schistosomiasis in non-endemic areas with low parasitic burden (Obeng et al. 2008; Pontes et al. 2003). When used to detect Schistosomiasis in various sample types, PCR consistently shows exceptional sensitivity and specificity (Gomes et al. 2010; Ten-Hove et al. 2008). PCR specificity is 99.9% and sensitivity 94.4% when using genus-specific PCR, and specificity is 98.9% and sensitivity 100 % when using species-specific (S. mansoni) PCR (Sandoval et al. 2006). Different types of PCR are used like ddPCR, RT-PCR, qPCR, and conventional PCR for the recognition of schistosomiasis from several specimens; stool, saliva, serum, and urine (Rahman et al. 2021).

3.2.2.2. REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (QRT-PCR)

Real-time quantitative polymerase chain reaction is a technique used to quantify the quantity of PCR products, typically by incorporating fluorophores into the reaction. During the qPCR process, the fluorescence signal produced is measured in each amplification cycle. Unlike traditional PCR, qPCR eliminates the need for gel electrophoresis to visualize the DNA bands, making it less labor-intensive. Moreover, qPCR offers advantages over conventional PCR as it enables the detection of lower concentrations of target DNA (Chala 2023).

3.2.2.3. DROPLET DIGITAL PCR (DD PCR)

Recent advances in PCR technology have resulted in ddPCR (digital droplet PCR) emerging as a more sensitive and precise alternative to qPCR (Yang et al. 2014). ddPCR is highly effective in a variety of applications, including the detection of cell-free DNA (cfDNA) and the diagnosis of infection and clinical conditions such as cancer (Olmedillas-López et al. 2017). Furthermore, its efficacy in diagnosing *S. japonicum* infections in animal models and various human clinical samples has been demonstrated, allowing for quantification of infection intensity via direct target gene copy number (Weerakoon et al. 2017).

ddPCR capability to test multiple targets renders it a valuable diagnostic tool for identifying numerous parasites in an infected individual (Jongthawin et al. 2016). This characteristic enables the simultaneous detection and quantification of multiple parasite species, providing a more comprehensive thorough approach to diagnosis. Overall, the improved sensitivity, precision, and multiplexing capabilities of ddPCR make it a promising technology for research, clinical diagnosis, and infectious disease surveillance (Weerakoon et al. 2018).

3.2.2.4. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

When compared to PCR-based methods, the LAMP technique provides a cost-effective and rapid means of detecting DNA, making it particularly useful for field use. Its ease of use stems from not requiring specialized equipment such as a thermocycler or electrophoresis apparatus, making it suitable for resource-limited settings once properly optimized (Weerakoon et al. 2018). The assay is highly specific to the target sequence and remarkably sensitive recognition to the use of specific inner and outer premier sets (Xu et al. 2010). However, multiple premiers are used, and the initial optimization process can be time-consuming. Furthermore, there is a risk of carryover contamination, which could result in false positive results when reamplifying previous LAMP products (Ma et al. 2017).

Table 1: Summary of strengths and shortcomings of Schistosomiasis diagnostic techniques

Assay Type	Test Name	Strengths of tests	Shortcoming of tests	Reference
Conventional methods	Direct Microscopy	Simple, cost-effective	Ineffective in early-stage diagnosis, time-consuming,	(Chala 2023)
	FECT	Simple, time efficient	Limited sensitivity, operator dependency	
	Kato-Katz	Cost-effective, gold standard	Low detection limit	
Immunological Methods	Intradermal Test	Low cost & highly sensitive	Low specificity	(Chala 2023)
	CCA Rapid Kit test	More sensitive than conventional method	Limited accuracy and costly	
	DDIA	Rapid, easy to use, no special equipment needed, high specificity	Limited sensitivity, potential cross-reactivity	
	DIGFA	Rapid, simple, highly specific, and sensitive	Limited availability, technical requirement	
	IHA	Sensitive, wide range application, simple	Cross-reactive, technical skill requirement	(Van Lieshout et al. 2000)
	ELISA	Sensitive, high throughput, highly specific	Time-consuming, complex, cross-reactivity	
Nucleic Acid Methods	PCR	High specificity and sensitivity, can detect a small amount of template	Expensive, time-consuming,	(Ullah et al. 2020b)
	qRT-PCR	Highly sensitive and specific	More expensive than other PCR methods	(He et al. 2018)
	dd-PCR	Highly sensitive and specific	Expensive equipment required	(Weerakoon et al. 2017)
	LAMP	Rapid, highly efficient, less equipment and reagent needed	Prone to carryover contamination, chances of false positive results	(Gandasegui et al. 2018)
	RPA	Cost-effective, highly sensitive, and specific	Risk of a false positive outcome	(Poulton and Webster 2018)

LAMP assays are effective at detecting *S. mansoni* and *S. haematobium* infections, especially in areas where both parasites co-exist. This suggests that they have the potential to be used as point-of-care diagnostics for rapid and accurate detection (Lodh et al. 2017). Early detection of pre-patent schistosome infections has been made possible by the successful application of the LAMP technique in animal models (Fernández-Soto et al. 2014). Field surveys have also shown that LAMP assays an effective in detecting *S. mansoni* infection in low transmission areas, using samples from snails, humans, and animals' feces. This demonstrates the molecular approach's ability to identify transmission foci and create risk maps, which can be used to support control programs (Gandasegui et al. 2018).

Current work has investigated the feasibility of developing multiplex LAMP assays to detect multiplex parasitic species, including schistosomes, in infected people. Multiplex LAMP enables various endpoint readout options for differentiating amplified products, such as melting curve analysis or distinct gel electrophoretic banding patterns (Liu et al. 2017), facilitating species detection. Multiplex LAMP, like qPCR, is a promising method for effectively diagnosing soil-transmitted helminths (STH) co-infection or the co-infection of intestinal protozoa and schistosomes in resource-limited endemic communities (Llewellyn et al. 2016).

3.2.2.5. RECOMBINANT POLYMERASE AMPLIFICATION (RPA)

RPA is a type of isothermal amplification that works at lower temperatures, typically around 40 °C. To amplify DNA sequences, it uses DNA polymerase, DNA binding proteins, recombinase protein, and

oligonucleotides nucleoprotein complexes (Poulton and Webster, 2018). The RPA technique, like LAMP, is simple to use and can be implemented in resource-constrained settings without the need for specialized equipment such as a thermocycler, electrophoresis apparatus, or gel documentation units. This novel technique has recently been combined with chips and lateral flow devices, transforming it into a convenient and portable tool for point-of-care diagnosis (Zanoli and Spoto 2013).

RPA has demonstrated success in the diagnosis of both intestinal and urinary schistosomiasis and has been thoroughly tested field. RPA has significant advantages over microscopy and serology in terms of convenience, shorter detection time, and improved diagnostic sensitivity (Poulton and Webster 2018). However, the RPA technique has some practical limitations. The requirement to transfer the amplified products to the detection device introduces the risk of nucleic acid contamination and can result in false positive results (Weerakoon et al. 2018).

A summary of the strength and shortcomings of Schistosomiasis diagnostic methods are enlisted in Table 1.

4. FUTURE PROSPECTIVE

The ongoing research to improve existing diagnostic methods, the discovery of novel biomarkers via proteomic and transcriptomic, and the progress of multiplex assays for the simultaneous finding of multiple schistosome species or infection stages are all possibilities for the future. Furthermore, the incorporation of diagnostics and new imaging technologies into disease control programs could improve disease surveillance and targeted interventions. Collaboration among researchers, healthcare professionals, policymakers, and stakeholders is critical for fully realizing the benefits of these advances. With continued efforts and a global approach, recent advances in schistosomiasis diagnosis have the potential to have a substantial influence on disease control and improve the lives of those affected by this neglected tropical disease.

5. CONCLUSION

Significant recent developments in the identification of schistosomiasis have been made, to address the limitations of traditional methods and improve diagnostic accuracy, sensitivity, and accessibility. The emergence of rapid point-of-care tests, molecular diagnostics using PCR and LAMP, serological assay, and antigen detection tests are notable developments. These developments have shown promise in detecting Schistosome molecules at various stages of life, providing valuable diagnostic candidates. Despite these accomplishments, achieving optimal diagnosis remains a challenge. Some test's sensitivity and specificity may still need to be improved, and cost and accessibility barriers may prevent widespread implementation, particularly in resource-limited settings. Accurate differentiation of different Schistosome species remains critical for appropriate treatment, and early detection remains an important aspect of disease management.

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