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Advances in the Diagnosis of Human Schistosomiasis

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SUMMARY

Schistosomiasis is a major neglected tropical disease that afflicts more than 240 million people, including many children and young adults, in the tropics and subtropics. The disease is characterized by chronic infections with significant residual morbidity and is of considerable public health importance, with substantial socioeconomic impacts on impoverished communities. Morbidity reduction and eventual elimination through integrated intervention measures are the focuses of current schistosomiasis control programs. Precise diagnosis of schistosome infections, in both mammalian and snail intermediate hosts, will play a pivotal role in achieving these goals. Nevertheless, despite extensive efforts over several decades, the search for sensitive and specific diagnostics for schistosomiasis is ongoing. Here we review the area, paying attention to earlier approaches but emphasizing recent developments in the search for new diagnostics for schistosomiasis with practical applications in the research laboratory, the clinic, and the field. Careful and rigorous validation of these assays and their cost-effectiveness will be needed, however, prior to their adoption in support of policy decisions for national public health programs aimed at the control and elimination of schistosomiasis.

INTRODUCTION

Schistosomiasis (also called bilharzia) is a major intravascular infection that has serious public health consequences, with significant socioeconomic impacts, in the developing world (1–4). It is one of the most prevalent, though neglected, of the tropical infectious diseases. More than 240 million people in 78 countries are infected, and close to 800 million are at risk (5). Schistosomiasis is caused by trematode parasites of the genus *Schistosoma*, of which three major species—*Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*—cause severe disease in humans (6, 7). *S. mansoni* and *S. japonicum* are responsible for intestinal schistosomiasis,

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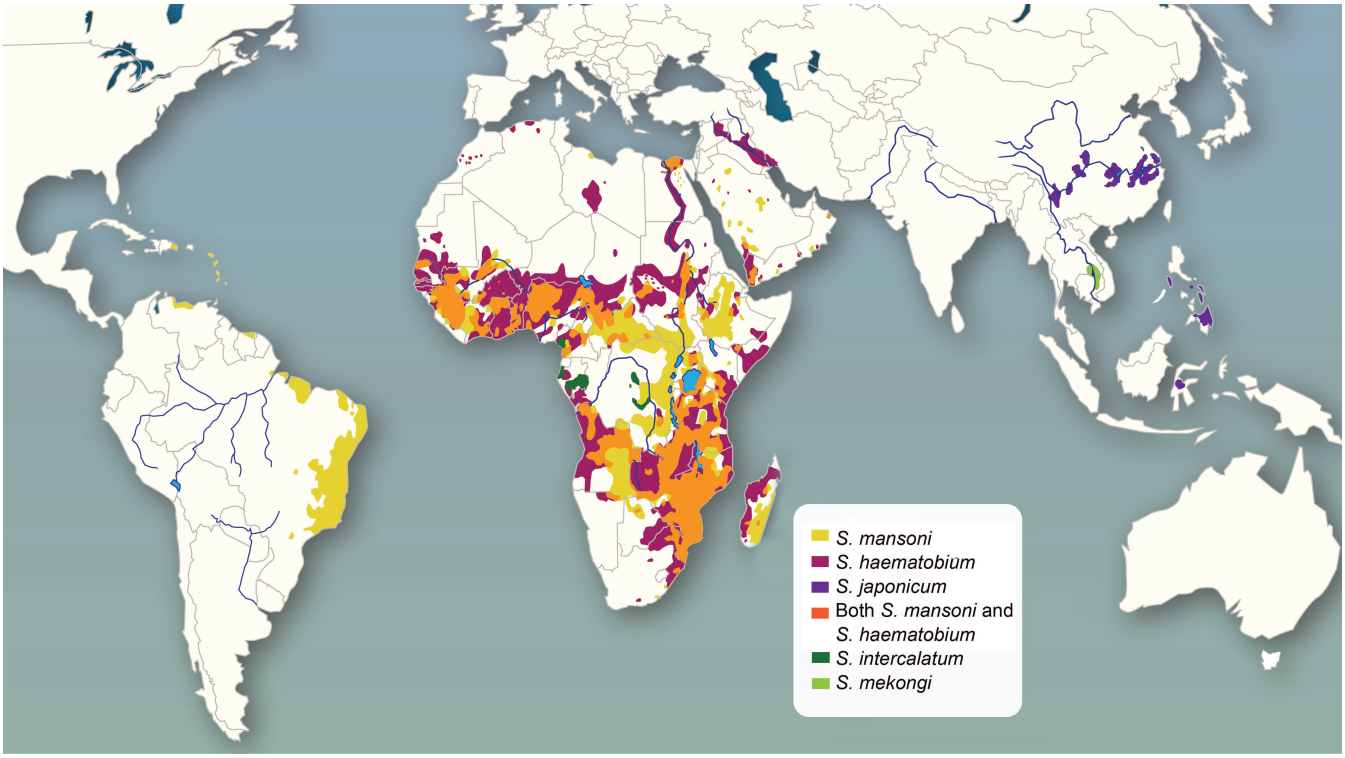

FIG 1 Global distribution of schistosomiasis. (Adapted from reference 8 with permission from Elsevier.)

while *S. haematobium* causes urinary schistosomiasis. *S. japonicum* is distributed in the People's Republic of China, Indonesia, and the Philippines, whereas *S. mansoni* has a wider spread involving Africa, the Middle East, South America, and the West Indies (8, 9). *S. haematobium* has a distribution similar to that of *S. mansoni* but does not occur in South America or in the West Indies (Fig. 1). In addition, *S. mekongi* and *S. intercalatum* are two species with local importance, causing intestinal schistosomiasis in the Mekong River basin of Southeast Asia and in Middle and West Africa, respectively (8).

As a disease of poverty and limited sanitary facilities, schistosomiasis has proved difficult to control for centuries (5–7). Disease burden assessments for schistosomiasis, based on the extent of end organ damage and the associated morbidities related to malnutrition and chronic inflammation, indicate that the annual number of disability-adjusted life years (DALYs) lost is around 70 million (10). The number of DALYs lost is almost equal to that of HIV infection and may exceed that of malaria or tuberculosis (10–12). Moreover, in Africa, around 300,000 deaths due to schistosomiasis are reported annually (12, 13).

Parasite Life Cycle

The schistosome life cycle is maintained in a mammalian definitive host and a freshwater snail intermediate host (Fig. 2). Humans acquire the infection following direct contact with water sources containing infectious cercariae. The fork-tailed larvae penetrate mammalian skin and enter the circulation via the capillaries and lymphatics. During penetration, they transform into schistosomula and migrate in the blood circulation. They are then carried around and throughout the body by blood flow for several days before becoming trapped in the hepatic portal vein leading to the liver. During this course of migration, they are found in the lungs in large numbers, as they are temporarily held up in capillaries of the lungs (14). Within the portal system, the male and female worms sexually mature and pair up, after which they migrate to mesenteric and vesical venous plexuses depending on the species: *S. japonicum* to the inferior mesenteric vein, *S. mansoni* to the superior mesenteric vein, and *S. haematobium* to the pelvic venous plexus. Oviposition takes place around 4 to 6 weeks postinfection in *S. mansoni* and *S. japonicum* and around 90 days in *S. haematobium*. The eggs penetrate the vasculature walls and enter either the bladder or intestinal lumen to be shed in urine (urinary schistosomiasis) or stool (intestinal schistosomiasis). The eggs hatch in freshwater sources and release free-swimming miracidia, which then infect a specific freshwater snail intermediate host—for example, with *S. mansoni*, this is generally *Biomphalaria pfeifferi* (Africa) or *Biomphalaria glabrata* (the Americas). Within the snail, the miracidia transform into sporocysts, and after two rounds of asexual reproduction, free-swimming cercariae are released after about 30 days. The cercariae continue the life cycle by penetrating the skin of the definitive mammalian host. Whereas *S. haematobium* and *S. mansoni*, in general, only infect humans, *S. japonicum* infects humans and more than 40 species of mammalian reservoir hosts (15, 16).

Pathogenesis, Clinical Manifestations, and Treatment

Each of the schistosome species gives rise to different disease spectra of various pathologies and severities. Cercarial skin penetration causes dermatitis with maculopapular eruptions (17). Generally the disease status can be classified as acute, chronic, or advanced schistosomiasis (18). Acute disease, or Katayama syndrome, occurs as a result of the host immune responses to migrat-

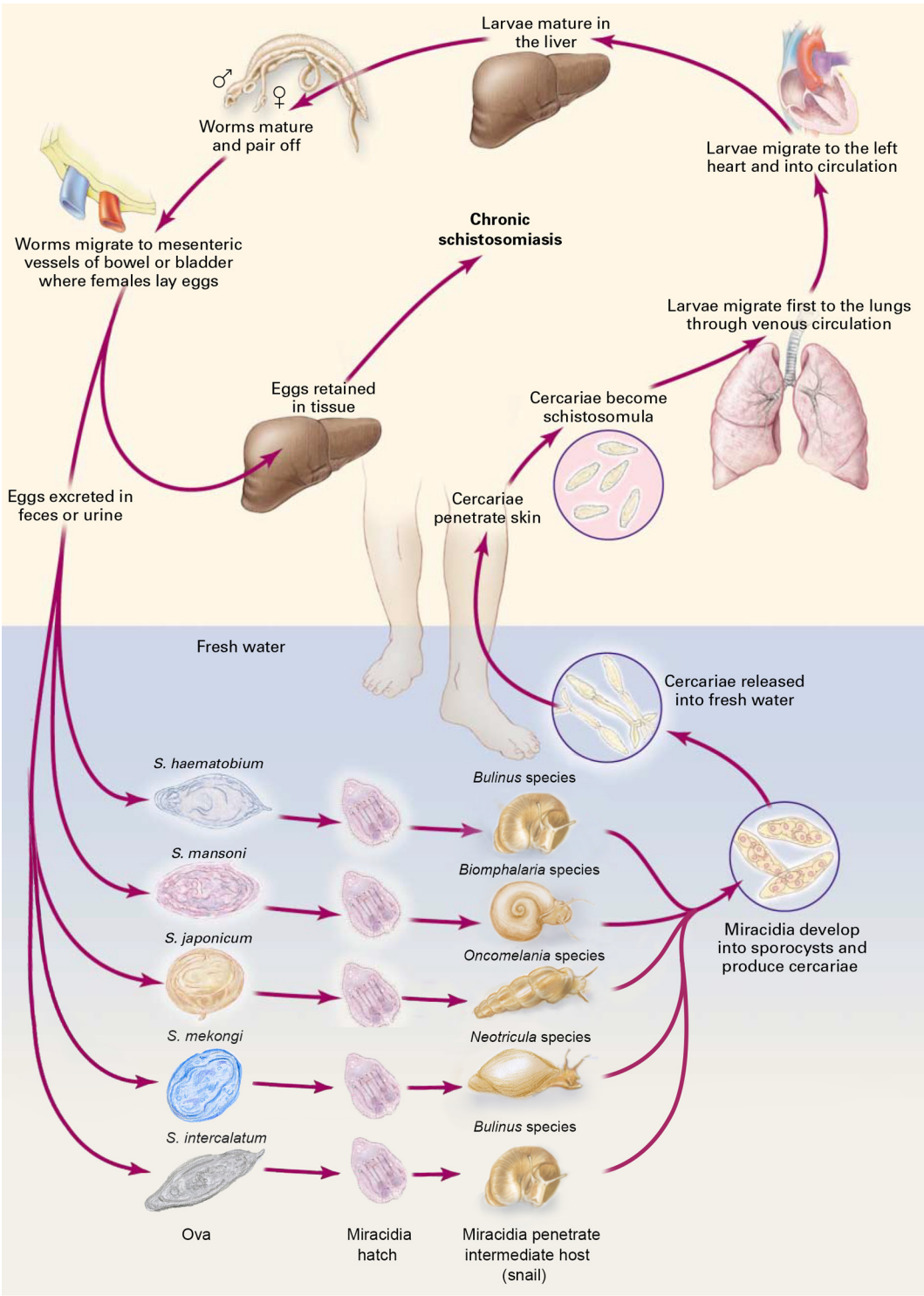

FIG 2 Life cycle of human schistosomes. (Adapted from reference 16 with permission. Copyright 2002 Massachusetts Medical Society.)

ing schistosomula, worm maturation, egg production, and the release of egg antigens (19). This phase is usually asymptomatic in individuals from areas of endemicity, but in people infected for the first time, such as immunologically naive travelers, symptoms include fever, headache, malaise, abdominal pain, and eosinophilia (20).

The chronic phase of the infection is mainly due to the granulomatous inflammatory reaction against the schistosome eggs deposited in different organs and tissues. In intestinal schistosomiasis, egg deposition occurs mainly in the liver and the intestinal wall and can lead to multiple-granuloma formation and tissue lesions in these organs. This causes intestinal mucosal hyperplasia,

polyposis, ulceration, and abscess formation, which manifest clinically mainly as abdominal pain, chronic diarrhea, and perrectal bleeding (21). Granuloma formation caused by egg deposition in the liver results in a periportal fibrosis extending to advanced disease, with portal hypertension and hepatosplenomegaly. Ascites and variceal bleeding are two serious and common complications at this stage, which can result in the death of the patient (18).

The chronic phase of urinary schistosomiasis occurs following egg deposition and granuloma formation, mainly in the urinary bladder wall, resulting in abnormalities in the mucosa (22). The disease manifests with lower urinary tract symptoms, such as hematuria, frequency, and dysuria. Further complications include bladder calcification, urinary tract fibrosis causing obstructive uropathy, and bladder malignancies (23). *S. haematobium* is a class 1 carcinogen and has also been shown to increase the risk of sexually transmitted infections, including HIV infection, especially in female genital schistosomiasis hematobia (24, 25). Female genital schistosomiasis affects the entire genital tract, manifesting as pain, contact bleeding, and infertility (26). Infection with *S. haematobium* can occasionally cause hepatic complications as well.

Schistosome egg deposition can occur in any ectopic site, giving rise to site-specific clinical manifestations, such as neuroschistosomiasis, which occurs following egg deposition in the central nervous system (CNS) (27). In addition to these specific morbidities, schistosomiasis is associated with debilitating generalized conditions, such as malnutrition, anemia, growth retardation, and impaired development in childhood (10).

Age-specific prevalences and intensities of schistosome infections are generally positively skewed, with the characteristic convex curve having a peak in adolescence (28, 29). This pattern is predominant for *S. haematobium*, while it is least seen with *S. japonicum*. The maximum prevalence and intensity of *S. haematobium* infections occur in children aged 10 to 14 years, while for *S. mansoni* infection, the corresponding age is 10 to 24 years. Schistosome prevalence and intensity gradually reduce with age for both these infections. Moreover, changes in *S. haematobium* infection prevalence and intensity at ages following adolescence are steeper than those for *S. mansoni*. However, this characteristic pattern is not clearly seen for *S. japonicum* infections. These differences in the pattern of reduction and prevalence of infection with increasing age can be attributed to multiple factors, such as differences in exposure to the parasites, the development of immune mechanisms against the infection, a lessening of exposure to contaminated water with increasing age, snail distribution patterns, and, in regard to *S. japonicum* infection, involvement of the animal reservoir hosts, all of which play important roles in transmission (28–31).

Altogether, the spectrum of clinical manifestations and complications in schistosomiasis mainly depends on the intensity of the infection and the magnitude of the host immune response. Once the disease is at an advanced stage, significant morbidity with lifelong disabilities or severe complications resulting in death can occur.

Currently, praziquantel (PZQ), a heterocyclic prazinoisoquinoline derivative, is used for the treatment of acute infection as well as for mass drug administration to at-risk populations for the control of schistosomiasis. The drug acts directly on adult worms, paralyzing them by damaging the tegument. Hence, PZQ treatment is aimed at controlling schistosome egg production by destroying the adult worms so that the resulting morbidity, including the associated complications and mortality, are minimized. Nevertheless, the treatment is not helpful in reversing complications associated with tissue fibrosis. Since PZQ is not effective against the immature, early schistosome stages, other treatment alternatives have to be considered. The use of artemisinin derivatives, such as artemether, is one such option which has the potential to kill the immature stages (8, 32, 33).

Overview of Schistosomiasis Diagnostics

Schistosomiasis can be cured without progressing to complications if there are an accurate diagnosis and prompt treatment and killing of worm stages during the initial stages of infection. Also, with prompt treatment, morbidity can be reversed during the early stages of chronic infection. Hence, the use of appropriate, sensitive diagnostic tools to identify infected individuals is imperative. Also, the application of sensitive and specific diagnosis would be extremely helpful in implementing strategies for the control and elimination of schistosomiasis. As enunciated in the recent World Health Organization (WHO) road map to overcome the global impact of neglected tropical diseases, including schistosomiasis, there will be an emphasis to provide regular treatment for at least 75% of children in need by 2020, with the eventual aim of disease elimination (34). The development and implementation of control strategies, including the provision of accurate diagnostic tests, to achieve these goals require careful consideration of available resources, financial undertakings, and requisite administrative and political support, particularly as successful elimination will require the application of complex interventions and rigorous monitoring measures. Different countries will need to set up their own national plans of action in this regard (7, 35, 36). It is a sobering thought that despite extensive efforts, as indicated earlier, the global disease burden of schistosomiasis still remains unacceptably high. This persistence of the disease despite massive and integrated control programs over the last few decades (35, 37, 38) may be due in part to the lack of accurate diagnostic tools for case detection and community screening in areas where schistosomiasis is endemic.

Clinical assessment of a particular disease generally comprises two approaches—diagnostic and screening tests. A diagnostic test is used to determine the presence or absence of a disease when a subject shows signs or symptoms of the disease. A screening test is used to identify asymptomatic individuals who may have the disease, such as in community-based screening. Usually, diagnostic tests are performed after a positive screening test to establish a definitive diagnosis, and also in clinical settings where patients present directly with clinical manifestations. Diagnostic accuracy or the validity of a test reflects its ability to discriminate between a particular disease condition and the healthy status of an individual. Hence, the most important factor in determining the value of a test is the measurement of its diagnostic accuracy or discriminative capability. This is usually measured by means of different indicators, such as sensitivity, specificity, likelihood ratios, and predictive values. However, each of these criteria for measuring the diagnostic accuracy of a test has to be determined carefully, as the application of these criteria may be different from situation to situation, especially in regard to the prevailing disease prevalence and the characteristics of the population for which the test is evaluated, and also in relation to the design of the survey in which the diagnostic test is applied (39, 40).

Among the indicators mentioned above, sensitivity and specificity are two important measures for determining the accuracy of a clinical test, and their calculation requires comparison with a suitable gold standard test. The sensitivity of a test is the ability of the test to correctly identify patients with a particular condition (the true positive fraction), whereas specificity is the ability of the test to correctly identify individuals who do not have the condition (the true negative fraction) (41). With regard to schistosomiasis, especially in areas with low transmission and low prevalence, where the aim of intervention is the elimination of the disease, the test that is used for case detection needs to have a high sensitivity rather than a high specificity. This is the same in the case of the assessment of cure rates following treatment. However, in the case of schistosomiasis, for which there is no accurate gold standard test, other approaches are required, such as the use of sequential or simultaneous multiple tests and the consideration of related epidemiological information (42, 43). Usually, for the diagnosis of a patient presenting in a clinical setting, a test with the highest possible combination of accuracy measures is important. However, when it comes to community-based evaluation in control programs, in addition to the accuracy of the test, it is imperative to consider other features, such as the time needed to perform the test and the expertise and training required for the test procedures, including costs, while maintaining adequate quality control (42).

It is imperative to develop more effective approaches for the prevention, control, and elimination of schistosomiasis. Morbidity reduction and parasite elimination are the two main pillars of current control programs (44). Effective diagnosis plays a key role in control strategies, with wide applications in case detection in areas with a high prevalence as well as those with a low prevalence, where the main aims are elimination of infection, evaluation of disease intensity, and assessment of therapeutic responses as well as the overall effectiveness of the interventions employed. Diagnostics with high specificity and sensitivity are required to promote transmission interruption leading to control and elimination. All these aspects need to be addressed separately, and appropriate diagnostic tools play a pivotal role in proper identification and monitoring of the issues involved. Diagnostic tests with low sensitivity may miss infected individuals who thus remain undiagnosed but continue to contribute substantially to disease transmission, thereby hindering the efficiency of control efforts. As high schistosome worm burdens are not always related to significant morbidity, missing those individuals shedding small numbers of eggs may mean that they will experience significant disease later in life. Current diagnostic tools for schistosomiasis have practical limitations despite their technical improvements. For example, the application of novel techniques in community and clinical settings will be limited unless the tests are inexpensive and field deployable, as schistosomiasis is generally endemic in resource-poor settings. Therefore, it is imperative to develop diagnostic tools that are capable of addressing these issues appropriately. Furthermore, it is important to consider that none of the diagnostic tests that are being used currently provide 100% accuracy. Whereas the sensitivities of tests that are commonly used in epidemiological surveys can be increased by the use of multiple tests and different sampling techniques (45–48), these procedures are not compatible with routine applications, as they are not cost-effective and are impractical. Also, the application of statistical modeling to optimize the sensitivity and specificity of diagnostic tests has been used in the absence of a perfect gold standard for comparison (48, 49).

In considering the costs of diagnosis, parasitological methods mainly involve labor costs, as reagents, especially, and equipment are relatively inexpensive. However, the requirement for each test to be repeated for a higher diagnostic accuracy does raise the costs involved. Laboratory-based serological and molecular diagnostic tests are substantially more expensive due to the costs of processing samples, the purchase and maintenance of requisite instruments, the provision of expensive reagents, and the expenditure associated with training staff in the relevant procedures. In addition, field-applicable, rapid immunodiagnostic tests, though having minimal requirements for instruments and labor, can involve significant production costs and hence can be expensive. Thus, the cost of undertaking a diagnostic test varies substantially depending on the number of repeat assays needed and the costs of specific reagents, instruments, and sample processing required (50, 51). Hence, the ideal diagnostic test should be cost-effective in terms of labor, sample processing, equipment, and reagents, and it should provide an accurate diagnosis by use of an assay ideally performed once.

An assortment of diagnostic techniques have been developed for detection of schistosomiasis over the past few decades, ranging from basic microscopic detection to sophisticated molecular approaches. The current diagnostic strategies can be grouped into the following four main categories: (i) direct parasitological diagnosis; (ii) immunological diagnosis; (iii) DNA and RNA detection; and (iv) use of cytokines, metabolites, and other schistosome molecules as biomarkers. In this review, we mainly discuss the diagnosis of schistosome infection in humans, but we have also considered the detection of parasite stages contaminating environmental sources, such as waterways, lakes, rivers, and streams, and the identification of snail infections, two aspects that are important for monitoring the elimination of schistosomiasis from a community.

DIRECT PARASITOLOGICAL DIAGNOSIS

Parasitological methods were the earliest diagnostic procedures developed and include the detection of eggs in stool samples, in the case of intestinal schistosomiasis, or in urine, for urinary schistosomiasis. Since oviposition (egg laying) in intestinal and urinary schistosomes commences around 4 to 6 weeks and 90 days postcercarial infection, respectively, none of the available direct egg detection tests are appropriate for early diagnosis of the disease prior to the parasite becoming patent, by which time serious symptoms can arise (52). The microscopy-based Kato-Katz (KK) thick stool smear technique, developed in 1972 (53), is still used widely and is the standard method recommended by the WHO for the diagnosis of intestinal schistosomiasis and for the quantitative assessment of infection intensity (7). The KK procedure has a high level of specificity, is simple, is less laborious than many other procedures, is inexpensive, and can readily be used under field conditions.

Nevertheless, despite these attributes, the KK test has several major drawbacks. When infection intensity is high, with a large worm load in the host, KK sensitivity is similarly high, since there is significant egg output. However, the sensitivity of the test is compromised for subjects with low-intensity infections and in areas with a low prevalence (54–56). Furthermore, there is daily variation in schistosome egg excretion, and the overdispersal of

egg output (57–59) results in high day-to-day variability in KK test results, especially for low-intensity infections (60) or after PZQ treatment (61). Also, there is the possibility that eggs clumping together in stool samples can lead to a significant variability in egg counts, resulting in underreporting of infection prevalence and/or infection intensity. Accordingly, the precise disease burden in a community can be grossly underestimated with the KK procedure, and this can also lead to misinterpretation in the assessment of therapeutic outcomes (59, 62–64). Furthermore, these limitations can also lead to inaccuracies in determining the efficacy of other diagnostic techniques, as the KK test is still regarded as the gold standard or reference diagnostic method for schistosomiasis (7, 46). The sensitivity of the test can be improved by using multiple stool samples over several consecutive days, but this can compromise its simplicity and cost-effectiveness, and this modified approach can suffer from low compliance due to the reluctance of individuals to provide more than one stool sample for examination (57). While modifications to the KK test to improve the test efficacy have been suggested (65, 66), the inherent drawbacks have yet to be resolved.

Other parasitological procedures, including formol-ether sedimentation, salt flotation and centrifugation, and the interaction of magnetic microspheres with eggs, have been developed to improve the microscopic examination of stool samples (67–71). The miracidium hatching test, based on the positive phototrophic behavior of schistosome miracidia, has also proved useful for diagnosis (72). These techniques have improved the diagnostic sensitivity (73), but the general disadvantages associated with the direct visualization of eggs by microscopy generally prevail. It is also notable that these modifications are laborious and are generally not optimal for routine or large-scale screening.

Urine filtration and concentration of *S. haematobium* eggs, with subsequent microscopic examination, form the main parasitological approach used in the diagnosis of urinary schistosomiasis. Due to its simplicity, syringe filtration is preferred in community and school surveys and requires less equipment than urine concentration with centrifugation. Similar to stool smear testing for fecal eggs, these tests are also prone to low sensitivity (74), although the eggs can generally be identified readily, as a relatively large volume of urine is used and there is an absence of the disturbing solid materials found in stool (42). Indeed, KK and urine egg detection techniques are particularly compatible in environments with high schistosome transmission levels, in light of their simplicity and cost-effectiveness. Also, these tests require little specialized training, and they can be used in large-scale population surveys.

In addition to the direct detection of eggs, changes in urinalysis provide important clues to the diagnosis of urinary schistosomiasis. Hematuria and proteinuria have been shown to clearly be associated with urinary schistosomiasis. These indicators, including macro- and microhematuria, have been applied conveniently in community studies by using rapid-detection reagent strips in combination with demographic information obtained using questionnaires (75–77). Moreover, urine heme dipsticks have proved useful for monitoring the impact of PZQ treatment on *S. haematobium* in African communities and have been suggested as the recommended method for monitoring mass treatment programs for this schistosome species (78).

A novel approach for the direct detection of schistosome eggs is by computer-directed visual identification using minimicroscopes constructed from inexpensive imaging devices, such as mobile phones or Web cams, with samples placed directly on the image sensor (79). The image analysis can be done locally or using a distally located computer for the interpretation of results, and these methods widen the possibilities of using copro-microscopic diagnostics in resource-poor settings.

The direct detection of schistosome parasite stages can be used in the clinical setting through organ biopsies and novel imaging techniques (80, 81). These tests are useful in the clinical management of patients, especially in situations where the clinical evidence may be suggestive of schistosomiasis but not confirmed by other diagnostic tests, such as the KK test (18). Such procedures are very sensitive and are extremely helpful in complicated case management, where a prompt diagnosis is critical (18, 82).

IMMUNOLOGICAL DIAGNOSIS

Immunological diagnostics include tests that detect antischistosomal antibodies or circulating schistosomal antigens in plasma, serum, urine, or sputum. An array of tests have already been formulated for the diagnosis of schistosomiasis, while others are currently being developed using advanced technologies (Table 1).

The immune response in schistosome infection progresses in different phases correlating with the time of infection. A T helper 1 (Th1)-like response is predominant at the initial phase of the infection, when migration of the immature parasitic stages occurs. With schistosome maturation, a strong Th2-like response predominates, regulated mainly by egg antigens, while the Th1 response decreases. The Th2 response then gradually declines with the formation of granulomas around the eggs. Later modulation of the granulomatous response occurs with the onset of T regulatory cell and B cell activation and antibody production (83–85). Different IgG isotypes are released predominantly during the acute phase of schistosomiasis, and IgA is produced mainly at the chronic granuloma formation phase. IgG production peaks around 20 weeks after infection, while IgM levels peak at 12 to 16 weeks postinfection (86–88). It has been shown, using a soluble egg antigen (SEA)-based enzyme-linked immunosorbent assay (ELISA) (described later), that serum IgG levels are significantly higher in chronic than acute *S. japonicum* infection, while IgM, IgA, and IgE levels are higher in the acute infection (89). However, intrinsic problems in schistosomiasis immunodiagnosis, such as the difficulty in accurately differentiating between active infection, past infection, and reinfection, remain unresolved. Also, there are inherent specificity problems due to antibody cross-reactivity with different species of schistosomes, including reactivity with cercarial antigens from animal schistosomes, and with other helminth parasites (89–91).

Immunological methods are particularly useful in cases where parasitological tests are negative for individuals with light infections (92). The development of immunological diagnosis has paved the way for less laborious rapid tests that are useful both in communities in areas of endemicity and at point-of-care facilities. With the reduction in infection prevalence and intensity following community PZQ treatment, direct parasitological techniques often fail to detect cases accurately, which leads to false-negative results. In such situations, indirect immunodiagnostic tests are favored because of their ease of performance and high sensitivity. These serology-based assays are especially useful for disease surveillance and for preliminary screening in communities with low infection rates. However, the targeted antibodies circulating in

TABLE 1 Parasite-derived biomolecules and their application in the immunological diagnosis of human schistosomiasis

Antigenic target (life cycle stage)	Diagnostic approach	Parasite	Application of the test with human samples	Reference(s)
Soluble egg antigens	ELISA—antibody detection in serum	*S. mansoni*	Yes	127–129
	ELISA—detection of specific IgG4 reactivity in serum		Yes	140
	ELISA—antibody detection in saliva	*S. mansoni*	Yes	340
		S. japonicum	Yes	341
	ELISA—circulating antigen detection in serum by using IgY isolated from egg yolk	*S. japonicum*	Yes	192
	Detection of antibodies by magnetic affinity enzyme-linked immunoassay (MEIA), using serum	*S. japonicum*	Yes	150
	Detection of antibodies by dot immunogold filtration assay (DIGFA), using serum		Yes	159
	Detection of antibodies by dipstick with latex immunochromatographic assay (DLIA), using serum		Yes	163
	Detection of antibodies by colloidal dye immunofiltration assay (CDIFA), using serum		Yes	162
	Detection of antibodies by dipstick dye immunoassay (DDIA), using serum		Yes	164
	Detection of antibodies by up-converting phosphor reporter (UCP-LF) rapid diagnostic kit, using serum	*S. mansoni*	No	165
Cercarial antigens	ELISA—antibody detection in serum	*S. mansoni*	Yes	127, 128
	Detection of antibodies by UCP-LF rapid diagnostic kit, using serum		No	165
Adult worm antigens	ELISA—antibody detection in serum	*S. mansoni*	Yes	127, 128
	ELISA—antibody detection in urine	*S. mansoni*	Yes	342
		S. haematobium	Yes	342
	Detection of antibodies by silver-enhanced colloidal gold metallo-immunoassay, using serum	*S. japonicum*	No	160
Adult worm microsomal antigen	ELISA—antibody detection in serum	*S. mansoni*	Yes	132
		S. japonicum	Yes	104
	Detection of antibodies by Falcon assay screening test (FAST) ELISA, using serum	*S. mansoni*	Yes	137
		S. haematobium	Yes	138
	Detection of antibodies in serum by FAST ELISA and immunoelectrotransfer blot (EITB)	*S. haematobium*	Yes	96
Components of soluble egg antigens	ELISA—specific antibody detection in serum	*S. japonicum*	Yes	139
Cationic fraction 6 antigen CEF-6 (egg)		*S. mansoni*	Yes	130, 131, 136
200-kDa tegumental protein Sm200 (worm/tegument)			Yes	141
Tegument antigen SmTeg (schistosomulum/tegument)	ELISA—antigen detection in serum/urine		Yes	146
Cathepsin B (Sm31)	ELISA—antigen detection in serum	*S. mansoni*	Yes	281
Secretory protein rSP13 (schistosomulum, adult worm, and egg)		*S. japonicum*	Yes	158
Glyceraldehyde-3-phosphate dehydrogenase (SjGAPDH) (adult worm)			No	271
Leucine amino peptidase (rSjLAP) (adult worm)			Yes	270
Fructose bisphosphate aldolase (rSjFBPA) (adult worm)			Yes	270
Tadem repeat protein Sj7TR (egg/schistosomulum)			Yes	273, 343
Thioredoxin peroxidase 1 (SjTPx-1) (adult worm/tegument)			Yes	343
Stress-induced phosphoprotein 1 (STIP1) (adult worm/tegument)			No	275
Cyclophilin A protein (SjCypA) (schistosomulum/subtegument)			No	277

(Continued on following page)

TABLE 1 (Continued)

Antigenic target (life cycle stage)	Diagnostic approach	Parasite	Application of the test with human samples	Reference(s)
Sj13 antigen (cercaria, adult worm, egg)	ELISA—specific antibody detection in saliva		Yes	274
SjP40 (cercaria, adult worm, egg)	ELISA—detection of SjP40 antigen/anti-SjP40 specific antibodies in serum		No	157
Hydrophilic domain of the 23-kDa membrane protein (Sj23HD) (schistosomulum/adult worm)	Detection of specific antibodies in serum by ELISA and immunoblotting		No	276
26-kDa glutathione *S*-transferase (rSj26GST)	Detection of specific antibodies in serum by MEIA		Yes	149
Sj14-3-3 protein (egg/adult worm)			Yes	151
Cercarial transformation fluid (SmCTF)	Detection of specific antibodies in serum by using a rapid diagnostic test in an ELISA format	*S. mansoni* *S. haematobium*	Yes	147
Recombinant calcium binding protein SjE16 (with SEA) (egg)	Detection of specific antibodies in serum by electrochemical immunosensor array (ECISA)	*S. japonicum*	Yes	166
Circulating cathodic antigen (adult worm)	ELISA—antigen detection in serum/urine	*S. mansoni*	Yes	172, 174, 175
	Detection of antigen in urine by rapid lateral flow cassette assay (POC-CCA)		Yes	173, 177, 183–185
Circulating anodic antigen (adult worm)	ELISA—antigen detection in serum/urine	*S. mansoni*	Yes	174, 175
	Detection of antigen by UCP-LF rapid diagnostic kit, using serum	*S. mansoni*	Yes	193, 194
		S. japonicum	Yes	188
	Detection of antigen by UCP-LF rapid diagnostic kit, using urine	*S. japonicum*	Yes	185, 188
SjCHGC06971 SjCHGC04754 BUD31 homolog RNase (adult worm)	Potential markers for direct antigen detection or specific antibody detection	*S. japonicum*	No	191

blood tend to remain for a considerable period even in the absence of infection (37, 46, 93). Intradermal tests, antigen detection tests, and antibody detection tests, including the circumoval precipitin test (COPT), indirect hemagglutination test (IHT), indirect immunofluorescence assay (IFA), and ELISA, are examples of methods that have been used for the diagnosis of schistosomiasis.

Intradermal Tests

Intradermal tests for skin reactions following injection of egg antigens, larval antigens, or adult worm antigens (AWA) were some of the earliest immunodiagnostic methods used for the diagnosis of schistosomiasis. The tests were simple and cost-effective and were largely used in prevalence studies in the early days (94, 95). However, it was subsequently shown that the tests remain positive for years following successful treatment, producing a high rate of false-positive results, and as such, these approaches are generally not used (45).

Antibody Detection

Antibodies developed against different schistosome life cycle stages can be detected in human plasma/serum by using a number of different techniques. Antibody detection has been used successfully for the diagnosis of the three main human schistosome species and is especially important for detecting infections in areas with low rates of endemicity, where patients have low egg burdens (45, 96–99). The majority of the antibody detection tests used in the diagnosis of schistosomiasis utilize *S. mansoni* antigens. Immunodiagnostic tests incorporating *S. mansoni* antigens have been applied for the diagnosis of *S. haematobium* and *S. japonicum* infections, with *S. mansoni* cercarial transformation fluid (SmCTF) being one such preparation used (100–102). However, the use of *S. mansoni* antigens in the diagnosis of *S. japonicum* infection can result in low sensitivity (98). Species-specific diagnosis of schistosomiasis has been achieved using immunoblotting procedures with an adult worm microsomal extract and other schistosomal antigens (98, 104, 105). Many of the antibody detection tests have been validated in large-scale field trials, some mediated by the WHO (102, 106, 107). We describe here the different methods used for antibody detection for the diagnosis of schistosomiasis.

COPT and CHR. The production of a precipitate after exposure of patient serum to lyophilized schistosome eggs is the basis of the COPT (99). The test has a high sensitivity and specificity (108), and it has been used for diagnosis, mainly for *S. japonicum* infection in China, and as a reference for comparison with other procedures (109, 110). It has also been combined with copro-microscopy to improve diagnostic accuracy (92, 111). Major drawbacks associated with the COPT are its labor-intensiveness, its rather complex and lengthy procedure, and the long gap in seroconversion following treatment, which can result in the misdiagnosis of a cleared infection (45, 92). This becomes important in the evaluation of schistosomiasis control programs involving mass drug administration with PZQ. The failure of antibody levels to wane slowly after PZQ treatment could be due in part to the drug affording only a partial cure (112). The cercarien Hüllen reaction (CHR) test is positive after live cercariae are mixed with patient

serum, there is precipitate formation on their surfaces, and the larvae become visibly immobilized. This test has seen limited application in the past and is currently very sparingly used, as it has disadvantages similar to those of the COPT (113, 114).

IHT. The IHT detects reactivity between antibodies in the serum of an infected individual and schistosomal antigen-coated red blood cells. Due to its simplicity and relatively high sensitivity, this test has been used in large-scale community surveys (115, 116) and as a surveillance tool in areas where schistosomes are endemic (117, 118). Moreover, a combination of a Bayesian statistical approach with the IHT has been used successfully for prevalence estimates of *S. japonicum* infection (43). Yet several weaknesses, including the fact that the test remains positive several years after successful treatment of active worm infections and the problem of antibody cross-reactivity with other trematode infections, resulting in reduced specificity (54, 115, 116, 119), restrict its wider use.

IFA. The reaction between parasite antigens and antischistosomal antibodies in patient serum or other bodily fluids is the concept behind the IFA, which is performed using adult worm paraffin sections, cercariae, and eggs (120–122). IFA detection of IgM and IgG antibodies in the acute and chronic stages of schistosomiasis has been used as a sensitive method of diagnosis, especially in low-prevalence areas (90, 123, 125), in some instances combined with other diagnostic procedures to increase the sensitivity and specificity (111, 126). Some limitations of the test, including the need for relatively expensive microscopes and reagents, with appropriate laboratory facilities and technical expertise, hinder its application in community surveys (123, 125).

ELISA. Reactivity between antibodies in patient serum and extracted antigens from different life cycle stages of schistosomes can be tested by ELISA; SEA, larval antigens, and AWA have been used effectively in this assay (100, 127–129). An *S. mansoni* cercarial antigen preparation (SmCTF) was successfully evaluated in an indirect ELISA for detection of antischistosomal antibodies in human sera (100). However, since the use of crude whole-parasite extracts increases the antibody cross-reactivity with other helminthic infections and leads to reduced specificity, purified antigens have been used to improve performance. Examples of purified antigens include the cationic fraction 6 (CEF-6) of eggs (130, 131) and adult microsomal antigens (132), which have increased test specificity (133–135); CEF-6 has also proved useful for monitoring the efficacy of PZQ therapy (136). One practical drawback of ELISA is the time required to perform the test. However, the introduction of the modified Falcon assay screening test (FAST) ELISA, which has a sensitivity and specificity similar to those of conventional ELISA, but with reduced time requirements, has been helpful in large-scale community screening initiatives (137, 138). The fraction antigen ELISA (FA-ELISA) is another modified ELISA method, which uses fractionated SEA and provides a high sensitivity and specificity in case detection, as well as providing effective assessment of the efficacy of therapy for *S. japonicum* infection (139). It has also been shown that IgG4 reactivity to SEA in ELISA can be used as an immune biomarker for the monitoring of infection with *S. mansoni* in areas of endemicity (140). Recently, the *S. mansoni* 200-kDa tegumental protein (Sm200) was used in an ELISA for the diagnosis of *S. mansoni* infection in a murine model, exhibiting more than 90% sensitivity and specificity, denoting it as a promising reagent for diagnosis (141).

In addition, antibody-based methods for the diagnosis of *S. mekongi* and *S. intercalatum* infections have been attempted using heterologous *Schistosoma* antibody detection assays, as specific serology-based assays are unavailable for both parasites. *S. mekongi* infection was diagnosed using a non-species-specific serological method, an SEA-based ELISA, but which *Schistosoma* spp. caused infection could not be determined (142). *S. mansoni* adult worm and soluble egg antigens were also recently used successfully in the diagnosis of *S. mekongi* infection (143). Adult *Schistosoma* worm membrane extracts have been applied in immunoblots for detecting antibodies against *S. intercalatum*, and an alkaline phosphatase immune capture assay (APIA) has also been developed for diagnosing this infection in patients (144, 145).

An ELISA detecting specific IgG against schistosomula tegument antigens (ELISA-SmTeg) has shown an improved performance compared to that for an assay detecting antibody against AWA, particularly in diagnosing early-stage acute *S. mansoni* infections among individuals in a travel group from an area in Brazil where the disease is not endemic, who were infected in a new focus of *S. mansoni* (146). Furthermore, the SmCTF-ELISA was later applied in an effective lateral flow device that was used as a point-of-care rapid diagnostic test (RDT). This SmCTF-RDT test was at least as sensitive as duplicate KK tests and a single urine filtration test for detection of *S. mansoni* and *S. haematobium*, respectively, and could potentially be a better alternative than the urine circulating cathodic antigen (CCA) test (described below) in the diagnosis of schistosomiasis in areas of endemicity (147, 148).

A magnetic affinity enzyme-linked immunoassay (MEIA), which is more rapid and simple than ELISA, has been used for the diagnosis of *S. japonicum*. Recombinant *S. japonicum* 26-kDa glutathione *S*-transferase (rSj26GST) (149), SEA (150) and Sj14-3-3 protein (151) have been used in the MEIA, and this test has proven more specific than ELISA; with its apparent reliability, MEIA could be used as an alternative to ELISA for the surveillance and monitoring of schistosomiasis in areas of low endemicity.

The pros and cons of antibody detection tests. There are drawbacks common to most of the available antibody detection tests for schistosomiasis. Despite the fact that combined approaches have been successful in diagnostic screening, whereby individuals are initially tested for the presence of antischistosomal antibodies and then those with positive results confirmed by copro-microscopy techniques (45), the approach can be logistically demanding and time-consuming. Furthermore, as antischistosomal antibodies tend to remain for several years, it is difficult to differentiate between an active infection and previous exposure to an infection that has been cleared (46). In addition, a study in an area of China with low schistosomiasis transmission showed that some *S. japonicum* egg-positive individuals had low levels of antischistosomal antibodies determined by commonly used immunodiagnostic kits, indicating the likelihood of underestimating the true prevalence of infection by serodiagnosis alone (152). Due to the limitations associated with antibody detection, misdiagnosis of active disease is often a problem, and the therapeutic response following PZQ treatment cannot be accurately assessed. Furthermore, the higher variability in the diagnostic threshold for antibodies lowers the likelihood of accurate diagnosis, and moreover, infection intensity cannot be determined by antibody detection tests (46). The intensity of infection is an important parameter in monitoring the effectiveness of control programs, in assessing epidemiological associations, and as an indicator of morbidity (153–155).

In many antibody detection assays, the antigen used is a crude parasite extract of multiple components. Consequently, high rates of cross-reactivity with other trematodes and soil-transmitted helminths occur, leading to a lower test reproducibility and reduced specificity (54, 119). Ideally, antibody detection should be performed using a specific, purified schistosome component or a schistosome-derived recombinant protein as the immunodiagnostic target (156, 157). The identification of putative secreted proteins of *S. japonicum* (SjSPs) and the subsequent development of the rSP13-ELISA are thus major advances in that this test can detect low-intensity infections (158). Additionally, sequence alignments of the *SjSP-13* gene with the genome sequences of other helminths found no homologs, which likely eliminates the possibility of cross-reactive antibodies generated by other parasites. Furthermore, antibodies generated against SjSP-13 are short-lived and decline quickly following treatment, indicating the potential of the rSP13-ELISA tool for detecting current *S. japonicum* infections and evaluating the effectiveness of drug therapy.

Other antibody detection approaches have been developed to address some of the practical limitations associated with community surveillance and point-of-care facilities. These include rapid diagnostic tests for accurate detection of antischistosomal antibodies, including a dipstick with latex immunochromatographic assay (DLIA), a dipstick dye immunoassay (DDIA), a dot immunogold filtration assay (DIGFA), and a colloidal dye immunofiltration assay (CDIFA) (159–162). A DLIA using SEA of *S. japonicum* has been shown to exhibit a high sensitivity, and cross-reactivity with other helminthic infections was minimal, making the test highly specific (163). The DDIA has also been shown to be an efficient, cost-effective, and rapid test and has been used for large-scale community screening in areas of low endemicity, with a high sensitivity and specificity (45, 164). Nevertheless, all these tests have failed to overcome the general issues associated with the entire repertoire of antibody-based tests, including the general inability to differentiate between current and past infections, especially in areas of low endemicity.

Lateral flow-based assays and up-converting phosphor reporters (UCP-LF) are now being introduced as rapid diagnostic tests for antischistosomal antibody detection; these kits allow the simultaneous detection of multiple targets on a single strip and hence facilitate the detection of different classes of antibodies specific to a particular infection (165). This cumulative information is important in making inferences about the presence of an infection or coinfection, as well as the stage of the infection. Furthermore, a novel electrochemical immunosensor array (ECISA) using a recombinant *S. japonicum* calcium binding protein (SjE16) and SEA as antigens to detect anti-*S. japonicum* antibodies in serum exhibited 100% sensitivity, with minimal cross-reactions evident. This rapid test is another promising tool for application in large-scale community screening in areas where schistosomiasis is endemic (166).

Antigen Detection

The detection of schistosomal antigens (from schistosomula, adult worms, or eggs) in blood, urine, or sputum is now a proven and highly effective method of diagnosis. Commonly detected antigens are AWA, SEA, and circulating antigens. Two major components that have been targeted are circulating cathodic antigen (CCA) and circulating anodic antigen (CAA). CAA and CCA are so named because of their characteristic biochemical migratory patterns in immunoelectrophoresis, and they are commonly incorporated into antigen capture immunoassays (96). These genus-specific proteoglycan antigens of the schistosomal gut epithelium are released in the vomitus of worms. CCA- and CAA-based tests can be used to evaluate active worm burdens as well as the therapeutic response (167–170). Both antigens can be demonstrated in blood at around 3 weeks postinfection (168, 171). CAA and CCA are also excreted in host urine and can be detected by use of different types of ELISA with serum and urine samples (172, 173); similar sensitivities are generally obtained with the detection of CAA in serum and CCA in urine. Hence, either can be used in areas where schistosomiasis is highly endemic, and both can be used in support of each other in areas of low endemicity (174–176).

CCA detection in urine has been developed as a rapid lateral flow cassette assay to diagnose intestinal schistosomiasis caused by *S. mansoni* (173, 177). The CCA test has some limitations in detecting *S. haematobium* infection but is effective in areas where only *S. mansoni* infections occur (178–180). The poor accuracy of the CCA test for diagnosis of urinary schistosomiasis means that the test may be unsuitable for rapid mapping of schistosomiasis in areas where *S. mansoni* and *S. haematobium* are coendemic (181). However, a technically improved CCA strip test has proved more successful in diagnosing urinary schistosomiasis (182). Nevertheless, despite its field applicability, the use of this antigen strip test in national control programs may be restricted due to its current cost. This point-of-care urine CCA assay has been used in community studies to estimate the prevalence of schistosomiasis (177) and for successful assessments of PZQ treatment (177, 183). Overall, compared to the KK test, this assay is a convenient and efficient method for screening and mapping schistosomiasis cases in communities with medium to high levels of endemicity (177, 183–185). However, day-to-day fluctuations in urine CCA dipstick test results have been observed, which has led to the suggestion that more than one urine sample collected on different days may be required for more accurate diagnosis. Also, it has been shown that the sensitivity of the CCA assay for diagnosing *S. mansoni* infection is reduced in areas of low endemicity and that CCA positivity is correlated strongly with infection intensity (178, 184, 186). Nevertheless, a recent study of patients from different areas in Uganda where *S. mansoni* is endemic reported that a single urine CCA assay had increased diagnostic accuracy compared to multiple KK tests and that the test sensitivity correlated with infection prevalence (187). Moreover, another recent study showed that low-intensity *S. mansoni* infections with negative KK results could be detected with the urine CCA test, highlighting the potential applicability of this test in control programs (64). Antigen detection tests have been applied for acute schistosomiasis case detection, but they are currently not used routinely in the clinical diagnosis of schistosomiasis (18, 188–190).

In regard to *S. japonicum*, four circulating antigens were identified in the sera of infected patients following their direct immune precipitation with an anti-AWA IgY antibody prepared in Hy-Line hens (191). These four proteins, SjCHGC06971, SjCHGC04754, BUD31 homolog, and RNase, share sequence identity with *S. mansoni* homologues. However, they have yet to be confirmed by a proteomic analysis of *S. mansoni* or *S. japonicum* adults or eggs and thus need further evaluation before they can be considered reliable diagnostic markers. Similarly, a sand-

TABLE 2 Schistosome-specific DNA and RNA targets and their application in the molecular diagnosis of human schistosomiasis

Parasite	Genetic target amplified (DNA/RNA)	Amplification technique	Type(s) of samples used for testing	Reference(s)
S. mansoni	121-bp tandem repeat sequence	Conventional PCR	Serum	202, 344
			Feces	201, 202, 204
			Urine	223, 224
		Touchdown PCR	Serum	225
		Real-time PCR	Serum	228, 345, 346
			Cerebrospinal fluid	27
		PCR-ELISA	Feces	208
	28S rDNA	Conventional PCR	Urine	203, 347
			Feces	204
	Cytochrome oxidase 1 (*cox1*)	Multiplex real-time PCR assay	Feces	197
	NADH dehydrogenase 1 (*nad1*)	Real-time PCR	Feces	55
	nad5	Multiplex PCR	Feces	206
	Specific regions between *nad6* and *cox2*	Multiplex PCR	Feces	206
	Mitochondrial minisatellite DNA sequence (620 bp)	LAMP	Feces	236
S. haematobium	DraI repeats	Conventional PCR	Urine	205, 223
		Real-time PCR	Serum/urine	199
	cox1	Conventional PCR	Serum/urine/saliva/semen	222
		Real-time PCR	Vaginal lavage fluid	348
		Multiplex real-time PCR	Feces	197
	Internal transcribed spacer rDNA (ITS)	Conventional PCR	Urine	203
	ITS 2	Real-time PCR	Urine	198, 210
S. japonicum	*SjR2*	Conventional PCR	Feces	216
			Serum/feces	158, 226
		Nested PCR	Serum	220
		LAMP	Feces/serum	52, 232
	SjCHGCS 19	Nested PCR	Serum	229
	28S rDNA	Conventional PCR	Urine	203
	cox1	Conventional PCR	Serum/urine/saliva	221
	cox2	Real-time PCR	Feces	200
	Specific regions between *nad6* and *cox2*	Multiplex PCR	Feces	206
	Specific regions between *nad1* and *nad2*	Multiplex PCR	Feces	206
	nad1	Conventional PCR	Feces	207
		Real-time PCR		196, 218
	nad6	Real-time PCR	Feces	200
	miR-3479, miR-10, miR-3096, miR-0001, miR-277, Bantam	Real-time PCR	Plasma/serum	245, 246
	miR223	Real-time PCR	Serum	249

wich ELISA incorporating chicken egg yolk IgY and other circulating *S. japonicum* antigens has also been described as having a high sensitivity and specificity in the diagnosis of both acute and chronic schistosomiases (192), and it may prove valuable for case detection.

Some recent advances in the detection of circulating schistosomal antigen have involved UCP-LF (165, 193, 194), as described above. Detection of an active, single-species worm infection by using different clinical samples in a UCP-LF CAA assay has been shown to be possible, although further technical improvements will be needed to make it convenient and field applicable (165). A CAA assay of this type was recently used in the diagnosis of *S. japonicum* infection in an area of low endemicity in China, using urine samples (188); the assay exhibited a higher sensitivity than that of the KK technique and detected a significant number of cases that were egg negative. Moreover, a recent proof-of-concept study showed promising results in the diagnosis of *S. japonicum* and *S. mekongi* with CCA and CAA tests using small volumes of banked, frozen urine samples; larger-scale community application should now be undertaken to further evaluate the accuracy of these tests (185).

In addition, newly established monoclonal antibody-based diagnostic assays for detection of *S. mansoni*, involving immunomagnetic separation and fluorescence microscopy, have been developed and have a high sensitivity and specificity for application in low-prevalence areas, but the need for specialized laboratory facilities would likely limit their wider use in field-based surveys (195).

DNA AND RNA DETECTION-BASED METHODS

The detection of schistosome DNA or RNA by conventional or more advanced PCR-based techniques (e.g., real-time quantitative PCR [qPCR] or multiplex PCR) is a promising adjunct to parasitological and serological diagnostic tests for accurate schistosomiasis diagnosis. Recent advances include the detection of egg DNA, circulating cell-free parasite DNA (CFPD), and circulating microRNAs (miRNAs) (Table 2).

TABLE 3 Summary of recent studies on amplification of cell-free parasitic DNA for the diagnosis of schistosomiasis

Parasite	Gene amplified	Type(s) of samples used for testing	Subjects	Reference(s)
S. mansoni	121-bp tandem repeat sequence	Serum/plasma	Animal model	149, 155,259, 260
			Humans	18, 132, 149, 158, 261
		Cerebrospinal fluid	Humans	27
	28S rDNA region	Urine	Animal model	347
			Humans	203
S. japonicum	Retrotransposon SjCHGCS 19 gene	Serum	Animal model/humans	229
	Retrotransposon SjR2 gene	Serum	Animal model	226
			Humans	158
	Cytochrome oxidase 1 (COX1) gene	Serum/urine/saliva	Humans	221
	28S rDNA region	Urine	Humans	203
S. haematobium	DraI gene	Serum/urine	Humans	199
	Cytochrome oxidase 1 (COX1) gene	Serum/urine/saliva/semen	Humans	222
	ITS rDNA region	Urine	Humans	203

Detection of *Schistosoma* DNA by PCR

The detection of schistosome DNA in host stool, urine, or organ biopsy samples has been performed using different PCR techniques (196–199). Conventional PCR amplifies a specific target gene segment and is a very specific method for the direct detection of schistosome DNA in fecal samples (200, 201). Conventional PCR is also more sensitive than microscopic egg detection, particularly for low-intensity infections (201–204). Sensitive detection of *S. haematobium*-specific DNA in urine samples has been successful with urine sediments on filter papers (205). In addition to the detection of nuclear DNA, mitochondrial gene segment amplification has been shown to provide both a high level of sensitivity, due to the availability of numerous copies within a single cell, and pronounced species specificity (55, 206), a feature important in differentiating between different schistosome species (197, 202). Improvements in conventional PCR in combination with other techniques, such as restriction fragment length polymorphism (PCR-RFLP) analysis (207) and PCR-ELISA (208), have also been applied for the detection of schistosome infections.

Real-time PCR (qPCR) has additional advantages over conventional PCR: it is able to detect lower concentrations of target DNA, it is quantitative, and it is less labor-intensive, since there is no need for electrophoresis to visualize products. The technique has been used for the identification of the different human schistosome species and in assessing infection intensity (197, 209, 210). An expansion on qPCR techniques is multiplex PCR, which amplifies more than one DNA target in a single reaction mixture and has been used successfully for case detection (197), for differentiating between *S. japonicum*, *S. mansoni*, and *S. haematobium*, and as an important tool in epidemiological studies and in monitoring schistosomiasis control programs (197, 206). In a recent advance in PCR technology, droplet digital PCR (dd PCR) was developed and has proven to be more sensitive and precise than qPCR (211–213). dd PCR has been used successfully for the detection of cell-free DNA and in the diagnosis of infections and other clinical conditions (211, 214, 215). Although not yet applied for the diagnosis of schistosomiasis, it may prove a useful alternative or adjunct for the sensitive detection and precise quantification of the disease in future.

Identification of an infected individual by using a copro-PCR-based method relies on the presence of parasite DNA in the analyzed stool sample, of which only a very small aliquot can be used in the assay (216). Moreover, inhibition of PCR by compounds within fecal samples can be a further problem, although diagnostic accuracy can be increased by combining parasitological or serological testing with PCR (217). With the advent of the loop-mediated isothermal amplification (LAMP) technique, early detection of *Schistosoma* mitochondrial DNA in stool samples has been demonstrated, as detailed below. The drawbacks of PCR-based tests are the high cost of reagents, the requirement for suitably trained staff, and the need for appropriate but expensive equipment (201, 218).

Detection of Cell-Free Parasite DNA in Serum and Other Body Fluids

To overcome some of the drawbacks of other diagnostic procedures, attempts have been made to detect *Schistosoma* CFPD in human serum/plasma and other body fluids (202, 219). CFPD is released into the circulation, originating from dead schistosomula, tegument shedding of worms, or the disintegration of inactive eggs (220). Further suggested origins of CFPD include schistosomula or juvenile worms as they move in the circulation during the early postinfection period (199). CFPD is uniformly distributed in plasma, unlike schistosome eggs in feces or urine, so one of the major limitations of egg DNA amplification, i.e., sampling, can be avoided with CFPD detection. CFPD can be excreted in urine (221), saliva (222), or other body fluids, such as cerebrospinal fluid, and is quantified using PCR (202, 219, 222). Infections with all three major human schistosomes have been identified with PCR-based CFPD assays using both species- and genus-specific target genes in animal models and patients (Table 3). Sandoval et al. (203) developed PCR assays based on ribosomal DNA (rDNA) that produced very sensitive and specific amplification of genus- and species-specific amplicons from five *Schistosoma* species (*S. japonicum*, *S. mansoni*, *S. haematobium*, *S. intercalatum*, and *S. bovis*); notably, they showed that urine could be used as the template for amplifying PCR products from both *S. mansoni* and *S. haematobium*. In a further advance, DNA isolation and specific PCR-based identification of *S. mansoni* and *S. haematobium* were achieved by use of urine sediments obtained by filtration (223).

Indeed, PCR identification of *S. mansoni* in urine sediments has proved superior in diagnostic accuracy to the KK and urine CCA tests in areas of endemicity (224).

PCR-based tests can detect CFPD in host serum from a very early schistosome infection (225, 226), even in the first week postinfection (227), thus representing a useful adjunct for the early diagnosis of schistosomiasis. In combination with real-time PCR, the approach has proven valuable for monitoring therapeutic responses (222, 228), as the amount of CFPD declines gradually following effective treatment. Moreover, detection of CFPD can play an important role in situations where diagnostic dilemmas occur, such as in neuroschistosomiasis cases, which normally present false-negative results by conventional techniques (27).

Xia and colleagues (226) successfully amplified the highly repetitive *SjR2* retrotransposon gene, which is specific for *S. japonicum*, in a rabbit model and demonstrated its detection in serum within the first week postinfection and its disappearance after 10 weeks of treatment. Previously identified *S. japonicum* retrotransposons were tested against *SjR2* in the rabbit model to detect further novel effective diagnostic markers. This identified a 303-bp region from the non-long-terminal-repeat retrotransposon *SjCHGCS 19* which had a high sensitivity and specificity in a nested PCR (229). Furthermore, the sequence was detected as early as 3 days postinfection but was undetectable at 17 weeks posttreatment. Testing of *S. japonicum*-infected patients resulted in >95% sensitivity and specificity, confirming its potential as a diagnostic target (229). Another study on *S. japonicum* involving CFPD detection in serum and urine in human cohorts from areas in the Philippines where the disease is highly endemic (221), using the mitochondrial gene CO1, confirmed the promise of the approach for the diagnosis of an active light schistosome infection. In further support of CFPD detection, Xu et al. (158) successfully PCR amplified the *SjR2* gene of *S. japonicum* from human serum and were able to validate the rSP13-ELISA that they developed.

It is important that the amount of schistosome DNA circulating in the serum or plasma of an infected individual is generally relatively low and is dependent on parasite load. Consequently, it is important to consider different strategies that use smaller blood volumes effectively and that may not require a concentration step. For example, testing a smaller volume of plasma could be attempted by using a larger input volume of extracted DNA for PCR (219). In this respect, a recent study achieved some diagnostic success by using smaller volumes of blood, saliva, and urine (221), but the approach needs further optimization prior to being employed on a large scale. Importantly, the possibility of detecting CFPD in urine or saliva can eliminate the risks and inconvenience of using blood samples. Since it is minimally invasive and has a good sensitivity and specificity, further development of this technique could be an important way forward in diagnosing schistosomiasis in all phases of clinical disease, including detection of Katayama syndrome and active infection, along with monitoring drug treatment. However, as for any PCR-based method, the requirements for relevant expertise and expensive reagents and equipment are major hindrances for the wider applicability of this approach in both field and clinical settings.

LAMP

Loop-mediated isothermal amplification (LAMP) was introduced recently as a cost-effective and feasible alternative to conventional PCR for the detection of schistosome DNA in fecal and serum samples. In the LAMP reaction, a large pyrophosphate iron byproduct is produced, which subsequently forms an insoluble salt on combining with a divalent metallic iron ion. In the one-step amplification reaction, manganous iron and calcein are added to allow the visualization of alterations in fluorescence. This is a very sensitive signal recognition method which enables naked eye detection of test endpoints and hence avoids the need for electrophoresis equipment (230). LAMP is generally a very specific and sensitive method, with its use of specific inner and outer primers (231, 232), and compared with PCR-based assays, LAMP has the advantages of simplicity, being more rapid, and having a higher amplification efficiency. Furthermore, as indicated, the results can be inspected visually, so the method has considerable potential for application in low-resource countries and is highly cost-effective. However, one important limitation is that the DNA amplification mechanism involved in the LAMP technique itself could lead to carryover contamination, giving rise to false-positive results (230, 233). Also, other possible problems, such as difficulties in optimization and the limitations of multiplexing associated with the use of increasing numbers of LAMP primers, need to be considered in applying this approach (234, 235).

A 301-bp sequence from the *SjR2* gene of *S. japonicum* was successfully amplified in a LAMP assay using blood samples from infected rabbits. The results indicated the possibility of early detection of *S. japonicum* infection, but the high sensitivity of the method may preclude its use in assessing the response to treatment (52).

A recent modification of LAMP technology produced promising results in a murine model of schistosomiasis mansoni and has raised hopes for its use in field settings as a rapid diagnostic test for human infection, with high sensitivity (236). A 620-bp sequence corresponding to a mitochondrial *S. mansoni* minisatellite DNA region was selected as the LAMP target, and the technique was able to detect infection as early as 1 week postchallenge, using stool samples.

Notably, detection of successful LAMP reactions by observing green fluorescence via the addition of SYBR green I to the reaction mixture clearly has practical value, as it can be used in field settings to provide test results without the need for electrophoresis. It is now important to develop this approach further by its validation in community settings as well as by determining whether it can be used for the diagnosis of the other human schistosome species.

Detection of Circulating miRNAs

MicroRNAs (miRNAs) are a group of noncoding RNAs that are mainly involved in posttranscriptional gene regulation (237). They are present in a wide range of body fluids, including blood plasma/serum. The release of miRNAs from cells into the circulation occurs via three main pathways: passive leakage from broken cells, active secretion of miRNAs enclosed in microvesicles (exosomes), and active secretion of miRNAs bound to RNA binding proteins (238).

Identification and characterization of a set of parasite-derived miRNAs in both *S. mansoni* (239, 240) and *S. japonicum* (241–244) provided the basis for their detection in the circulation. The presence of schistosome-specific miRNAs was first reported for the plasmas of *S. japonicum*-infected rabbits, by Cheng et al. (245) and then by Hoy et al. (246), who demonstrated elevations of several parasite-derived *S. mansoni* miRNAs, including sma-miR-277, sma-miR-3479-3p, and bantam, in a mouse model at 8 weeks

postinfection. These parasite-derived miRNAs were detectable at different infection intensities, with a high sensitivity and specificity, denoting their potential as novel diagnostic markers (246). Recent evidence has shown that only sja-miR-277 and sja-miR-3479-3p can reliably be detected in the sera of two mouse strains infected with *S. japonicum* (P. Cai, G. N. Gobert, H. You, M. Duke, and D. P. McManus, unpublished observations).

In addition to targeting schistosome-specific miRNAs, dysregulation of host miRNA profiles in different tissues following infection has been investigated, emphasizing their involvement in regulatory functions in the host microenvironment. Variations in miRNA profiles during disease progression have been demonstrated in murine models even during very early acute infection (247, 248). As the alteration in miRNA expression profiles is often correlated with numerous human diseases, including liver diseases, He et al. investigated the serum levels of host miRNAs in mice, rabbits, buffaloes, and humans infected with *S. japonicum*, and circulating miR-223 was suggested as a potential new biomarker for the detection of schistosome infection and the assessment of the response to chemotherapy (249). In contrast, another study showed that host-derived miRNAs could not differentiate between uninfected and *S. mansoni*-infected individuals, suggesting a limited potential of host-derived miRNAs for detecting disease prevalence (246). Inconsistent serum levels of host miR-122, miR-21, and miR-34a in different murine models have also been reported during *S. japonicum* infection, which will likely impair their value as individual diagnostic biomarkers for schistosomiasis (Cai et al., unpublished observations). However, the serum levels of these miRNAs as a panel in combination may correlate with hepatic immune responses in schistosome-infected hosts, thereby serving as a novel biomarker assay to indicate the degree of hepatopathology caused by schistosomiasis.

These advances in determining schistosome-specific and host miRNA profiles provide some insight as to their future potential as early diagnostic markers of infection, in the evaluation of disease progression, and in determining therapeutic responses. However, they need to be applied in clinical settings, and the costs of the required reagents and technical resources required may limit their wide-scale application.

CYTOKINES, METABOLIC PRODUCTS, AND OTHER PARASITE MOLECULES AS BIOMARKERS

In addition to the detection of circulating antigens and antibodies and nucleic acid targets, specific host cytokines and different schistosome metabolites have also been assessed as biomarkers for the diagnosis of schistosomiasis. Metabolic and cytokine biomarkers are not particularly specific to schistosome infection and have limited diagnostic value in isolation. Cytokines and schistosomal metabolomic products have been identified in blood and different biofluids of humans and animal models. Profiling of these biomarkers has been attempted using different techniques, including basic immunological and molecular methods and advanced approaches, such as proton nuclear magnetic resonance (NMR) and mass spectrometric (MS) methods, including matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS).

Cytokines

Various cytokines are released during different stages of a schistosome infection, reflecting the host immune response (84, 250, 251). Generally, in the early acute infection phase, predominated by the juvenile schistosomulum stage, a Th1 cell-mediated response is generated, which releases proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), interleukin-1 (IL-1), and IL-2. Then, with the deposition of schistosome eggs in tissues, there is a switch to a Th2-mediated immune response, characterized by the production of IL-4, IL-5, IL-10, and IL-13, along with IgE antibodies. However, it was recently demonstrated that less common cytokine combinations, such as IFN-γ with IL-13 and IFN-γ with IL-4, occur during the initial hepatic pathology of *S. mansoni*-infected mice (252). In regard to human schistosomiasis, despite the predominance of different cellular response activities at different stages of the infection, there is an overlap of Th1 and Th2 cell activities which can complicate the diagnostic interpretation of cytokine profiles in subjects with the disease (83, 84).

IL-18 has been shown to be induced during early hepatic pathology in *S. mansoni* infection (253), whereas soluble TNF receptors I and II and intercellular adhesion molecule I (ICAM-I) have been identified as important hepatic fibrosis markers associated with *S. japonicum* infection at both the acute and advanced stages (254). In addition, a recent study of a focus with coendemicity demonstrated the relationship between *S. mansoni* and *S. haematobium* infection intensities and related changes in cytokine profiles (235); with increased *Schistosoma* infection intensity, there was a decrease in cytokine responses, with the Th2 phenotype becoming more prominent (255).

With regard to chronic schistosomiasis, different markers for the identification and evaluation of hepatic fibrosis related to *S. japonicum* and *S. mansoni* infections have been identified. Changing levels of these markers in blood and urine, correlating with the severity of the pathology, have been identified, and some of these important markers were recently reviewed (256); they include collagen types I, III, and IV and their metabolic products, hyaluronic acid, chitinase 3-like protein 1, matrix metalloproteinases, and laminin.

Metabolic Markers

Changes in the levels of different basic metabolic by-products in mammalian hosts can occur due to the presence of particular pathogens, and such changes have been tested to identify diagnostic markers of schistosomiasis. In this context, metabolic signatures of *S. mansoni*-infected mice have been identified by NMR spectroscopy (257). Among the changes in urinary, fecal, and plasma metabolite compositions, urinary changes in particular were noted from day 41 postinfection, with hippurate, phenylacetylglycine (PAG), and 2-oxoadipate being important urinary markers, while D-3-hydroxybutyrate and glycerophosphorylcholine were consistent plasma markers (257). Similarly, in other studies, analyses of urinary metabolomic profiles of schistosome-infected mice revealed possible parasite-induced effects on host metabolic pathways, including amino acid metabolism and glycolysis, and changes in the gut microbial flora (258, 259). Moreover, changes in human urinary biochemical profiles from areas where *S. mansoni* is endemic have been used to differentiate between infected and uninfected individuals. These findings were important in demonstrating possible changes in human liver function, gut microflora, and energy metabolism due to schistosome infection (260). However, the specificity of these metabo-

lites was not assessed, so their use as accurate diagnostic markers has not been determined.

Urinary metabolic profile changes in hamsters infected with *S. japonicum*, including increased levels of short-chain fatty acids, have been described (261). Possible disturbances to lipid metabolism have also been identified in mice, with reductions in cholesteryl ester and triacylglycerol levels due to acute schistosomiasis mansoni (262).

Some other changes in metabolic by-products have also been identified and used to differentiate schistosome-infected from uninfected individuals. Alterations in urinary metabolite profiles of mice infected with *S. mansoni* have been demonstrated by NMR spectroscopy, ultraperformance liquid chromatography, and capillary electrophoresis fingerprinting. Changes in 3-ureidopropionate, *p*-cresol glucoronide, PAG, isocitrate, and indoxyl sulfate levels were prominent markers capable of differentiating infected from control animals. Moreover, an increase of the PAG level in urine was significant after 30 days postinfection, highlighting it as an early urinary metabolic marker candidate that needs further testing for specificity so as to develop it as a valid diagnostic probe (259, 263). MALDI-TOF MS has been used for the diagnosis of *S. japonicum* infection in a rabbit model, and the proteomic pattern in plasma provided a high sensitivity and specificity compared with those of other serological and conventional diagnostics (264). In addition, significant rises have been noted in oligosaccharides and the oxidative stress marker monoaldehyde following *S. mansoni* infection in mice and human subjects (265, 266).

Other Antigens and Proteins as Diagnostic Markers

Current advances in proteomics and transcriptomics have led to the identification of numerous schistosome molecules produced by different life cycle stages, including proteins and other components, with potential as new diagnostic targets. Transcriptomic and proteomic analysis revealed that most *S. japonicum* genes share pairwise orthologs with *S. mansoni* (267). The study further identified *S. japonicum* components expressed in different life cycle stages, including a specific tegument protein (SjTs4) and an eggshell protein (MF3) (267), which may prove valuable as diagnostic targets, although further testing of these components for diagnostic application is required. A proteomic study of excretory/secretory (ES) proteins of adult *S. japonicum* worms identified more than 100 proteins, among which a fatty acid binding protein (FABP), which had previously been localized in the subtegumental region and vitelline glands (268), was the most abundant. FABP has the potential to induce immunogenic reactions in the host circulation, raising the possibility of a role for an anti-FABP antibody in serodiagnosis of *S. japonicum* (269). In another, similar study, Zhong et al. (270) used proteomics in combination with Western blotting and identified four putative diagnostic protein candidates, namely, leucine aminopeptidase (LAP), fructose bisphosphate aldolase (FBPA), glutathione *S*-transferase, and a 22.6-kDa tegumental antigen. Of these, recombinant SjLAP (rSjLAP) and SjFBPA (rSjFBPA) were successfully applied in an ELISA for the diagnosis of *S. japonicum* in humans, with high levels of sensitivity and specificity (270). In another study, IgG response patterns for ES antigens of *S. japonicum* were assessed in rabbits (271). ES antigens inducing short-lived antibody responses were identified by MALDI-TOF MS, and their importance for diagnosis and therapeutic evaluation was determined. *S. japonicum* glyceraldehyde-3-phosphate dehydrogenase (SjGAPDH) and fructose 1,6-bisphosphate aldolase (Sj-FBA) were identified as the major antigens involved. Furthermore, it was shown that SjGAPDH induced short-lived antibody responses in the host and that detecting IgG against this antigen may provide the basis for developing a method for diagnosing early infections of *S. japonicum* (271). Furthermore, SjP40, an *S. japonicum*-specific antigen, was identified as another potential early diagnostic marker by Zhou et al. (157), who measured the rise of anti-SjP40 antibody levels in rabbits during the course of infection. Moreover, the *S. japonicum*-specific tandem repeat antigen Sj7TR, thioredoxin peroxidase 1 (SjTPx-1), and the Sj13 protein are other molecules identified as potentially important biomarkers for use in antibody detection (272–274).

A recent immunoproteomic analysis of *S. japonicum* tegument proteins identified multiple highly immunoreactive antigens, which were also tested against patient sera (275). Of these, stress-induced phosphoprotein 1 (STIP1) was shown to be the most immunoreactive tegument protein, with good antigenicity, making it a potential vaccine target or biomarker for the diagnosis of *S. japonicum* infection (275). In another study, the hydrophilic domain of the *S. japonicum* 23-kDa membrane protein (Sj23HD) was identified as an important early diagnostic marker in comparison with soluble egg antigen (276). Moreover, the *S. japonicum* subtegumental protein cyclophilin A (SjCypA) has also been identified as an immunogen and a potential diagnostic (277). In addition, aquaporin, which was shown by proteomic studies to be the most abundant transmembrane protein of the *S. mansoni* tegument (278), could also be a possible diagnostic target. Furthermore, recent mass spectrophotometry-based studies detected multiple antigenic proteins of *S. mansoni*, including annexin, major egg antigen, and troponin T, and highlighted the possibility of using these components in improved diagnostic tests for schistosomiasis (279). In addition, cathepsin B (Sm31) from *S. mansoni* adult worms has shown promise as a useful serum diagnostic marker (280, 281). Other schistosome proteins and antigens from different life cycle stages which may be of value as diagnostic markers include protease inhibitors, such as α macroglobulin, a serpin, the MEG 2 and 3 egg secretory proteins, and the 18-kDa, 31/32-kDa, 38-kDa, Sm29, and Sm21.7 proteins (282–284).

DIAGNOSIS AND EVALUATION OF SCHISTOSOME-INDUCED LIVER DISEASE

Schistosome-induced hepatic damage is due mainly to the formation of granulomas and fibrosis around the schistosome eggs lodged in presinusoidal portal venules, and these immunologically based outcomes are responsible for most of the serious sequelae associated with chronic schistosomiasis (16, 285). This pathological process results in structural and biochemical changes in the liver. Detection of such changes is helpful in the diagnosis and evaluation of schistosome-induced hepatic disease. Liver biopsy, hepatic imaging, and the detection of biomarkers are the main methods of assessing the condition.

Liver biopsy, with accompanying histology, can provide the most informative direct evidence of local hepatic damage, but its invasiveness, poor acceptance by patients and physicians, associated complications, such as bleeding, and possible sampling error on collection have limited its routine applicability in clinical practice (286, 287).

Ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) are commonly used scanning modali-

ties in evaluating the hepatic pathology in schistosomiasis. Since US is a convenient and reliable method, it is routinely used in the diagnosis and evaluation of patients with schistosomiasis (288–290). US can be used to demonstrate classical features of schistosomal hepatic damage, such as periportal fibrosis, appearing as "bulls-eye" lesions, a network echogenic pattern, hepatic granulomas, and gallbladder thickening (291, 292). Furthermore, the use of portable US equipment has widened the applicability of imaging in community field settings in areas where schistosomiasis is endemic. US is applied in grading schistosomiasis disease status (grades I to III), based on criteria published by the WHO (293). CT and MRI are not routinely used for schistosomiasis diagnosis in resource-poor settings, due to their associated risks and costs, but as their diagnostic characteristics are similar to those of US, disease-associated complications can be assessed by using these imaging techniques, if available.

The main noninvasive biomarkers in evaluating schistosome-induced liver damage include the levels of hepatic extracellular matrix components in the circulation and urine (183, 219). Detectable extracellular matrix components, mainly collagen and its metabolic products, are broadly classified into three categories, as indicators of matrix deposition, indicators of matrix degradation, and molecules for which a specific role has not been determined. Serum levels of these biomarkers are significantly elevated with the progression of hepatic fibrosis and are hence important in qualitative and quantitative assessments of pathological status, including related complications, and also in monitoring the response to treatment. Procollagen III amino-terminal peptide, laminin, hyaluronic acid, chitinase 3-like protein 1, procollagen I carboxy-terminal peptide, procollagen IV C and N peptides, and metalloproteinase inhibitors are some examples of these biomarkers (294–296). In addition, different indices, based on routine investigative findings, are relevant in the assessment of hepatic disease. The aspartate amino transferase/platelet ratio index (APRI) is one such commonly considered indirect tool (296, 297). Furthermore, a recent study suggested the possible application of the international normalized ratio (INR) and hyaluronic acid in estimating the degree of hepatic damage (298).

Although these markers provide supportive evidence in determining the degree of hepatic pathology in schistosomiasis, they are neither unique nor specific to the disease and hence need to be evaluated in combination with other clinical and investigative parameters in clinical practice. Furthermore, the applicability of using these biomarkers in routine practice is additionally limited due to the costs of the tests and the resources required to undertake them (296, 299).

DIAGNOSIS AND EVALUATION OF SCHISTOSOME-INDUCED GENITOURINARY DISEASE

The damage and pathology in the genitourinary system caused by *S. haematobium* are due primarily to the granulomatous inflammation provoked by egg deposition in the genitourinary tract, which can result in polyposis, ulceration, bladder calcification, carcinomas of the bladder, and ureteral strictures. Such structural changes in the genitourinary tract can be detected and evaluated by direct observation through cystoscopy, microscopic and histological examination of biopsy specimens, and use of imaging techniques (16, 18, 300).

Indeed, imaging approaches are commonly used to assess the morbidity caused by genitourinary schistosomiasis and related complications. These techniques include X-ray imaging, CT, MRI, and US scanning (18, 300). US imaging, which is noninvasive and convenient, is commonly applied for the detection and evaluation of pathological lesions in the urinary tract. As defined in WHO protocols and subsequent revisions, ultrasonic assessment allows the bladder damage by *S. haematobium* to be classified into grades (grades 0 to III) according to severity, based on the changes in bladder wall thickness, bladder wall irregularities, bladder shape, calcifications, the presence of polyps and tumors, and hydronephrosis (293, 301).

S. haematobium eggs are deposited in the bladder, and resulting histopathological changes, such as a roughened mucosa, are characteristically seen as sandy patches, which can be detected in biopsy specimens examined by cystoscopy (16, 18). This approach can provide comprehensive information on the egg-induced pathology, but it is invasive, which is a major limitation of the procedure.

As malignancies are commonly associated with urogenital schistosomiasis, cancer biomarkers have been tested for early detection of such severe complications. Catechol estrogen quinine (CEQ)-derived metabolites or CEQ-DNA adducts (302), the nuclear matrix protein BCLA-4 (303), the oncoprotein p53, and sialylated glycans (304) are biomarkers that have been identified in the serum and urine of patients with urogenital schistosomiasis-associated bladder cancer.

DIAGNOSIS AND EVALUATION OF NEUROSCHISTOSOMIASIS

Neuroschistosomiasis is a disorder resulting as a severe complication of schistosome infection with the spread of eggs to the central nervous system (CNS). This leads to the formation of granulomas in nervous tissue, leading to cerebral and spinal schistosomiasis. Involvement of the brain usually results in seizures, and involvement of the spinal cord causes myeloradiculopathy (18, 305). The gold standard for diagnosing neuroschistosomiasis is the direct detection of parasite eggs and related pathological changes following biopsy (18, 305, 306). However, this is a highly invasive and dangerous procedure. Involvement of the CNS can be detected by neuroimaging techniques, such as CT and MRI; these are helpful in detecting evidence of lesions, such as masses and tissue edema (81, 305, 307). Moreover, PCR amplification of schistosome DNA from cerebrospinal fluid has also been used successfully in the diagnosis of neuroschistosomiasis (27, 221). It is also logical to confirm the diagnosis of suspected neuroschistosomiasis cases by looking at direct and indirect evidence of systemic infection in patients with acute neurological complications. With regard to specific immunodiagnosis of neuroschistosomiasis, the combined use of different immunological tests, such as indirect hemagglutination assay (IHA) and ELISA, is also useful in supporting the clinical diagnosis. This combined approach results in a higher sensitivity and specificity (305, 308). In addition, the detection of immune complexes with schistosomal antigens in cerebrospinal fluid has been useful in diagnosing neuroschistosomiasis (309). Also, as in routine diagnosis of schistosomiasis, direct detection of schistosome eggs in fecal or urine samples is also helpful in the final diagnosis of the condition. In short, it is important to emphasize the value of using multiple strategies in the diagnosis of complicated schistosome infections, such as neuroschistosomiasis (305–307).

DETECTION OF INFECTED INTERMEDIATE SNAIL HOSTS

In addition to the detection of schistosome infection in mammalian hosts, environmental monitoring of schistosomiasis is important for control efforts to lead to disease elimination. Of the available environmental monitoring methods, the detection of infected snails and the identification of miracidia in water sources are two important entities that require further advances.

Bulinus, *Biomphalaria*, and *Oncomelania* act as the intermediate hosts of *S. haematobium*, *S. mansoni*, and *S. japonicum*, respectively. Xeno-monitoring, the identification of the presence of schistosomes in intermediate host snails, is an important indirect indicator of the state of infection prevalence in a particular community, particularly when the prevalence of schistosomiasis is low. This can indicate the extent of environmental contamination and is an effective way of monitoring the disease. Moreover, determining the prevalence of schistosome species within snail hosts, including prepatent infections, is especially important for identifying specific transmission sites with seasonal and spatial patterns (310, 311). These findings are important for detecting risk areas to guide surveillance and the use of interventions, as well as to determine the efficacy of ongoing control strategies (312). Hence, this approach is extremely helpful in monitoring control and elimination efforts. Different techniques, ranging from basic laboratory methods to advanced molecular diagnostics, are used to identify infected snails.

One of the first developed and widely used methods for detecting schistosome infections in snails was the observation of cercarial shedding induced by artificial light exposure. Another basic technique is the crushing of snails between glass slides and inspection for sporocysts and cercariae. However, there are detection limitations with these techniques in situations where there is a low parasite burden, where there is aborted development of sporocysts, and, especially, with a prepatent infection, as cercarial shedding does not occur until 25 to 30 days after a snail is infected with a miracidium. Also, there are reports of focal and low-frequency cercarial shedding in areas of high *Schistosoma* transmission (313). Furthermore, the labor-intensive nature of these procedures, including the collection and maintenance of snails and the associated costs, are both major hindrances in their application (314).

Due to the limitations of these conventional techniques, a number of advanced approaches have been developed. As with the developments in diagnostics for human case detection, different molecular techniques are proving valuable in the identification of schistosomes in specific snail hosts. Various DNA amplification methods are the mainstay of such approaches and are recognized as being more efficient, especially when large-scale screening is required (315). It has been shown that PCR-based assays can detect prepatent schistosome infections and infection with a single miracidium (316–318). Amplification of different species-specific schistosome gene segments in snails, including the 18S rDNA gene of *S. mansoni* and the DraI 121-bp repeat sequence of *S. haematobium*, has been tested successfully and applied in community studies on a large scale (310, 312, 319, 320). Modified PCR methods, including multiplex and nested PCRs, have also been used successfully for the identification of infected snails (314–316, 320–322).

With regard to further advances in the application of molecular diagnostics for xeno-monitoring, real-time PCR and LAMP-based techniques have proven very effective. Real-time PCR assays using fluorescence resonance energy transfer hybridization probes combined with melting curve analysis (FRET-PCR) have been used to detect *S. japonicum* infection in snails and have proved successful. This is reflected in the capability of detecting a cercaria artificially introduced into a pool of 10 noninfected snails, highlighting the possibility of using this method in epidemiological surveys of snail intermediate hosts (323). In addition, PCR with an oligochromatographic dipstick for the detection of amplicons has been shown to reduce the extensive technological requirements for real-time PCR and has also been used effectively as a simple, rapid method for snail diagnosis (324, 325).

Due to its convenience of application, LAMP is now widely used to detect schistosome-infected snails in epidemiological surveys. Tong et al. (326) successfully amplified the 28S rDNA gene of *S. japonicum* from pooled field samples of *Oncomelania hupensis* from areas of low endemicity; furthermore, using the positive proportion of *O. hupensis* snails infected with *S. japonicum* as determined by the LAMP assay, effective geographical risk mapping of the transmission of schistosomiasis was undertaken, as this approach is important to guide surveillance and response strategies in high-risk areas (326). In an earlier study targeting *S. japonicum* 28S rDNA, it was shown that LAMP was able to detect a single infected snail in a pooled sample of 100 snails (327). Furthermore, the highly repetitive 121-bp gene sequences Sm1-7 in *S. mansoni* and DraI in *S. haematobium* were successfully amplified by LAMP in very early prepatency, in that infection was detected 1 day after exposure of snails to miracidia (313).

A recent study in Uganda investigated the genetic diversity and microepidemiology of *S. mansoni* infection in snails by using DNA bar coding. The study revealed a complex spatial distribution of multiple miracidial infections within snails of a particular region, indicating the extent of possible genetic diversity of the schistosome parasites within infected humans (328).

Some early studies revealed that snail infections correspond to the prevalence and intensity of human schistosomiasis. Furthermore, a high schistosome infection prevalence has been shown for snails when human PZQ drug treatment is ineffective (329). Metabolomic analysis of snails following miracidial infection has shown that malic acid and other carboxylic acids are important biomarkers for detecting *S. mansoni* at different stages of infection in the hemolymph and digestive glands of *Biomphalaria alexandrina* (330). Changes in lipid levels and concentrations of metabolic by-products, such as pyruvic acid, fumaric acid, and malic acid, in the digestive gland gonad complex of *B. glabrata* snails infected with *S. mansoni* have also been identified (331, 332). Although the latter approaches are important for understanding the biology of intermediate hosts infected with schistosomes, their application in detecting infection would be limited due to their nonspecificity and the extensive technology required to undertake the analysis.

Evaluating the presence of cercariae directly in water sources is a useful way of identifying possible schistosomiasis transmission sites. Conventional microscopic quantitation is used to detect cercariae in natural water sources and has been applied to describe the diurnal variation, seasonal patterns, and spatial distribution of cercariae (333, 334). However, this approach has the limitations common to the other conventional techniques discussed above. Infection of sentinel mice has also been used to identify transmission sites and to assess the risk for schistosomiasis in China (335).

PCR-based molecular tools are now increasingly being applied to this area of surveillance (336). Importantly, real-time PCR has been used successfully for detection of *S. japonicum* cercariae in water samples and has the potential to be used for rapid and high-throughput analysis of environmental samples (337). Despite the fact that novel molecular techniques in snail diagnosis are more accurate and sensitive, earlier traditional methods are still widely used in practice because of their technical simplicity, field applicability, and ease of application in resource-poor settings.

CONCLUDING COMMENTS AND FUTURE DIRECTIONS

Advances in improved diagnosis will play a central role in the control and eventual elimination of schistosomiasis, a disease of poverty that affects communities of the lowest socioeconomic status. Considering the current global situation with regard to schistosomiasis, as well as the goal of elimination, an effective and practical diagnostic test should be inexpensive and easy to use. It should also retain the requisite level of technical accuracy and be readily available for use at the community level and in point-of-care facilities. Of the different methods currently available, parasitology-based techniques are logistically challenging and have the major limitation of low diagnostic accuracy, especially with low-intensity infections in low-prevalence areas. Difficulties in differentiating between current and past infections and the inability to assess therapeutic responses are the major limitations of conventional antibody detection methods, although recent advances, such as the development of the rSP13-ELISA, have shown potential in overcoming some of these challenges. Circulating antigen (CAA and CCA) detection has improved considerably and is now an accurate and practical approach that can be applied noninvasively by using urine samples. In addition, the recently developed novel lateral flow-based rapid serodiagnostic assays add practical significance in that they can be applied in the field setting for community surveillance and monitoring of schistosomiasis prevalence. Moreover, the detection of circulating schistosome DNA and RNA is proving to be a highly sensitive diagnostic approach that, as with the detection of circulating antigens, can be applied to noninvasively collected biosamples, such as urine and saliva. The use of metabolomics and the quantification of cytokines are also important in assessing different disease stages of schistosomiasis, but they lack specificity, which restricts their value for diagnosis. Moreover, the direct detection of infected snails and other environmental surveillance methods to identify sites of transmission are effective ways of monitoring schistosomiasis in different geographical locations, especially in areas such as China, where disease elimination is now on the immediate horizon. Also, taking into consideration the current elimination goals of the WHO, it will be important to focus on the target product profiles for the diagnostic tools required for different stages of a schistosomiasis control program (338).

Novel PCR-based tests with high diagnostic accuracy for detecting cell-free parasitic DNA in conveniently collected clinical samples will likely be an important future option. Such assays, however, will require the simplification of DNA extraction and PCR procedures applicable to field settings. Moreover, further optimization of expediently applicable rapid diagnostic strip tests for both antigen and more accurate antibody detection tests could prove invaluable. The bottom line is that such test advances will need to consider costs and applicability in field settings, balanced by diagnostic accuracy, if they are to replace the currently widely used but less accurate conventional parasitological diagnostic tests.

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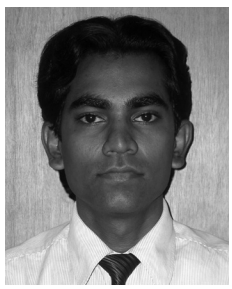

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