REVIEW

New diagnostic tools in schistosomiasis

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Abstract

Schistosomiasis is a water-based parasitic disease that affects over 250 million people. Control efforts have long been in vain, which is one reason why schistosomiasis is considered a neglected tropical disease. However, since the new millennium, interventions against schistosomiasis are escalating. The initial impetus stems from a 2001 World Health Assembly resolution, urging member states to scale-up deworming of school-aged children with the anthelminthic drug praziquantel. Because praziquantel is safe, efficacious and inexpensive when delivered through the school platform, diagnosis before drug intervention was deemed unnecessary and not cost-effective. Hence, there was little interest in research and development of novel diagnostic tools. With the recent publication of the World Health Organization (WHO) Roadmap to overcome the impact of neglected tropical diseases in 2020, we have entered a new era. Elimination of schistosomiasis has become the buzzword and this has important ramifications for diagnostic tools. Indeed, measuring progress towards the WHO Roadmap and whether local elimination has been achieved requires highly accurate diagnostic assays. Here, we introduce target product profiles for diagnostic tools that are required for different stages of a schistosomiasis control programme. We provide an update of the latest developments in schistosomiasis diagnosis, including microscopic techniques, rapid diagnostic tests for antigen detection, polymerase chain reaction (PCR) assays and proxy markers for morbidity assessments. Particular emphasis is placed on challenges and solutions for new technologies to enter clinical practice.

Keywords: Antibody detection, circulating antigens, control, diagnosis, elimination, Kato–Katz, polymerase chain reaction, *Schistosoma*, serology, urine filtration

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Introduction

From a global public health perspective, schistosomiasis is the most important water-based disease [1]. Schistosomiasis is a neglected tropical disease (NTD) that is caused by chronic infection with blood flukes of the genus *Schistosoma*. Three species are of particular relevance; *Schistosoma haematobium*, which occurs in Africa and the Arabian Peninsula, *Schistosoma mansoni*, which is endemic in Africa, the Arabian Peninsula, South America and the Caribbean, and *Schistosoma japonicum*, which is restricted to China, the Philippines and Indonesia [2,3]. Humans become infected when they contact freshwater bodies infested with schistosome cercariae that are released from intermediate host snails [3]. Schistosomiasis is a disease of poverty and is associated with a lack of clean water at home, which forces people to contact unprotected natural open freshwater sources where transmission occurs [4–6].

The symptomatology and severity of disease depend on the schistosome species, the total number of blood flukes harboured in a host, length of infection, level of host immunity, age and gender [3,7]. Almost 800 million people were at risk of schistosomiasis in mid-2003 [1]. Estimates for 2010 suggest that over 250 million people were infected and the disease caused

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11 700 deaths and a global burden of 3.3 million disability-adjusted life years [8,9]. Water resources development and management (e.g. large dams and irrigation systems) are key risk factors for the spread and intensification of schistosomiasis [1]. Climate change is likely to exacerbate transmission or extend the disease further north or into higher altitudes [10–15]. High mobility of people and migration bear the risk of (re-)introducing schistosomiasis transmission into areas that had been considered to be non-endemic or that managed to locally eliminate the disease, despite the presence of intermediate host snails [16]. A recent example is infection of people with *S. haematobium* after bathing in the Cavu River in southern Corsica. The life cycle might have been initiated by an infected individual excreting *S. haematobium* eggs into the river and the presence of suitable intermediate host snails (i.e. *Bulinus truncatus*) [17,18]. Hence, there is a risk of acquiring schistosomiasis among travellers and expatriates from non-endemic areas who might occasionally contact contaminated freshwater through leisure bathing or water sport activities [19].

Although travellers and tourists are usually diagnosed with schistosomiasis only upon return to their home country and are managed according to specific guidelines there, the control of schistosomiasis in countries where schistosomiasis is endemic emphasises large-scale administration of praziquantel without prior diagnosis; a strategy phrased 'preventive chemotherapy' (PCT) [20]. Countries that have effectively reduced morbidity due to schistosomiasis are encouraged to move towards elimination of this infection as a public health problem by reducing heavy infection intensities in at-risk populations to below 1% [21]. For this purpose, it is recommended that PCT should be combined with other interventions, such as improved access to clean water, sanitation and hygiene (WASH), information, education and communication (IEC) and snail control [22]. Interventions should go hand-in-hand with effective surveillance–response mechanisms, readily tailored to social–ecological systems [21,23,24].

For individual patient management of returning travellers and for different stages of a schistosomiasis control programme, appropriate diagnostic tools are required, as specified in target product profiles [25–29]. Tools for the rapid identification of high-risk communities at the onset of a control programme (e.g. simple school-based questionnaires) and for an assessment of prevalence and intensity of infection in morbidity control settings (e.g. microscopy) are readily available and recommended by the World Health Organization (WHO) [30]. However, diagnostic assays that are highly sensitive and specific, and hence appropriate for monitoring and surveillance, post-transmission control and verification of elimination have yet to be standardised and recommended for wider use. Here, we review and discuss widely employed tests and new promising assays to diagnose schistosomiasis. Particular emphasis is placed on the challenges for new technologies to enter clinical practice.

Diagnostic tools

For decades, innovation, research and development of drugs and vaccines for schistosomiasis and other NTDs have been neglected. For instance, an analysis of 1393 new chemical entities that were brought to market between 1975 and 1999 revealed that only 16 (1.1%) were for NTDs and tuberculosis [31]. In a more recent systematic assessment covering the period 2000–2011, it was found that among 850 new therapeutic entities, 37 (4.4%) had an indication for NTDs. However, most of them pertained to existing products that have been repurposed [32]. With regard to diagnostics for NTDs, the situation might be even worse. Indeed, innovation has often been stalled because of a general belief that the control and elimination of schistosomiasis and other NTDs could be achieved with the available tools [33].

At the 54th World Health Assembly in May 2001, PCT was endorsed as the global control strategy against schistosomiasis (and soil-transmitted helminthiasis) [34]. Subsequently, WHO presented a vision with a progressive shift from morbidity control to interruption of transmission and finally elimination [35]. However, little attention was paid to the role of diagnostics. This issue had been emphasised in 2009, along with an attempt to highlight different diagnostic tools that can be used for different stages of a schistosomiasis control programme [25]. In 2012, the fight against NTDs was reinforced by the London Declaration and the WHO Roadmap for NTDs. As a consequence, the elimination of schistosomiasis transmission became the declared objective [16,36,37] and the need to develop and apply new, highly sensitive and specific diagnostic tools that are adapted to low-transmission settings was stressed [21,27].

Target product profiles, standard protocols and preferred reporting

Recognising the pivotal role of diagnostics, there is a need for target product profiles that are readily adapted to specific stages of a control programme [26]. Table 1 summarises two target product profiles for diagnostic tools for schistosomiasis at two distinct stages of control: (a) at an early stage when the focus is on mapping (establishing baseline disease prevalence and facilitating spatial targeting of interventions) and impact monitoring (determining prevalence after interventions have commenced); and (b) at a late stage during the post-elimination surveillance (rigorous surveillance after interventions have ceased).

TABLE 1. Key characteristics of target product profiles for diagnostic tools that are readily adapted to two specific stages of a schistosomiasis control programme[a]

Characteristic	Onset of a control programme (mapping and impact monitoring)	Late-stage of a control programme (post-elimination surveillance)
Possible target population	School-aged children	Children who are born after transmission has been interrupted
Possible sample type	Questionnaire, urine or blood spot (if possible, avoid stool)	Urine or blood spot (if possible, avoid stool)
Ideal diagnostic marker	Species-specific antigen	Species-specific antigen or antibody
Ideal test format	Point-of-care rapid diagnostic test	High-throughput laboratory assay
Required performance characteristics	Sensitivity: >50% Specificity: >95%	Sensitivity: >99% Specificity: >99%
Current reference standard	Kato–Katz thick smear (*Schistosoma mansoni* and *Schistosoma japonicum*) Urine filtration (*Schistosoma haematobium*)	Kato–Katz thick smear (*S. mansoni* and *S. japonicum*) Urine filtration (*S. haematobium*)

[a]Table adapted from Solomon et al. [26].

In recent years, efforts have been made to standardise diagnostic protocols and to improve the accuracy and completeness of reporting on diagnostic studies. Of particular note is the STARD initiative ('**STA**ndards for the **R**eporting of **D**iagnostic accuracy studies') that came forward with a 25-item checklist that should be included for reporting diagnostic accuracy studies. The use of a flow diagram detailing the design of the study, methods for recruitment, order of test execution, flow of participants and presentation of decisive and indecisive test results is strongly encouraged [38,39]. Moreover, the validation of existing and new tools for schistosomiasis diagnosis is making progress [40–42], most prominently in multi-country studies [43].

Table 2 summarises a host of methods that are being used for schistosomiasis diagnosis. The tests are listed in the order in which they might be most useful at specific stages of a control programme; from rapid screening of high-risk communities using simple school-based questionnaires to molecular approaches in reference laboratories that might be necessary for post-transmission surveillance.

Tests for rapid assessment, mapping and monitoring

Chronic infection with *S. haematobium* can cause inflammation of the urogenital tract, resulting in ulceration and bleeding into urine [44]. Populations characterised by high *S. haematobium* infection levels can be identified rapidly and at low cost, using simple school-based questionnaires that assess for self-reported blood in urine [45–47]. Visible haematuria declines after antischistosomal treatment, and hence, self-reported haematuria has only limited diagnostic value as schistosomiasis control progresses [48].

Reagent strips for detecting microhaematuria in urine, in contrast, are useful tools for the indirect diagnosis of *S. haematobium* infection also in low-prevalence settings and in populations subjected to PCT [44,48,49]. Reagent strips are therefore suggested as appropriate adjuncts for monitoring urogenital schistosomiasis control programmes [44,48].

For the rapid identification of communities at high risk of intestinal schistosomiasis, questionnaires assessing blood in the stool showed a lower accuracy than self-reported blood in urine for community diagnosis of *S. haematobium* [46]. A commercially available lateral flow immune-chromatographic reagent strip test detecting circulating cathodic antigen (CCA) in urine has been applied in the past as an early indicator of *S. mansoni* infections, particularly in young children, who have yet to become egg- or antibody-patent [50]. Since 2008, a more sophisticated point-of-care (POC) test detecting *Schistosoma* CCA in urine has been developed and is now commercially available and recommended by the authors for *S. mansoni* prevalence mapping. Indeed, this POC-CCA urine cassette test has been shown to accurately detect *S. mansoni* infections in different age groups, countries and evaluations [43,51–55]. The test is also able to diagnose the two Asian schistosome species; *S. japonicum* and *Schistosoma mekongi* [41,56]. The evidence accrued so far suggests that a single POC-CCA cassette test is considerably more sensitive than conventional stool microscopy using Kato–Katz thick smear for *S. mansoni* diagnosis, particularly when infection intensities are low and after anthelminthic treatment [50,54,57,58]. Hence, the POC-CCA cassette test is suggested as an appropriate tool for monitoring schistosomiasis control programmes that progress from morbidity control towards elimination [57,58].

Urine and stool microscopy

S. haematobium eggs excreted in urine can be trapped on polycarbonate filters with a fine pore size of 8–30 μm after 10 mL of a well shaken mid-day urine sample is passed through a syringe [59]. Eggs on the filter are usually stained with Lugol's iodine and counted under a microscope. Infection intensity is expressed as the number of eggs per 10 mL of urine with a threshold of 50 eggs distinguishing between light and heavy infection [34]. Although this method is easy to perform, it lacks sensitivity to detect very light infection intensities and is affected by day-to-day variation in egg output [60–62]. Hence, to accurately detect light infections, multiple filtrations over consecutive days are needed. In a recent proof-of-concept study, urine sample preparations in the field

TABLE 2. Accuracy and applicability of different tests for the diagnosis of *Schistosoma* infections in humans

Method	Unit of diagnosis	Sensitivity	Specificity	Quantification	High throughput	PHCU	Laboratory	Reference laboratory	Commercially available	Cost	Reference(s)
Colour of urine											
Questionnaire	Red urine	x	x	x	xxx	xxx	xxx	xxx	N/A	x	[46]
Visible haematuria	Macrohaematuria	x	x	x	xxx	xxx	xxx	xxx	N/A	x	[46]
Rapid tests											
Reagent strip	Microhaematuria	x	x	xx	xxx	xxx	xxx	xxx	xxx	xx	[44]
POC-CCA	Circulating cathodic antigen	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx	xx	[43]
Microscopy											
Urine filtration	*S. haematobium* eggs	xx	xxx	xxx	xx	N/A	xxx	xxx	N/A	xx	[59]
Direct faecal smear	*S. mansoni* eggs, *S. japonicum* eggs	x	xxx	x	xx	N/A	xxx	xxx	N/A	xx	[64,65]
FECT	*S. mansoni* eggs, *S. japonicum* eggs	x	xxx	x	xx	N/A	xxx	xxx	N/A	xx	[65,67]
Kato-Katz	*S. mansoni* eggs, *S. japonicum* eggs	xx	xxx	xxx	xx	N/A	xxx	xxx	xx	xx	[30,68,69]
FLOTAC	*S. mansoni* eggs, *S. japonicum* eggs	xx	xxx	xxx	x	N/A	xxx	xxx	N/A	xx	[76]
Mini-FLOTAC	*S. mansoni* eggs, *S. japonicum* eggs	xx	xxx	xxx	xx	N/A	xxx	xxx	N/A	xx	[77]
Antibody detection											
ELISA	Anti-*Schistosoma* antibody	xxx	x	x	xxx	N/A	x	xx	N/A	xxx	[61]
IHA	Anti-*Schistosoma* antibody	xxx	x	x	xxx	N/A	x	xx	N/A	xxx	[61]
Antigen detection											
UCP-LF CAA	Circulating anodic antigen	xxx	xxx	xx	xx	N/A	x	xxx	N/A	xxx	[92]
DNA detection											
PCR	*Schistosoma* DNA	xxx	xxx	xx	xx	N/A	x	xx	N/A	xxx	[95]
LAMP	*Schistosoma* DNA	xxx	xxx	xx	xx	N/A	x	xxx	N/A	xxx	[95]

The following grading system was used: x, low; xx, moderate; xxx, high; N/A, not applicable/not available.
ELISA, enzyme-linked immunosorbent assay; FECT, formalin–ether concentration technique; IHA, indirect haemagglutination assay; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; PHCU, primary healthcare unit (without microscope, centrifuge and other technical equipment); POC-CCA, point-of-care circulating cathodic antigen; UCP-LF CAA, up-converting phosphor-lateral flow circulating anodic antigen (urine-based).

were carried out successfully using readily available paper products (e.g. newspaper, school workbook paper and single-ply paper towels) to gravity-filter urine [63].

The direct faecal smear (also known as wet-mount preparation) is routinely used in many laboratories for the detection of intestinal protozoa and helminth infections. Its preparation is straightforward. In brief, approximately 2 mg of fresh stool are added to a drop of saline (or iodine for intestinal protozoa), mixed and directly examined under a microscope [64,65]. Although the simplicity and concurrent detection of multiple parasite species is an advantage and the method has a reasonable sensitivity to detect intestinal protozoa infections, it shows low sensitivity for helminth infections, including *S. mansoni* [66].

The formalin-ether concentration technique (FECT) is often used in combination with the direct faecal smear in hospital laboratories in high-income countries. In brief, the procedure includes four steps: (a) homogenisation of approximately 1.0–1.5 g of stool in formalin; (b) filtration of the stool–formalin suspension through a 400-μm sieve or surgical gauze; (c) addition of ether and centrifugation of the stool–formalin–ether mix; and (d) microscopic examination of the sediment layer [65,67]. Although more sensitive than a direct faecal smear, FECT is characterised by low sensitivity for detecting *S. mansoni* infections [40,66].

The Kato–Katz technique is the standard method to assess the prevalence and intensity of infection with *S. mansoni* (as well as *S. japonicum* and soil-transmitted helminths) in endemic countries [30,68,69]. In brief, fresh stool is pressed through a fine 60- to 105-μm mesh and filled in a plastic or stainless steel template designed to transfer 41.7 mg or 25 mg of stool, respectively, on a microscope slide. The resulting small stool cylinder on the slide is covered with glycerol–methylene blue-soaked cellophane, evenly spread and examined under a microscope. After a clearing time of at least 30 min, but ideally about 24 hours, the number of *S. mansoni* (or *S. japonicum*) eggs in the thick smear can be counted under a microscope and is expressed as the number of eggs per gram of stool (EPG) after multiplication with the appropriate factor (24 or 40, depending on the amount of stool). Of note, Kato–Katz thick smears are usually examined for concurrent soil-transmitted helminth eggs. The EPG values are used as a proxy for infection intensity. The Kato–Katz technique is relatively simple and inexpensive and sufficiently sensitive to detect moderate and heavy *S. mansoni* infections [28]. However, its ability to detect light infections is compromised by its relatively low detection limit (around 20–50 EPG, depending on the templates used). Hence, it might (a) underestimate the true prevalence, particularly in low-prevalence settings; and (b) confound confirmation of cure after treatment [28,57,70]. A latent Markov modelling approach confirmed that the sensitivity of the Kato–Katz method is significantly lower in the weeks post-treatment with praziquantel compared with pre-treatment assessment [71], which is probably due to a reduced worm load, and hence a low egg output after treatment. A commercially available portable light microscope (Newton Nm1-600 XY) showed good diagnostic accuracy for *S. mansoni* detection using Kato–Katz thick smears, whereas a novel mobile phone microscope only resulted in moderate sensitivity [72].

FLOTAC is a technique that has been developed by a veterinary parasitologist for the diagnosis of intestinal parasites in different domestic animal species [73]. Subsequently, FLOTAC has been broadly validated for the diagnosis of human nematodes and trematodes, including *S. mansoni* [40,74–76]. Mini-FLOTAC is a further development of the initial FLOTAC technique [66,77,78]. Of note, the principle of the FLOTAC family is homogenisation of approximately 1 g of stool in formalin, filtration to remove debris, and addition of a flotation solution with a specific gravity to bring helminth eggs into flotation. There are specifically designed FLOTAC devices that enable the flotation of eggs, translation and subsequent visualisation under a microscope. Although the original FLOTAC technique bears the advantage that the complete 1 g of stool can be examined, it requires a large-bucket centrifuge that might not be available in resource-constrained settings. The Mini-FLOTAC is designed to overcome the restriction of centrifugation and still has a detection limit of 10 EPG [66]. First applications in the field showed that it detects *S. mansoni* infections with a higher accuracy than the direct faecal smear, FECT and Kato–Katz thick smear [66,79].

Serological tests

Serological detection of anti-schistosome antibodies in human blood is commonly employed to determine whether or not an individual has been previously exposed to schistosomes. In most cases, antibodies develop within 6–8 weeks after infection and are frequently detectable before *Schistosoma* eggs can be found microscopically in stool or urine samples. However, very early infections may still be missed [7]. At a later stage of infection, antibody detection has a good sensitivity, but is only moderately specific and cannot distinguish between the different *Schistosoma* species [80]. Positive results indicate active or past infection, but fail to readily distinguish between both conditions, because high antibody titres may persist for long periods after schistosomiasis has been successfully treated and cured. Hence, antibody detection is of low diagnostic value for the detection of active infection in highly endemic areas where exposure to schistosomes is common. One exception is the application of serology to detect initial active infection in young children who are not yet egg-patent but might require special care [50,81].

In settings where interruption of schistosomiasis transmission is assumed, serology plays an important role as a confirmatory diagnostic tool to prove that people living in formerly endemic areas are indeed no longer exposed to the infective agent [28]. Moreover, serological tests are a powerful tool for the detection of schistosomiasis in travellers returning from endemic settings (e.g. in Western travel clinics). Indeed, signs and symptoms in individuals with light-intensity infections are unspecific and often absent, so that clinical examination and egg microscopy alone do not reach satisfactory sensitivity. Recent investigations built around the acquisition of *S. haematobium* infections in individuals during travel to Corsica confirmed this observation: serology identified several infections that were missed by urine microscopy [17]. In clinical practice, a positive serology in people from non-endemic countries who have been exposed to freshwater in schistosomiasis-endemic areas is usually sufficient to prescribe treatment with praziquantel [82,83].

Different test methodologies have been developed for the serodiagnosis of schistosomiasis, among which enzyme-linked immunosorbent assay (ELISA) techniques using soluble *S. mansoni* egg antigens and indirect haemagglutination assays (IHAs) with adult *S. mansoni* worm antigen are commonly used [84]. However, although there are a couple of commercially available antibody detection assays, they are not standardised among the companies and users. Most laboratories employ their own assays and/or have their fixed suppliers. Combining assays with different antibody-isotype or antigen combinations may lead to an improved diagnostic accuracy. Cross-reactions between antibodies directed against schistosomes and other trematodes are frequent, which is why some guidelines recommend performing concurrent serological testing for *Fasciola* spp. in case of positive schistosome serology [85].

Although the available serodiagnostic tools use antigen originating from *S. mansoni*, also infections due to the other *Schistosoma* spp. are detected with high sensitivity [86]. Obtaining *S. mansoni* worm and egg antigens from infected animals is a mandatory prerequisite for the development of the most commonly employed serodiagnostic tools. However, this process is rather laborious, expensive and may limit the suitability of these serological tests for use in resource-constrained settings. More recently, efforts have been made to develop antibody detection assays that use *S. mansoni* cercarial antigens—commonly referred to as *S. mansoni* cercarial transformation fluid—which are easier to produce because they can be obtained from infected intermediate host snails. The diagnostic performance is comparable to that of routinely employed serological tests [87]. Indeed, a rapid diagnostic test (RDT) format using *S. mansoni* cercarial transformation fluid antigen has been developed and validated in different settings, reporting high sensitivity, which calls for further investigation [80,88,89].

New diagnostic tools

Antigen detection tests. Novel tools showing a very high diagnostic accuracy, namely monoclonal antibody-based antigen detection techniques, have recently been developed [90,91]. These assays, using an up-converting phosphor-lateral flow (UCP-LF) reporter technology, detect parasite-excreted circulating anodic antigen (CAA) and CCA in serum or urine at very low levels, which are indicative of single worm infections [92]. Using urine as a non-invasive sample methodology, the UCP-LF CAA assay has recently been used to evaluate advanced schistosomiasis japonica screening and control programmes in China, showing evidence that triplicate Kato–Katz thick smears might underestimate the prevalence of active *S. japonicum* infections by a factor of 10 compared with the UCP-LF CAA assay [41]. Applying the UCP-LF CAA test for *S. haematobium* diagnosis in a close-to-elimination setting in Zanzibar, United Republic of Tanzania, showed that the empirical prevalence revealed with the UCP-LF CAA was several-fold higher than the prevalence detected with a single urine filtration [42]. Noteworthy, the IHAs applied as the first-line screening tool in China fell short in about 50% of active cases. The specificity of the UCP-LF CAA assay is warranted by the complete uniqueness of the antigen, by highly specific monoclonal antibody detection, as well as enrichment of the CAA following sample pretreatment by trichloroacetic acid. Experimental infections of mice, rabbits and baboons with *Schistosoma* and post-treatment studies in humans indicate the correlation of serum CAA levels with the number of living adult worms [93,94]. The UCP-LF CAA assay was recently adapted to a dry reagent format that allows convenient storage at ambient temperature and worldwide shipping without the need for a cold chain [91]. The possibility of concentrating larger urine volumes allows the approach using pooled samples for a more cost-effective way of screening specific population groups in close-to-elimination settings [92].

DNA detection tests. For the detection and quantification of *Schistosoma*-specific DNA in clinical samples, a number of molecular techniques and a range of DNA targets have been described, elegantly summarised in a recent review [95]. All studies claim a specificity of virtually 100%, but sensitivities range from equal to, up to substantially higher than traditional microscopy techniques. This range largely reflects the quality and quantity of the microscopy procedures used in the different studies [96–98]. For the detection of DNA in urine, the sensitivity further increases by the use of concentration procedures such as sedimentation or filtration, the latter with

potential application for population-based surveillance in more remote endemic regions [99,100].

An advantage of using molecular diagnosis is the option to detect *Schistosoma* DNA in alternative samples besides faeces and urine, including semen and vaginal lavages, which may facilitate the diagnosis of genital schistosomiasis [101]. Moreover, polymerase chain reaction (PCR) on gynaecological samples seems to be a powerful diagnostic research tool in further exploring the increased susceptibility of *Schistosoma*-infected women to acquiring an human immunodeficiency virus infection [102].

In endemic settings, PCR techniques can also be used to type strains, which allows for the detection of hybrids between human and animal schistosomes. Indeed, recent studies have shown that natural interaction between *S. haematobium* and *Schistosoma bovis* (a bovine *Schistosoma* species) does occur [103]. Zoonotic hybrid schistosomes may develop when humans and livestock are in contact with the same water bodies, and such hybridisation may profoundly alter the parasite's biological characteristics, its transmission patterns, the clinical course of infection and the sensitivity to anti-schistosomal drugs [81,104]. New research is needed using these molecular strain typing methods, as the results may directly impact on current schistosomiasis control efforts.

For the diagnosis of early-stage infections, in particular in travellers, there is a need for ultra-sensitive blood-based diagnostic tests, which can detect *Schistosoma* infection even before serology and microscopy become positive. Detectable plasma and serum levels of *Schistosoma* DNA have been reported during acute human infection [105,106]. However, following the administration of praziquantel, patients stayed positive for many months [105–107]. It needs to be elucidated whether this finding reflects continuous release of parasite DNA from tissue-trapped eggs.

Despite the fact that PCR-based technology is highly specific and sensitive, and despite its potential for high throughput analysis (e.g. for population-based screening), DNA detection tests are hardly used for clinical diagnosis within *Schistosoma*-endemic countries because they require expensive laboratory equipment and highly skilled personnel [108]. An interesting alternative could be the use of loop-mediated isothermal amplification (LAMP) technology, which can be relatively easily adapted to a basic laboratory under resource constraints. So far, LAMP assays have been mainly used to study *Schistosoma* infection in animal models or for the monitoring of *Schistosoma*-infected snails [109].

Tests for morbidity assessment

While chronic inflammation of the affected organs occurs in any type of schistosomiasis, symptoms are often unspecific and associated morbidity may not easily come to clinical attention. Hence, in addition to the parasitological diagnosis of a *Schistosoma* infection, there is a need for comprehensive clinical assessments to identify even subtle signs of disease. Although quantification of haematuria reliably indicates morbidity due to urogenital schistosomiasis, the identification of simple, field-applicable proxy markers for intestinal schistosomiasis is more challenging. Recently, two faecal POC assays have been shown to be strongly associated with heavy *S. mansoni* infection, namely (a) faecal occult blood tests and (b) faecal calprotectin (a calcium-binding protein of human neutrophils) [110]. It follows that these markers may be used to reveal bowel morbidity caused by intestinal schistosomiasis.

In clinical settings, ultrasound imaging of affected organs (*S. haematobium*: urinary tract; *S. mansoni*: liver and spleen) is a well established, widely accepted and accurate parameter to assess morbidity due to chronic *Schistosoma* infection [111].

Challenges for new technologies to enter clinical practice

Diagnostic tests are central for adequate clinical patient management of *Schistosoma*-infected individuals and for successful control programmes aiming at schistosomiasis elimination. Despite the significant progress made in the field of diagnostic test development, several barriers and obstacles exist that may delay the widespread use of new diagnostic assays in clinical practice.

Potential of new diagnostic technologies to enter clinical practice

In Western travel clinics and hospital settings where schistosomiasis is encountered as an imported disease and infections are usually of light intensity, early diagnosis and excellent diagnostic accuracy are important test priorities. PCR techniques provide a very high sensitivity and a rapid turn-around time, but are technically demanding, require laboratories equipped for molecular examinations, well-trained laboratory technicians and are rather expensive so that these assays will likely be limited to specialised laboratories. However, PCR examinations for *Schistosoma* detection are increasingly available in national reference laboratories, allowing their use not only for research purposes, but also in clinical practice as a diagnostic tool to guide individual patient management. In standard microbiology laboratories, however, microscopy using a concentration technique currently remains the cornerstone to prove active schistosomiasis. Given the low sensitivity of microscopy for egg detection and the widely declining expertise regarding microscopic parasite recognition outside endemic

areas, the addition of a sensitive RDT such as the POC-CCA for *S. mansoni* holds promise to improve detection rates for schistosomiasis with minimal turn-around times. Such RDTs have been previously developed for detection of *Giardia intestinalis* and *Cryptosporidium* spp. and have now become integral parts of diagnostic testing algorithms in many clinical laboratories [112].

In resource-constrained settings where the highest disease burden is concentrated, most patients present to district hospitals or rural health dispensaries where no or only limited diagnostic laboratory infrastructure is available. In these settings, referral to better equipped institutions is the exception. The availability of RDTs, which require only minimal infrastructure, such as POC-CCA urine cassette test for the detection of intestinal schistosomiasis, would much improve diagnosis and at the same time surveillance in peripheral settings [28]. If stool/urine microscopy is feasible in local laboratories in endemic areas, direct faecal smears and Kato–Katz thick smears may be combined with concentration methods such as FECT or Mini-FLOTAC for an improved diagnosis of schistosomiasis and potential parasitic co-infections. The Mini-FLOTAC is particularly promising because its technical procedure does not require constant power supply. Both UCP-LF CAA and PCR are highly sensitive, and allow for relatively high throughput of samples. One trained technician can manage several dozen UCP-LF CAA or PCR assays per day, provided there are sufficient centrifugation capacities, and a UCP-Quant reader and a PCR thermal cycler, respectively, are available. Such a throughput capacity would allow the use in large screening programmes for near-elimination surveillance, as well as the use as reference assays if modestly equipped centralised laboratories are available [108,113].

Current obstacles to successful implementation of new diagnostics

The development of new diagnostic tests for schistosomiasis has experienced similar challenges and barriers as previously described for other NTDs, e.g. lack of funding and limited commercial interest by private companies to invest in development of tests that mainly target poor populations in low-income countries [114]. Moreover, as long as morbidity control by PCT is the declared objective, diagnostics will remain neglected because, economically, the costs of testing will outweigh the costs of treatment. However, the drive towards schistosomiasis elimination calls for developing novel diagnostic tools with a high sensitivity and high specificity [16,21]. Reliably identifying individuals with light-intensity infections that might still contribute to the maintenance of transmission will be crucial to achieve elimination. A test-and-treat strategy in close-to-elimination settings or for post-elimination surveillance might be more cost-effective, less drug- and resource-wasting, and ethically more appropriate than the large-scale distribution of drugs to mostly uninfected people.

Although considerable funding to develop and standardise highly sensitive and specific diagnostic techniques is still needed, significant investments by the public sector and innovative product development partnerships have been established over the past years [115]. The POC-CCA for diagnosing schistosomiasis is a sensitive assay that is now commercially available and can also be deployed in endemic areas without any laboratory infrastructure. This RDT fulfils several of the 'ASSURED criteria' that were elaborated to describe the characteristics of an ideal diagnostic test in resource-constrained settings [114]. The acronym ASSURED specifies the following requirements that should be met by a test: A = affordable by the affected individuals; S = sensitive; S = specific; U = user-friendly; R = rapid turn-around time and robust performance (e.g. reagents tolerate tropical climate); E = equipment-free; and D = delivered to those in need. While the POC-CCA test meets most of these criteria, the current price (US$ 1.00–1.66 per test, depending on amount ordered) may prevent its use in endemic settings, particularly in peripheral healthcare centres where the test would be of greatest value.

In high-income countries where the individual test cost would be of lesser importance, high investments are still required to obtain official approval by the competent regulatory authorities, namely the Medical Device Safety Service in the European Union and the Food and Drug Administration in the USA [116]. Test implementation at a larger scale is less likely to occur without such approval. In addition, there is currently only one single company from which the POC-CCA is commercially available (Rapid Medical Diagnostics; Cape Town, South Africa).

Standardisation and appropriate handling of available tests are crucial to obtain accurate, reliable test results. Indeed, the diagnostic performance of malaria RDTs under operational field conditions has often been reported to be much lower than initially expected [117], which underscores the need for adequate training of the handling personnel. While this observation is true for all test formats, it is of particular relevance for PCR-based diagnostics that are technically demanding and should only be employed by well-trained personnel, including rigorous quality control in each PCR run. To date, PCR diagnosis of schistosomiasis is based on in-house assays as no PCR kits for helminth detection are commercially available. Hence, cross-laboratory comparability is difficult and the method is restricted to a few reference laboratories. For a wider application of PCR-based methods in developed countries, it would be desirable to further stimulate combined testing of *Schistosoma* spp. with other helminths into stool-based multiplex PCR assays [118].

Shortcomings of new diagnostic tests

Among the tests that are used for the diagnosis of schistosomiasis, only the urine filtration, Kato–Katz, FLOTAC and Mini-FLOTAC allow the quantification of eggs in a given amount of urine or stool, which provides information about the intensity of infection and is a proxy for the number of worms harboured and the morbidity experienced by the human host. The quantification of egg excretion also helps to assess the transmission potential of populations living in endemic areas and guides recommendations for the frequency of PCT interventions in the frame of morbidity control initiatives [20]. Moreover, quantitative diagnostic methods are used to determine the efficacy of praziquantel treatment in terms of egg reduction rates. The colour intensity of POC-CCA tests, the amount of CAA measured with the UCP-LF CAA and the amount of DNA measured with PCR are reported to be correlated to the number of eggs excreted and can give a certain estimate regarding the intensity of infection [42,51,119]. In addition, both CAA and CCA levels in serum and urine are reported to correlate with worm numbers in experimental animals [120,121]. However, a standardisation and clear indication is still missing. It is widely assumed that antibodies remain detectable after successful clearance of infection and that antibody tests are therefore not applicable to assess treatment outcomes. Also, DNA does not seem to clear for several months after praziquantel administration; whether this is because of continuous release of parasite DNA from eggs that are trapped in the host or because of surviving adult worms remains to be determined [105–107]. CAA and CCA are produced by living worms and have been shown to clear within a few days or weeks after successful treatment [55,58,122]. The POC-CCA and UCP-LF CAA tests are therefore deemed suitable techniques for assessing drug efficacy in terms of cure rates.

A drawback for most diagnostic methods except stool microscopy and stool-based multiplex PCR assays is that only a single pathogen species can be determined and that infections with multiple intestinal parasites cannot be differentiated. Ideal diagnostic approaches would allow the concurrent detection of several pathogens in one biological sample (e.g. urine or stool).

Outlook: new diagnostic tests in the next 5 years

Schistosomiasis control programmes

As intensities and the prevalence of infection decrease in many areas of the world, it will become more and more important for schistosomiasis control and elimination programmes to adopt the most sensitive (for detection of very light infection intensities) and highly specific tests (to minimise the number of false-positive samples in areas approaching elimination of schistosomiasis). In parasitological laboratories where microscopy is readily available, it will be important to evaluate new techniques such as Mini-FLOTAC in comparison to standard techniques (Kato–Katz, FECT) under operational field conditions to assess their applicability and diagnostic accuracy in settings of light infection intensity. However, microscopy alone—unless performed on multiple samples—is unlikely to be sensitive enough and will need to be supplemented by tests detecting antibodies, antigen and/or DNA, particularly in areas where elimination of schistosomiasis is the declared objective. The further development of new RDTs holds promise to overcome shortcomings of currently available rapid tests. At present, a urine-based RDT able to detect CAA is anticipated for development and, once commercialised, it might be used for rapid and exact mapping of urogenital schistosomiasis. Serological tests may also play a role to prove interruption of transmission and would be most suitable if available in an RDT format [123]. As shown by examples from Japan and China, where schistosomiasis elimination has been achieved or is in sight, respectively, elimination can only be attained with a rigorous adaption of diagnostic tests to the current stage of control, thereby reliably identifying also the last transmission pockets [124,125].

Individual patient management

In peripheral healthcare centres, the use of POC-CCA and, perhaps, additional RDTs could be highly beneficial for improved patient management. Indeed, the non-invasive test procedure, the short turn-around time (results available within less than 30 min) and the high sensitivity of the urine-based POC-CCA may also attract interest in Western travel clinics to employ this RDT as part of diagnostic algorithms.

PCR is available in reference laboratories in high-income countries and its application for individual patient management (e.g. in travel clinics) is likely to increase. If integrated into commercially available multiplex PCR assays, the use of molecular helminth diagnostics may steadily rise across microbiology laboratories in Europe, North America and elsewhere. However, the technical limitations as well as the significant costs associated with the technical equipment, maintenance, quality control and training of personnel will prevent its wider application in most endemic areas of low- and middle-income countries, at least for the foreseeable future.

Concluding remarks

After many years of general neglect, schistosomiasis and other NTDs have come into sharper focus with real prospects for escalating control and an ambitious roadmap towards

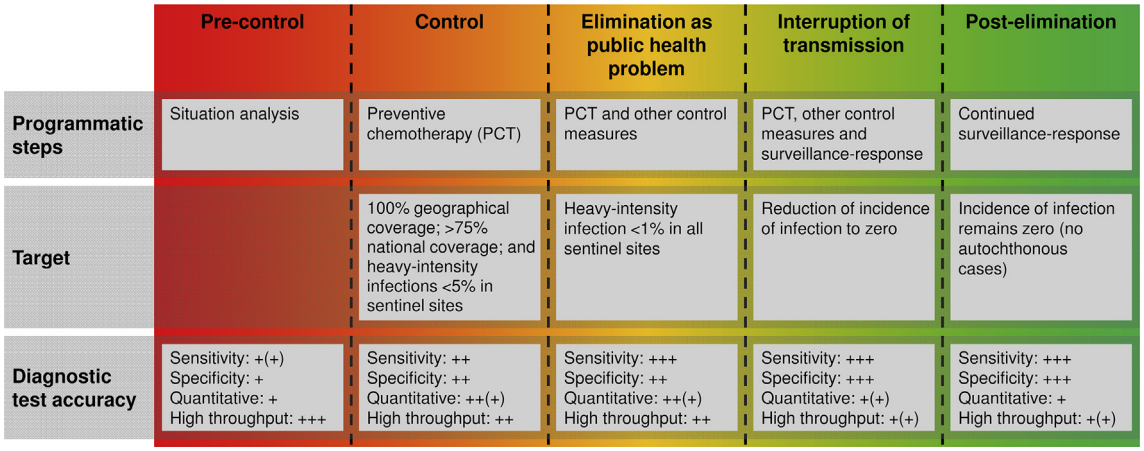

FIG. 1. Different stages of a schistosomiasis control programme (from pre-control to post-elimination), highlighting stage-specific programmatic steps, target and diagnostic test accuracy. (Note: the following grading system was used: +, low; ++, moderate; +++, high).

elimination in 2020. Only in recent years has the pivotal role of diagnostics been acknowledged, particularly with regard to diagnostic tools that are fit for specific stages of a control programme. In the current review, we highlight programmatic steps, targets and diagnostic characteristics for the main stages of a schistosomiasis control programme (Fig. 1), covering the entire cycle from the pre-control stage (reconnaissance of the extent of the programme, rapid mapping) to the post-elimination phase (continued surveillance after verification of transmission interruption). The current arsenal and characteristics of diagnostic tools for schistosomiasis are summarised (Table 2) and allow juxtaposing with the various stages of control. In view of growing efforts to integrate the control and elimination of multiple NTDs, there is a pressing need to develop, standardise and validate novel diagnostic platforms that allow for mapping, monitoring and surveillance of multiple NTDs. At the onset of control, rapid, inexpensive, high throughput tools are required, gradually replaced by POC assays and finally ultra-sensitive tools that might be operated in reference laboratories. Clearly, elimination can only be achieved if highly sensitive and specific diagnostic techniques become available for people exposed to infection and remaining transmission foci.

Transparency declaration

The authors declare that they have no conflicts of interest.

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