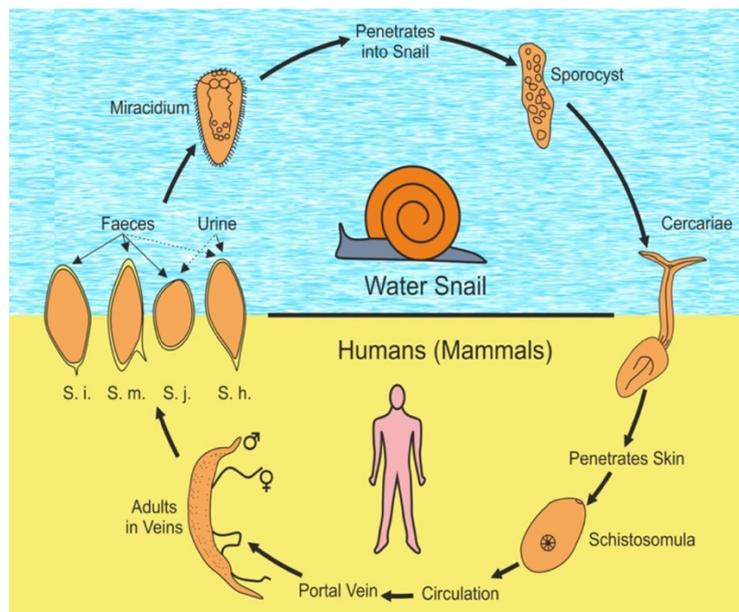


Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology

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Caption: *Schistosoma mansoni* life cycle (1) Paired adult worms (female is thin, male is bigger), (2) Eggs (see note below), (3) Ciliated miracidium, (4) *Biomphalaria* intermediate host snail, (5) Sporocyst, (6) Cercariae, the infective stage, and (7) Schistosomula, the young parasite.

Abstract

Schistosomiasis is a neglected invasive worm disease with a huge disease burden in developing countries, particularly in children, and is seen increasingly in non-endemic regions through transfer by travellers, expatriates, and refugees. Undetected and untreated infections may be responsible for the persistence of transmission. Rapid and accurate diagnosis is the key to treatment and control. So far, parasitological detection methods remain the cornerstone of *Schistosoma* infection diagnosis in endemic regions, but conventional tests have limited sensitivity, in particular in low-grade infection. Recent advances contribute to improved detection in clinical and field settings. The recent progress in micro- and nanotechnologies opens a road by enabling the design of new miniaturized point-of-care devices and analytical platforms, which can be used for the rapid detection of these infections. This review starts with an overview of currently available laboratory tests and their performance and then discusses emerging rapid and micro/nanotechnologies-based tools. The epidemiological and clinical setting of testing is then discussed as an important determinant for the selection of the best analytical strategy in patients suspected to suffer from *Schistosoma* infection. Finally, it discusses the potential role of advanced technologies in the setting near to disease eradication is examined.

Keywords: schistosomiasis, rapid, accurate, POC, diagnosis, immunoassays, PCR, LAMP, microfluidic, microarray.

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Purpose and Rationale:

The limitations of available diagnostic tests for schistosomiasis contribute to our current failure in disease control and eradication of this important poverty disease. This paper therefore aims at delineating specific requirements and desirable characteristics for a new generation of diagnostics based on the micro- and nanotechnologies, driven by the conviction that new technologies should benefit all segments of humankind, including the poorest.

Introduction

Schistosomiasis is one of the most prevalent and widespread [1] parasitic neglected tropical diseases in the world. It is caused by fluke worms of the genus *Schistosoma* and affects humans and various other animals [2, 3]. The 6 species involved in human infections are *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. guinensii* and *S. intercalatum*. The respective distribution, the population at risk and the main clinical manifestations are summarized in Table 1.3. Infection starts when cercariae released by snails penetrate through the skin exposed to infested water [4]. More details on the life cycle are given in Figure 1. Schistosomiasis affects 221 million people where children between 5 and 14 years of age which represents 45.8% of the affected patients [5] and puts at risk 800 million people in 74 countries worldwide [6, 7]. In sub-Saharan Africa, the number of affected people is 54 million and 393 million are at risk.[7] Worldwide, the disease kills about 300,000 patients annually [8] and results in approximately 25 million disability adjusted life years lost [9] despite the implementation of control measures [10].

Selective therapy of school children or mass drug administration (MDA) are widely used to control schistosomiasis, but fail to prevent rapid reinfection in endemic areas [8]. A substantial decrease in morbidity and mortality is observed in some of these areas, but the disease continues to spread to new geographic regions. It is increasingly imported into non- or low-endemic countries due to the increased migration of the human population (e.g., refugees, international tourism, and/or international projects) and additionally, environmental changes that result from development of water resources and population growth and migration can facilitate

the spread of schistosomiasis [11, 12]. Diagnostics address both the individual and population needs. At the individual level, they first can detect disease and identify patients at risk of severe disease, which will permit good clinical decision making and good case management.

The use of diagnostics also supports (1) the detection and prevention of drug resistance, (2) the surveillance of a disease, and (3) the assessment of the efficacy of drugs and vaccines in clinical studies [13]. Rapid and accurate diagnosis of schistosomiasis is a challenge [14]. It remains a key requirement for treatment and control [15-19]. There is still a need to determine the existence of infection more simply and reliably, both, in hospital settings where severe pathological effects are suspected, as well as in the field [20, 21].

The current diagnosis of schistosome infection is based mainly on clinical symptoms, and therefore low-level and chronic or asymptomatic/atypical infections are often missed [22, 23]. Various procedures to detect this infection have been validated and include direct parasitological techniques, immunologic methods, and molecular approaches [24]. The sensitivity of parasitological tests decreases when parasite egg numbers are low or eggs are absent [25] and specificity of rapid diagnostic tests (RDTs) is variable [24]. Routine serological tests such as enzyme-linked immunosorbent assay (ELISA) have good sensitivity but may cross-react with intestinal nematodes [26]. Methods based on the amplification of a highly repeated deoxyribonucleic acid (DNA) sequence by polymerase chain reaction (PCR) in fecal and serum human samples [27] show high sensitivity, high specificity, and good predictive value. Yet, the test cost and the need for specific laboratory equipment and conditions limit the use of these assays in many laboratories. The real-time polymerase chain reaction (RT-PCR) method, in spite of its high sensitivity and specificity [24], has remained only as a confirmatory diagnostic test, since it is intensive work and expensive. To reduce the costs loop mediated isothermal amplification (LAMP) of DNA was developed. Both LAMP and microarray methods have been tested and so far these methods promise to be excellent tools in epidemiological and clinical screenings

[1] but, the diagnostic performance of these techniques in areas with different schistosomiasis prevalence is so variable that it is difficult to declare a “gold standard” test [28, 29]. Also, the amplification mechanism of LAMP may easily lead to carryover contamination and therefore false-positive results.

The rapid evolution of micro- and nanofabrication technologies opens a door for the development and evaluation of new methods and tools for the diagnosis of schistosome infection. In this chapter we will first present the currently available diagnostics, followed by the latest developments in the field and end with a discussion. With a view on clinical application, the value of diagnostic tests for patients in different epidemiological scenarios is discussed. As a comprehensive review of the huge body of work that is being performed is outside the scope of this paper, we aim to provide an update of current relevant achievements in the field. The cited articles are collected by cross-referencing online keyword searches (“*Schistosoma* diagnostics methods” “Schistosomiasis” “*S. mansoni*” “*S. haematobium*” “*S. japonicum*” “POC”) in citation and database searching on Pubmed from January 1990 to November 2019. The works perceived as most important are exemplarily presented in this review. The

emphasis of literature citations is on the development of point-of-care diagnostics for parasitic infections.

Established diagnostic assays for schistosomiasis

Current laboratory tests for schistosome infection are based on direct parasitological detection, the detection of circulating antigens and/or specific antibodies, and molecular methods. More detailed information can be found in focused literature [13, 30]. As the background of evaluation for emerging and future test modalities, the current clinically available tests are summarized in the next paragraphs.

Microscopy: direct detection of parasite eggs

The reference method for the laboratory diagnosis of a schistosome infection is parasite egg detection in the infected individual’s stool, urine, or tissues [31, 32]. Such parasitological detection (PD) is widely used because it is relatively efficacious and moderately cost-effective for case management, screening or surveillance. The success of control measures against schistosomiasis has been difficult to determine because the sensitivity of PD decreases when there is no egg excretion, or it diminishes, principally in areas of low endemicity [25]



Figure 1 : Current diagnostics of intestinal schistosomiasis relies on the microscopic detection of *S. mansoni* eggs in stool samples, a laborious, time consuming process relying on infrastructure (microscopes) and specific expertise. Low-intensity infections are frequently missed [Nigo MM, PhD thesis, Basel 2020]

Other direct parasitological tests for intestinal schistosomiasis are the Kato-Katz (KK) [33] technique, the miracidia hatching test (MHT) [34], the salinity gradient [35] and on the recently developed FLOTAC [36-41] and Helmintex assay [42-47]. The KK technique has the advantage of egg quantification in fresh

stool samples. An amount of approximately 41.7 mg or 50 mg of feces is placed in a 1.5 mm × 6 mm template hole. The number of eggs is then counted and extrapolated to 1g [48, 49], yielding infection density (Table 2). This method is still recommended by the WHO for diagnosis at the community level. MHT can be

done using the miracidia hatching device [50] or by sieving stool sample through a nylon tissue bag for concentrating the eggs. Then, hatching is carried out in a well-lit room at a temperature of $25 \pm 3^\circ\text{C}$ followed by counting of the swimming miracidia [34]. The FLOTAC technique is more sensitive when compared to the KK technique, but it requires specific equipment, flotation solution, a suitable preservative medium that can affect the outcome of the test [41]. The principle of the Helmintex test is based on conjugates that are formed between the magnetic particles and the iron that is present in the eggshell pores of *S. mansoni*. Other factors such as electrostatic

forces or the surface ornamentation of the eggs play also a role [51].

In urinary schistosome infections, direct microscopy of filtered urine is used. After gentle homogenization, 10 mL of urine is filtered through a membrane on which the number of eggs is counted in the microscope at $40\times$ magnification [52]. These methods are still considered as reference methods [53], in spite of their limited capability to identify schistosome infection consistently in low-endemic areas. The development of better diagnostic tools for case management and disease control is therefore required [13].

Table 1: Expression of the intensity of schistosome infection [52]

Types of Schistosomiasis	Intensity expression	Light	Moderate	High
Intestinal schistosomiasis	Eggs per gram of stool	1 – 99	100 – 399	≥ 400
Urogenital schistosomiasis	Eggs per 10 mL of urine	< 50		≥ 50

Parasite-derived material detection

Adequate differentiation between past and current infection requires assays, which detect circulating parasite antigens. The existence of such antigens was first described by [54-65], and an inventory has been published [66]. The most thoroughly investigated antigens are the circulating anodic antigen (CAA) [67], a gut-associated proteoglycan (GASP) [68] and the circulating cathodic antigen (CCA), originally named the M-antigen [69], which is a polysaccharide antigen emanating from the worm gut [70]. They are named relatively to their electrophoresis migration [71]. Their presence in patients corresponds well with active infections [56, 61]. Detection of circulating antigens could be used as well as for the evaluation of chemotherapy efficacy [6].

Other antigens as targets for diagnosis have been reported: schistosome adult worm antigen (SAWA), schistosome egg antigen (SEA), enzymes, parasite proteins, and tegument antigens; they can be detected in patient serum [72, 73]. As some antigens are cleared by kidneys, urinalysis can produce evidence of

their presence in schistosomiasis due to *S. japonicum* [74], *S. haematobium* [20] and *S. mansoni* [75]. Antigens from virtually all stages of the schistosome life cycle have been tested for immunodiagnostic potential and methods for their direct detection in blood, stool, or urine had been developed. Methods for detecting circulating antigens generally involve the capture of the antigen by a monoclonal or polyclonal antibody, with specificity for repeated epitopes on the antigen. In most cases, specific monoclonal antibodies are used [74]. Examples of these immunoassays are ELISA [31], radio-immuno-assay (RIA) [76, 77], and direct fluorescent antibody tests [78-80].

Lateral flow immuno-chromatographic assays or RDT are used for diagnosing antigens in serum [81], and in urine samples [82, 83]. They provide rapid results, are a non-invasive technique and are easy to use because they are typically cassettes or dipsticks [84, 85]. Details on technical procedures can be found in the cited literature [86]. Figure 2 shows an RDT for CCA in urine for the detection of *S. mansoni*. RDTs permit detection of active infections and

are efficient in testing of large numbers of samples. However, they are frequently false negative due to inhibitory host antibodies [77] and often cannot distinguish between infections of different species because they are not species specific [14]. Diagnostic specificity of these tests is high because few false-positive are



Figure 2: Rapid diagnostic test for circulating cathodic antigen allows point-of-care diagnosis of *Schistosoma* infection. Field studies [Nigo MM, PhD thesis, Basel 2020] have shown very good sensitivity when prevalence and parasite load is high, but sub-optimal sensitivity for low-grade infection.

Antibody detection (AbD)

Schistosoma infection activates specific immunoglobulin-dependent responses against several parasite antigens [90]. Most AbD assays measure serum immune reactivity to SAWA and SEA preparations for diagnostic purposes, using crude extracts, or parasite tegument proteins. AbD assays exhibit a modest sensitivity and a limited specificity although several antigens are available for diagnostic purposes, and some studies have shown their superiority to parasitological methods in low-endemic areas (LEAs) [91]. Nonetheless, AbD is more attractive for monitoring areas of controlled transmission and are important for the diagnosis of atypical forms like schistosome neuro-infection [22, 23]. Screening and diagnosis should rely on the use of 2 or more immunological tests to improve sensitivity because the test result depends on worm burden.

found outside endemic areas and circulating antigen levels generally correlate with excreted egg counts [87-89]. AgD assays need to be evaluated further and are not currently considered as a suitable replacement for traditional diagnostic tests [28, 29].

AbD assays include various forms of RIA and ELISA, indirect immunofluorescence (IIF), complement fixation, indirect agglutination of erythrocytes or other tests, of which each has their specific weaknesses [92]. Many of these diagnostic techniques were miniaturized. Detection of antibodies in patient serum or cerebrospinal fluid (CSF) became possible [93]. Most AbD assays exhibit high sensitivity; however, they show limited specificity due to crude antigen preparations. They allow early diagnosis before oviposition of the worm [94, 95]. With the interpretation of the results, one has to take into account a possible persistence of host antibodies after the patient is cured [96, 97].

Alternative methods

Hematuria and proteinuria are associated with *S. haematobium* infection and are therefore often used for indirect diagnosis or as screening tool for schistosome infection [97, 98]. The cheap urine dipsticks for macroscopic hematuria and proteinuria detection are being used to guide treatment and public health interventions for *S. haematobium*. Hematuria is relatively specific for infection in a *S. haematobium* endemic area. However, hematuria has a variable sensitivity [99].

Molecular approaches

Although PD methods have proven to be inadequate as a gold standard, advances in the development of antigen and antibody detection assays are not sufficient to allow their use either universally or as a reliable single detection approach in schistosomiasis diagnosis, particularly in areas of low endemicity [24]. To overcome the shortcomings of both parasitological and immunological methods, the development of more sensitive and more specific molecular diagnostic tools diagnosis is desirable. The availability of DNA detection techniques may evolve to potentially valuable tools in the diagnosis of a schistosome infection. DNA-based assays have also proven to be useful for cure assessment [24]. The PCR

technique has shown its clinical value in a wide variety of infectious diseases and in a variety of sample types including blood, urine, and saliva [100]. It has been successfully used for the sex determination of the cercariae in *Schistosoma* sp. studies and in the development and application of new techniques to generate expressed sequence tags. Hamburger et al. [101, 102] developed a PCR protocol that was based on the amplification of a highly repetitive DNA sequence for monitoring *S. mansoni* infestation in water-based on DNA sequences from *S. mansoni* cercariae. According to Reithinger et al. [103], PCR assays can be divided into 3 distinct formats: namely the mid-tech approach, represented by conventional PCR, the high-tech represented by real-time PCR and the low-tech, represented by LAMP. PCR-based methods are considered to be highly sensitive and a detection limit is 1 fg of *S. mansoni* of genomic DNA has been reported [24]. PCR can be performed on feces, urine or serum [27, 73, 104]. In other studies, the use of PCR has been reported for diagnosing female genital *S. haematobium* infection and low intensity of *S. japonicum* infections in stool samples [105, 106], and for detection and quantification of *S. mansoni* and *S. haematobium* when present with other parasitic co-infections [107]. Superior accuracy, sensitivity, and specificity in areas with a low intensity of infection have been observed using antigen detection by PCR-based methods. It can be used to efficiently detect an active infection in almost 60% of IgG positive individuals who do not excrete eggs [104, 108]. Different studies demonstrated that the absence of amplification of other helminth DNA in a PCR assay is a strong indicator of its species-specificity [109]. Thus, PCR is more sensitive than the KK technique. However, Gomes and Enk [104, 110] found different results in samples tested by conventional PCR and microscopy. They observed that up to 41.6% of samples negative by KK are positive by PCR and that PCR assays for *S. mansoni* diagnosis increase prevalence estimates to above 38%, whereas positivity on K-K assay ranges from 18 to 30.9%. A high degree of specificity may render it preferable to serological techniques. Even though these studies have demonstrated that PCR-based technologies are reliable, specific and sensitive tools, they are not widely

used in low-income countries because highly skilled personnel and expensive cyclers are needed. The use of thermocycler machines to amplify DNA is severely hampered in field situations where electric power is unstable, intermittent or absent. There is a real need to render these techniques easier to use and technically more robust, such that obvious advantages of these tests can be translated into broad clinical utility even in resource-constrained locations [111, 112].

The value of standardized quantitative PCR-based methods for the detection of *Schistosoma* infection was shown in experimental settings [94]. RT-PCR was found highly sensitive and specific in the detection of active schistosomiasis [109]. Wichmann [113] found that blood-based RT-PCR detected 95% of true positive patients when, in contrast, anti-schistosome antibodies and microscopy were positive only in 72% and 25% of cases respectively. Moreover, RT-PCR also permits the determination of infection intensity in samples with a low parasite burden. Obeng [114] examined urine specimens from children to detect CCA, and then performed RT-PCR on urine sample after storage at -80°C using internal-transcribed-spacer-2 (ITS2) sequences for *S. haematobium*. When compared with eggs in the urine, the RT-PCR test was 100% sensitive, yet specificity was low. The detectable product was dependent on the number of eggs passed in the specimen and the authors postulated that the template DNA was derived from the eggs.

Currently, all PCR variants demand more sophisticated laboratory equipment and a greater operational effort when compared with the KK technique, in terms of low costs and ease of operation [27].

New DNA detection using new technologies, such as LAMP (Figure 3), have been developed. LAMP amplifies a few copies of DNA to 10^9 copies in less than an hour with high specificity and efficiency under isothermal conditions. LAMP method employs a *Bacillus stearothermophilus* (*Bst*) DNA polymerase and a set of 4 [115] to 6 or more specially designed primers: inner forward primer (FIP), inner backward primer (BIP), outer forward primer (F3), outer backward primer (B3), loop forward (LF) and loop backward that recognize a total of 6 distinct sequences on target DNA. A

standard LAMP reaction consists of a large number of reaction components, namely the enzyme and its buffer, 3 sets of primers of varying concentrations, and a detection dye. Significant progress has been made with the modification of the LAMP method. The LAMP

reaction produces large amounts of magnesium pyrophosphate ($Mg_2P_2O_7$) and double-stranded (dsDNA) avoiding post DNA amplification manipulation. LAMP has shown high sensitivity, detecting 0.08 fg of genomic DNA [116].

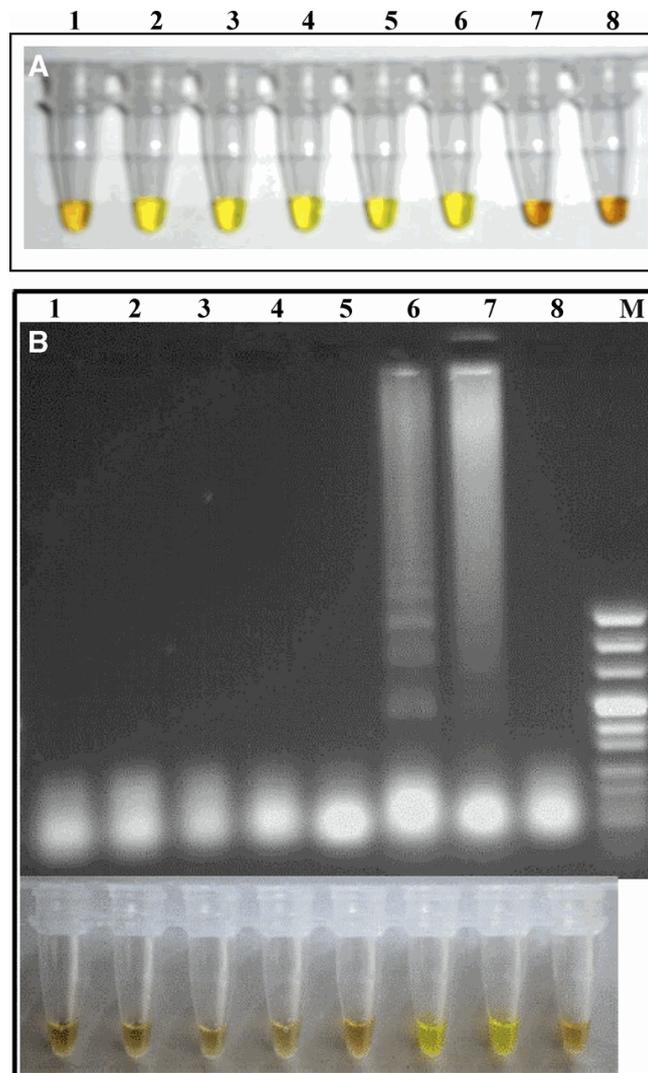


Figure 3: Isothermal loop-mediated amplification (LAMP) for the detection of *Schistosoma* DNA. Yellow colour indicates detection of specific DNA. A1-6: *Schistosoma* positive snails; A7-8: negative controls. B: evaluation of LAMP result with agarose electrophoresis: B1-5,8: negative control. B6-7: *Schistosoma* positive snails. The reduced hardware requirements for LAMP compared to conventional PCR and the simple, colorimetric readout may lead to field-applicable high-sensitivity molecular testing. From Hamburger et al [118].

The LAMP method has the advantage of being able to amplify target DNA from partially processed and/or non-processed samples [117]. It can be conducted in less advanced field laboratories with much less intensive training of local laboratory personnel. When a large

number of examinations are undertaken, the use of LAMP method could save time and financial resources [118]. This method has demonstrated superior accuracy when compared to the conventional PCR in detecting several pathogens such as viruses, bacteria, protozoa in

humans [119]. In the diagnosis of schistosomiasis, LAMP was found highly sensitive for the detection of *S. japonicum* infection pre- and post-chemotherapy in experimental settings [120]. LAMP was recently used for the detection of *S. japonicum* in human serum samples. It revealed high analytical sensitivity (96.7%) detecting 0.08 fg of parasite DNA and a specificity of 100% [121]. Fernandez-Soto [1] are leading in development of a sensitive, specific, cost-effective and easy to perform LAMP assay for early diagnosis of *S. mansoni* in feces. However, the amplification mechanism of LAMP may easily lead to carryover and therefore false-positive results.

Emerging diagnostic technologies for schistosomiasis

Advances in micro- and nanofabrication technologies have greatly contributed to improving POC laboratory diagnosis [122]. Benefits of miniaturization include low consumption of costly reagents and power, minimized handling of hazardous materials, short reaction times, portability and versatility in design, and capability for parallel operation, all of which are particularly important for POC diagnosis in the tropical and developing countries where schistosomiasis is endemic [123]. Important developments in this field are presented below in Table 2.

Table 2: Comparison of the main characteristics of diagnostic tests for schistosome infection

Characteristics						
Test	Methods	Advantages	Disadvantages	Price (US\$)	On market	Reference
Microscopy	Egg detection	Simple Quantification	Limited sensitivity Laborious	0.4	Yes	[77]
ELISA; DDIA; IHA	Antibody Detection	Sensitive	Limited specificity	5.0* *	Yes	[52] [124]
RDTs	Antigen Detection	Rapid; Large scale testing; Specific	Variable sensitivity; False positives	2.6	Not yet	[77] [78]
ELISA DDIA*** IHA****	Antigen Detection	Sensitive Specific	Variability in test results		Yes	[126] [127] [128]
PCR	DNA detection	Highly sensitive Specific	Laborious; Expensive; Instrumentation; Not quantitative	6.4	Yes	[17] [106]
RT-PCR	RNA detection	Highly sensitive Quantitative	Laborious; Expensive; Instrumentation;	7.7	Not yet	[114] [128]
LAMP	DNA detection	Highly sensitive Accurate	Some Instrumentation, false positive		Not yet	[1] [75] [120] [129]

* Price without including equipment use and personnel labor [24]

** Estimated price

DDIA = disperse dye immunoassay; DNA = deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; IHA = indirect hemagglutination test; LAMP = loop mediated isothermal amplification; RNA = ribonucleic acid.

Song et al. [129] presented an instrument-free disposable microfluidic POC device for the on-site detection of schistosome parasite infection in blood samples obtained from a finger prick. On board a reaction chamber is present and once filled with

water, it is heated to its operating temperature, which is used to incubate a LAMP reaction. The emission of fluorescent light is detected by eye or with the camera of a smartphone.

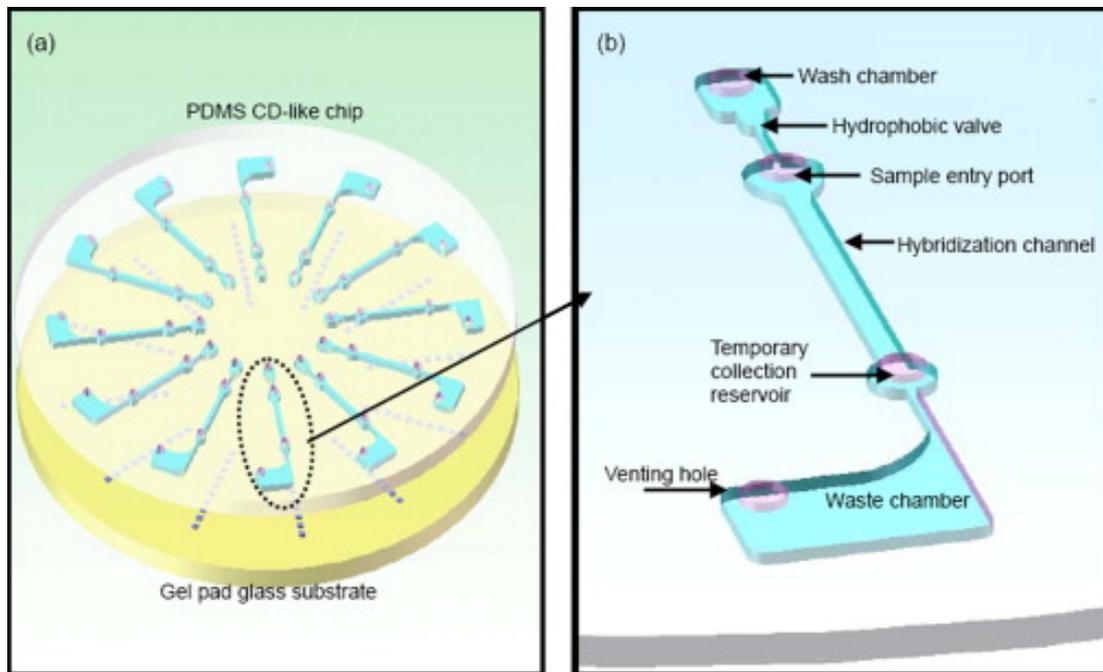


Figure 4: A compact disc (CD)-like microfluidic rotating disc for DNA hybridization assays in nanoliter samples proposed by Li et al [128, reprinted with permission]. Fluid handling is driven by differential rotation of the disk, allowing flow control without the need to interact with the disk. Parallel processing can be performed on multiple channels.

This device has potential, however, the discussed crossover contamination when using LAMP needs to be taken into account and thus a second test confirming an infection might be necessary.

A microfiltration device for the diagnosis of urogenital schistosomiasis was developed by Xiao Y et al. [130], shown in Figure 5

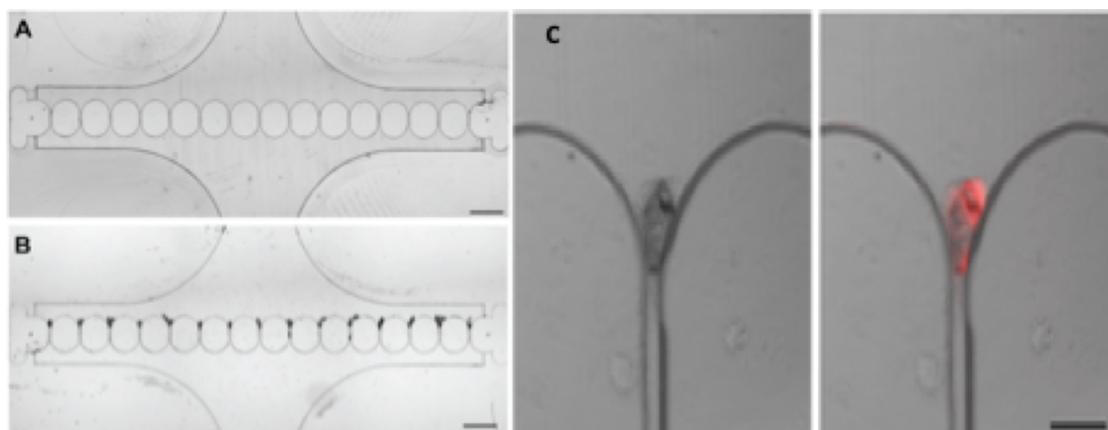


Figure 5: Microfluidic device for trapping and fluorescence-based identification of *Schistosoma hematobium* eggs from urine. A: microfluidic trap. B: trapped *Schistosoma* eggs in brightfield image. C: with fluorescence microscopy overlay. Adapted from Xiao Y et al [130, reprinted with permission].

In this device *S. haematobium* eggs are trapped and analysed by using brightfield microscopy. For POC use, it might be possible to design this device such that a smartphone camera can be used.

Capillary test strips or LF assays can also be considered as fairly simple microfluidic systems and are the most successful POC tests to date. LF devices can be reduced to their bare ingredients - patterned filter paper impregnated with reagents resulting in microfluidic paper-based analytical devices (μ PADs). Paper-based techniques allow performing multiple, simultaneous, and independent assays with multiplexed detection. Fast prototyping and production techniques for paper-based diagnostic devices have been proposed [123]. μ PADs are inexpensive, easy to use, lightweight to transport, compatible with biological samples and may be handled in remote settings by non-trained personnel.

Ishii [131] developed microfluidic quantitative PCR (qPCR) on environmental samples in a quadruplicate with a dynamic array under specific detection conditions. They found that the quantitative performance of microfluidic qPCR was similar to the conventional qPCR. However, it specifically detected pathogens in stool, demonstrating that this method may be applied to the rapid identification of disease causing-agents for diagnostic purposes. They also quantified multiple targets simultaneously in water samples. Therefore, they concluded that the microfluidic qPCR systems can be applied to the quantification of multiple pathogens in environmental samples. Despite the efforts made in developing microfluidic POC diagnostic devices, microfluidic systems for detection of schistosome infection are developed but still in its infancy due to issues as device sensitivity, multiplex analysis capabilities, assay stability/shelf life, and fabrication cost remain. Standardized evaluation of the performance microfluidic versus other POC technologies in a real-world setting is needed.

Microarrays

Microarrays are usually a glass slide but may also be a microtitre plate or on board of a microfluidic device. Small areas or micro-wells

are coated with different probes or biological material for the simultaneous detection of multiple targets. In this way, thousands of biomolecular interactions can be probed in parallel [132]. Microarrays can offer multi-component information, dynamic compensation for sample matrix effects, detection of malfunction/deterioration, and improved selectivity through signal pattern analysis. Recent progress has brought microarrays to the forefront of clinical diagnostics and medical research. DNA microarrays appear suited for highly parallel detection and identification of microorganisms from clinical samples [133] and therefore promise more rapid, accurate, and cost-effective detection of pathogens compared to culture techniques or conventional immunoassays.

Genomes of all 3 major schistosome species have been sequenced [134], and several postgenomic approaches and high throughput methods have been developed to take advantage of this wealth of information [135]. One such approach is a schistosome-specific microarrayed protein, containing 232 unique antigens [136]. Many of these arrayed proteins are novel molecules and the majority is from *S. japonicum*, with the remainder from *S. mansoni*. They can be then probed with antibodies from immune hosts as a powerful new technology for vaccine antigen discovery [137]. Using this approach, McWilliam [138] identified several novel antigens, which may be important targets for vaccine development.

Nanotechnology enabled diagnostics

Nanotechnology is based on structures and materials in the nanometre size range. Such nano-sized structures and materials differ from microscale structures and materials in various aspects, for example in the very high surface per weight, the occurrence of specific physical effects like particle surface plasmon effects not readily observed in the micro- or macro scale. Gold nanoparticles are stable, can bind biomolecules, and are already used widely in many different kinds of applications such as imaging and biomedical diagnosis [139]. An example is the amperometric immunosensor for the detection of *S. japonicum* antigen [140]. This sensor is made of carbon paste coated with a layer of chitosan on which a monolayer of

gold nanoparticles was formed. Antibodies against *S. japonicum* antigen were linked to the gold monolayer and used to detect this antigen with a detection limit of 0.06 µg/mL [141]. More recent work is the highly sensitive detection of *S. mansoni* DNA [142], where aminated magnetic particles and gold nanoparticles are immobilized on monolayers of mercaptobenzoic acid. Thiolated DNA probes are immobilized on the surface of the nanoparticles. Evaluation of the surface was performed by using an atomic force microscope and the electrochemical processes (binding of the antigens) were measured by using electrochemical impedance spectroscopy and cyclic voltammetry. *S. mansoni* DNA from cerebrospinal fluid and serum could be detected with a detection limit of 0.685 and 0.781 pg/µL respectively. Such sensors will require further development to render their application suitably simple and robust for application in the field [143].

Diagnostic tests in the “real world” of field practice

Parasitological methods

In most locations worldwide and in particular, in endemic areas, laboratory diagnosis of schistosomiasis is still based on parasite egg detection in feces or urine. Direct or indirect microscopy remains in use in POC although being laborious, and of limited sensitivity because of the daily egg count fluctuation. However, negative microscopy does not disprove the possibility of infection in people living in or coming from endemic areas. Results need to be validated by improved diagnostic techniques. Urine microscopy after centrifugation or filtration is required for urogenital schistosomiasis [144]. It presents a good specificity but a low sensitivity consecutive to variable egg release. Nevertheless, in endemic settings, efficiency could reach when urine is collected between 10 am and 2 pm according to the egg excretion pattern [73]. However, during field surveys, urine microscopy presents some difficulties.

Immunoassays

Immune host response against schistosome antigens has been an alternative way for diagnosis. For many years several immunodiagnostic techniques have been developed. AbD immunoassays are widely

used. However, since CCA and CAA can be detected by substituting other antigens, both processes can indiscriminately be used. Immunoassays based on CAA, SAWA or SEA circulating antibodies detection are already in use. Their validity is usually variable. Immunoassays complete direct microscopy by detection of specific antibodies in serum and cerebrospinal fluid. AbD is the best tool for field diagnostic activities. However, they usually exhibit a weak specificity. Frequently, reaction with other parasites antigens compromises the specificity. Nevertheless, antigens used in immunoassays have proven crucial test sensitivity and specificity element [145].

Molecular methods

Molecular methods present high performance in use [104, 111]. PCR is highly accurate, highly sensitive and specific for the detection of *S. mansoni*. They also permitted *S. mansoni* increased prevalence estimates, whereas KK assay underestimated them. However, [107] reported discordant results in samples tested by conventional PCR and KK techniques, and negative PCR results in KK-positive samples.

RT-PCR was found highly specific and sensitive in *S. japonicum* and *S. haematobium* detection [114]. Compared to microscopy and immunoassays, RT-PCR can be the best marker after chemotherapy [104]. Although owning these qualities, PCR and RT-PCR are cumbersome and costly methods. Nucleic acid extraction stages improvement could perhaps lead to cost reduction [104]. DNA detection development by LAMP technologies promises to be the best alternative [116, 120].

Emerging technologies

The persistent transmission and widespread distribution of schistosome infection call for the development of new “gold standard” diagnostic assays. Identification and detection of infected hosts as well as new diagnostic tests for field applications [118] are essential for case management and disease control. These tests must be more sensitive, more specific, and affordable for point-of-care diagnostic strategies (see also Table 3.3). There are 2 important challenges: (1) although parasitological tests remain central for schistosomiasis diagnosis, they have proven their accuracy inability, (2) immunoassays and usual molecular techniques have demonstrated

their weaknesses. To overcome these shortcomings, resorting to statistical indirect methods to assess the accuracy is frequently required. For example, the use of latent class analysis (LCA) [21, 84, 146] has proven its capability for validation of the sensitivity and specificity of the tests [21].

With the goal of the development of an automated and/or integrated device, microfluidic platforms procure hope. Miniaturization could enable affordable POC diagnostic tests for end-users in low resource settings [147, 148]

Table 3: Criteria/wish list for future diagnostic tests [116, 148, 149]

<p>ASSURED criteria:</p> <ul style="list-style-type: none">Affordable by those at risk of infection (less expensive for patients and for health services);Sensitive (few false negatives): able to diagnose light infections (1 egg per gram for intestinal and 1 egg/10 mL for urinary schistosomiasis);Specific (few false-positives);User-friendly (simple to perform in a few steps with minimal training);Robust (Field-adapted: stable in ambient temperature conditions - from 4 to 30°C) - does not require refrigerated storage) and rapid (results available in 30 minutes);Equipment free (less instrumentation, no computer use);Deliverable to the end usersInexpensive to produce (cost target of less than 1 US\$ per test to ensure commercial viability)Producing a visual readout (results can be read by naked eyes)Using non-invasive samples (urine, stool, saliva, sputum)
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New challenges and uncertainties for more adapted tests in different settings

Clinical scenarios for schistosomiasis test vary widely: For disease control in communities with a very high prevalence, a test with imperfect sensitivity will predictably be associated with a significant number of false negatives, rendering treatment of everybody the most rational approach and implying that individual testing only adds cost without adding benefit and is therefore not required. In contrast, in a low-prevalence situation near the eradication threshold, where infection severity typically is also low, a test with low sensitivity may miss many of those with persistent, but low parasite load, so that the infection cycle is not interrupted and completing eradication becomes infeasible. In this situation, a test with near perfect sensitivity like LAMP would be needed to identify affected individuals, but because most individuals undergoing the test will be negative, such testing may become unaffordable for developing countries. The situation is yet different in travellers from affluent countries, where optimal sensitivity is

desired, and even relatively costly tests are economically feasible.

Despite the impressive advances on the technology front, an integrated approach with practical, adapted, affordable rapid diagnostic tools in combination with suited clinical and epidemiologic testing strategies is still to be defined. Recent clinical observations of lower than expected current cure rates with the mainstay of therapy, praziquantel, particularly when sensitive testing is used for treatment success monitoring, also raise the concern of slow, but progressive drug resistance development. This would add to the challenge for novel tests that not only detect a parasite but also determine its sensitivity to standard drug treatment.

Reliable access to electricity for diagnostic devices, even simple microscopes, is a pervasive problem in developing countries. Fortunately, recent progress in miniaturization, battery technology, low power embedded high performance computing[149] and solar cell technology has led to development of portable, battery driven diagnostic devices for parasitic disease, e.g. portable microscopy [150] and portable molecular diagnostics like LAMP [151] that are suited for use in very low

resource environments. Thus, alternative developing countries and basic healthcare energies have the capability to benefit profoundly.

Table 4: Needs of schistosomiasis tests according to different settings

Schistosoma infection diagnostic methods	Needs
Parasitological	Improved microscopy
Immunoassays	RDTs; Dipsticks
Molecular	Miniaturized LAMP
Emerging	Microfluidic point-of-care devices (mobile phone based)

LAMP = loop mediated isothermal amplification; RDT = rapid diagnostic test.

Conclusion

Current parasitological methods have well-documented weaknesses. They are often too resource-consuming, laborious or expensive for broad use in endemic areas. In addition, they often do not allow distinction between latent versus recent infection. Immunoassays that have long been perceived as replacement or second option because of their high sensitivity, often fail due to their low specificity. However, combination of several test modality may improve reliability of testing, but this involves increased cost and time consumption. Use of molecular techniques offers a real alternative because they offset the weaknesses of the other methods while enhancing their strengths. Nevertheless, they remain laborious and expensive. The LAMP approach to nucleic acid detection appears to be a valuable platform but needs further development in terms of miniaturization, reduction of carryover contamination risk and develop instrumentation that is field-ready. New microfluidic devices have a significant potential for improving diagnosis based on protein and nucleic acid detection. The integration of multiple test modalities into a robust point-of-care device that is easy to handle and fulfills the ASSURED criteria appears technically feasible and when applied to the diagnosis of *Schistosoma* may revolutionize clinical diagnosis and greatly enhance public health efforts in this important disease.

Thus, there remains a significant unmet need for new tests that are highly sensitive, highly specific, adapted to field conditions and allow point-of-care diagnosis, are inexpensive and are suited to contribute either to mass treatment in high prevalence areas, eradication in low-prevalence areas, or individual diagnosis in symptomatic individuals outside such scenarios. The rapid evolution observed in nanotechnologies and microfluidics, as well as in molecular diagnostics, will hopefully render technologic progress also beneficial for the poorest in regions where testing may contribute most to humankind.

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