Target Product Profile

Schistosomiasis Surveillance Diagnostic

Use Case: Preventive chemotherapy reduction or stopping decision

Platform: Lateral flow test

Biomarker: CAA antigen

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Executive Summary

Neglected tropical diseases (NTD) affect the poorest populations. Several NTDs including schistosomiasis are controlled by preventive chemotherapy in the form of periodic mass drug administration (MDA). In areas with insufficient sanitation, schistosomes and soil-transmitted helminth are transmitted by eggs excreted in human stool and/or urine that contaminates the environment. Around 200 million individuals are infected with schistosomiasis, resulting in an estimated 1.7 to 4.5 million disability-adjusted life years (DALYs) lost, and 14,000 to 280,000 deaths per year.[1]

Control programs based on MDA have four designated stages: mapping disease distribution, impact monitoring of MDA interventions, stopping decisions for MDA, and post-elimination surveillance.[2] Current diagnostic tools including the Kato-Katz technique are thought to be sufficient for mapping disease distribution (see Appendix A: Common diagnostic tools). As the most commonly used method for schistosomiasis detection, its main strength is its extensive validation and familiarity all over the world. Requiring nothing more than a microscope and a good light source or power, the simplistic technology allows easier use at lower infrastructure levels. However, the major limitations of the Kato-Katz technique are its need for a trained microscopist and low sensitivity for detecting light intensity infections, which diminish its utility in later disease control stages. To support schistosomiasis control programs to continue to move toward elimination, a more sensitive, field-deployable diagnostic is needed.

This report proposes a target product profile (TPP) for the development of a new diagnostic technology that facilitates an accurate **stopping decision** for MDA. Each attribute has an "acceptable" standard that must be met and an "ideal" standard that if met would maximize the target product's value. This TPP focuses on the development of a lateral flow rapid diagnostic test that detects a *Schistosoma*-specific antigen.

Overview of Target Product Profile

Attribute	Acceptable	Ideal
1. Context (Use Case)		
1.1 Clinical and/or surveillance need (value proposition)	More sensitive than current microscopic methods, field-deployable, rapid diagnostic test to inform control programs.	More sensitive than current microscopic methods, field-deployable, rapid diagnostic test to inform control programs.
1.2 Intended use (use case)	Monitoring prevalence following mass drug administration (MDA) and informing the decision to adjust the treatment strategy to support elimination.	Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.
1.3 Target populations	Primary school children 6 to 14 years old and other high-risk populations.	Primary school children 6 to 14 years old and other high-risk populations.
1.4 Target countries/ geographic coverage	Schistosomiasis-endemic countries.	Schistosomiasis-endemic countries.
1.5 Location of use (infrastructure level)	Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.	Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.
1.6 Target user	Surveillance teams made up of technicians from the regional level, such as community health workers, with minimal training.	Surveillance teams made up of technicians from the regional level, such as community health workers, with minimal training.
1.7 Fit with clinical workflow/ linkage to action (process map)	Inform schistosomiasis control programs by estimating community-wide prevalence.	Inform schistosomiasis control programs by estimating community-wide prevalence.
1.8 Desired stability, storage, and cold chain requirements	45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.	45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.
2. Design		
2.1 Analyte (diagnostic marker)	Circulating anodic antigen (CAA).	CAA.
2.2 Sample type and volume	Clean-catch urine (~4 mL) or finger stick blood < 100 µL.	Urine (~4 mL) or finger stick blood < 10µL.

Attribute	Acceptable	Ideal
2.3 Sample preparation	Minimal collection or processing steps.	None.
2.4 Sample transport stability	≥ 2 hours at ambient temperature, or time necessary to collect and analyze specimen.	≥ 6 hours at ambient temperature, or time necessary to collect and analyze specimen.
2.5 Waste management (hazardous materials/chemicals)	Minimal or no hazardous materials, per World Health Organization (WHO) and country standards.	Minimal or no hazardous materials, per WHO and country standards.
2.6 Nature of result	Qualitative.	Qualitative and quantitative.
2.7 Time to result	Same-day result, < 24 hours.	Same-day result, < 15 minutes.
2.8 Throughput	> 50 samples per user per day.	> 100 samples per user per day.
2.9 Instrumentation format and complexity level	Field-based, rapid diagnostic test, few timed steps, no technically difficult techniques, CLIA-waived.	Field-based, rapid diagnostic test, no more than one timed step, automatic result reading, no technically difficult techniques, CLIA-waived.
2.10 Infrastructure requirements	Minimal, consistent with Tier 2 facility.	None.
2.11 Test-specific training requirements	Minimal, consistent with Tier 2 facility.	None.
2.12 Instrumentation size and weight	Small, easily deployable in the field.	No instrument.
2.13 Ancillary supplies	Minimal supplies to ensure optimal test performance, packaged as a kit.	None.
2.14 Mean time between failures	Minimal for instrument, not applicable for single use test.	No failures.
2.15 Quality control	Positive and negative control.	Positive and negative control.
2.16 Calibration	No run-to-run calibration required, instrument calibration not required in field.	None.
2.17 Product shelf life	12-month shelf life.	36-month shelf life, packaging should include thermal indicator.
3. Performance		

Attribute		Acceptable	Ideal
3.1	Analytical limit of detection (LOD)	Concentration of CAA corresponding to the number of worm pairs equivalent to the desired clinical sensitivity.	Concentration of CAA corresponding to a single worm pair.
3.2	Analytical specificity	Detects *S. mansoni* and *S. haematobium*, does not detect other helminths.	Detects *S. mansoni*, *S. haematobium, S. japonicum, S. mekongi, S. intercalatum*, and/or *S. guineensis*, does not detect other helminths.
3.3	Clinical sensitivity	> 70%.	> 95%.
3.4	Clinical specificity	> 95%.	> 99%.
3.5	Reproducibility and robustness	Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.	Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.
3.6	Comparative reference method	Kato-Katz (multiple slides and multiple days) and/or urine filtration/egg counting.	An appropriate composite reference standard.
4.	Commercialization		
4.1	Desired end-user price	To be determined.	To be determined.
4.2	Channels to market	To be determined.	To be determined.
4.3	Supply, service, and support	To be determined.	To be determined.
4.4	Product registration path and WHO prequalification	Not required for surveillance tests.	Not required for surveillance tests.

Rationale

1. Context (Use Case)

1.1 Clinical and/or surveillance need (value proposition)

Acceptable: More sensitive than current microscopic methods, field-deployable, rapid diagnostic test to inform control programs.

Ideal: More sensitive than current microscopic methods, field-deployable, rapid diagnostic test to inform control programs.

Neglected tropical diseases (NTD) affect the poorest populations. Several NTDs including schistosomiasis are controlled by preventive chemotherapy in the form of periodic mass drug administration (MDA). In areas with insufficient sanitation, schistosomes and soil-transmitted helminth (STH) are transmitted by eggs excreted in human stool and/or urine that contaminates soil and water sources. For schistosomiasis as well as STH control, the school infrastructure is essential to administer MDA, as school-aged children have the greatest burden of infection and morbidity.[3] Around 200 million individuals are infected with schistosomiasis, resulting in an estimated 1.7 to 4.5 million DALYs lost, and 14,000 to 280,000 deaths per year.[1]

Control programs based on MDA have four designated stages: mapping disease distribution, impact monitoring of MDA interventions, stopping decisions for MDA, and post-elimination surveillance.[2] Based on stakeholder opinions solicited at the Schistosomiasis Diagnostics Meeting (hosted by the Task Force for Global Health, Decatur, GA, August 2013), current diagnostic tools including the Kato-Katz technique are thought to be sufficient for mapping disease distribution (see Appendix A: Common diagnostic tools). However, as disease prevalence decreases through effective control strategies, a more sensitive diagnostic will be necessary to inform control programs.[4]

User needs assessments in the form of stakeholder interviews and field observations examined the strengths and limitations of the Kato-Katz technique. As the most commonly used method for schistosomiasis detection, its main strength is its extensive validation and familiarity all over the world. Requiring nothing more than a microscope and a good light source or power, the simplistic technology allows easy use at lower infrastructure levels. Major limitations are the need for a trained microscopist, and its low sensitivity for detecting light intensity infections, diminishing its utility in later disease control stages. Additional challenges include the need to collect, process, and read fresh stool specimens within a limited time frame, which adds logistical constraints such as transport of equipment and technicians.

Since schistosomiasis is endemic in some remote areas, a test that can function in the field with minimal infrastructure is necessary. It is anticipated that surveillance activities would be performed alongside control programs, which distribute praziquantel in the school setting through moderately trained school teachers or community health workers.[5] Therefore, an acceptable test would be relatively simple and ready to use, requiring minimal training.

1.2 Intended use (use case)

Acceptable: Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.

Ideal: Monitoring prevalence following MDA and informing the decision to adjust the treatment strategy to support elimination.

The surveillance needs for this diagnostic are to both monitor the impact MDA and inform the decision to reduce or discontinue MDA. The stopping decision in particular is a key part of program management—stopping too early can result in recurrence of transmission years later, while stopping too late wastes resources on unnecessary MDA. In the monitoring phase, the efficacy of the intervention also needs to be understood. The nature of these phases of elimination is that early monitoring is at relatively high prevalence, but as the stopping decision nears, the prevalence, and likely the intensity of infection, is low. The diagnostic test must be appropriate to both scenarios.[4]

The use case can be described as a field-based test being performed by surveillance teams, with involvement of school teachers or community health workers. These teams may be of varied training and technical expertise, and the conditions where they perform the testing may be without basic infrastructure. The data collected will be used to guide ongoing MDA programs, as well as inform the decision to stop MDA. The target population will primarily be school-aged children, both as a population of convenience in schools, as well as a population with potentially high relative prevalence. Delivery will be through high-level elimination programs. As a surveillance test, individual patient follow-up will not be required, but a mechanism to store and transfer surveillance data will be needed. The introduction of portable instrumented readers as well as low-cost tools would facilitate accurate results and reliable data handling.

Even though drug resistance to anthelminthic medicines have not been demonstrated in humans, the occurrence of resistance in livestock suggests it may be possible. Tools to identify and monitor drug resistance are important; however, more research is necessary.[6]

1.3 Target populations

Acceptable: Primary school children 6 to 14 years old and other high-risk populations.

Ideal: Primary school children 6 to 14 years old and other high-risk populations.

Schistosomiasis can affect all age groups, but the highest prevalence and intensities of infection are typically found in younger people. Additionally, primary school-age children are an important high-risk group for schistosomiasis and STH infections because the infections occur[3]:

- During a period of intense physical growth and rapid metabolism resulting in increased nutritional needs; when these needs are not adequately met, growth is impaired and children are more susceptible to infection.
- During a period of intense learning; when children are infected, learning capacities are significantly diminished.

- In a setting of continuous exposure to contaminated soil and water; children generally lack awareness of the need for good personal hygiene and like to play with soil and water.

As a result, current WHO guidelines for helminth control involve school-based surveillance. School-based treatment is efficient because school infrastructure reduces distribution costs and provides the opportunity to reach both enrolled and non-enrolled school-age children.[3]

By measuring the intensity of infection in treated areas over time, we expect to see reductions in intensity of infection and in the percentage of heavily infected individuals. Cohorts of 6- to 14-year-olds from sentinel primary schools are tested to assess their parasitological and clinical status.[7] WHO guidelines suggest evaluating children in the third year class, while monitoring new cohorts of 6-year-olds may provide evidence of transmission levels within the community.[1]

Table 1: Recommended treatment strategy for schistosomiasis.[8]

Category	Baseline prevalence among school-age children	Action to be taken[b]	
High-risk community	50% by parasitological methods[c] (intestinal and urogenital schistosomiasis) or 30% by questionnaire for history of haematuria	Treat all school-age children (enrolled and not enrolled) once a year	Also treat adults considered to be at risk (from special groupsd to entire communities living in endemic areas)
Moderate-risk community	10% but <50% by parasitological methods (intestinal and urogenital schistosomiasis) or <30% by questionnaire for history of haematuria	Treat all school-age children (enrolled and not enrolled) once every 2 years	Also treat adults considered to be at risk (special groupsd only)
Low-risk community	<10% by parasitological methods (intestinal and urogenital schistosomiasis)	Treat all school-age children (enrolled and not enrolled) twice during their primary schooling age (e.g. once on entry and once on exit)	Praziquantel should be available in dispensaries and clinics for treatment of suspected cases

[b] Equivalent to: high-risk community – all school-age children and adults require preventive chemotherapy annually; moderate-risk community – 50% of school-age children and 20% of adults require preventive chemotherapy annually; low-risk community – 33% of school-age children require preventive chemotherapy annually.
[c] For urogenital schistosomiasis, detection of haematuria by chemical reagent strips gives results equivalent to those determined by urine filtration
[d] Special groups: pregnant and lactating women; groups with occupations involving contact with infested water such as fishermen, farmers, irrigation workers or women in their domestic tasks, to entire communities living in endemic areas.

1.4 Target countries/geographic coverage

Acceptable: Schistosomiasis-endemic countries.

Ideal: Schistosomiasis-endemic countries.

Schistosomiasis is endemic in 76 countries and territories. Of these countries, 54 are endemic for *S. mansoni*, 55 are endemic for *S. haematobium*, and Philippines, China, and parts of Indonesia are endemic for *S. japonicum*.[1]

Table 2: Schistosomiasis-endemic countries in the WHO regions.[8]

Group	Countries and territories
Countries requiring preventive chemotherapy	African Region: Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritania, Mozambique, Namibia, Niger, Nigeria, Rwanda, Sao Tome and Principe, Senegal, Sierra Leone, South Africa, Swaziland, Togo, Uganda, United Republic of Tanzania, Zambia, Zimbabwe Region of the Americas: Brazil, Venezuela (Bolivarian Republic of) Eastern Mediterranean Region: Egypt, Somalia, South Sudan, Sudan, Yemen South-East Asia Region: Indonesia Western Pacific Region: Cambodia, China, Lao People's Democratic Republic, Philippines
Countries requiring updating for planning and implementation purposes	Region of the Americas: Saint Lucia, Suriname Eastern Mediterranean Region: Iraq, Libya, Oman, Saudi Arabia, Syrian Arab Republic
Countries requiring evaluation in order to verify if interruption of transmission has been achieved	African Region: Algeria, Mauritius Region of the Americas: Antigua, Dominican Republic, Guadeloupe, Martinique, Montserrat, Puerto Rico Eastern Mediterranean Region: Djibouti, Iran (Islamic Republic of), Jordan, Lebanon, Morocco, Tunisia European Region: Turkey South-East Asia Region: India, Thailand Western Pacific Region: Japan, Malaysia

Source: http://www.who.int/neglected_diseases/ntddata/sch/sch.html

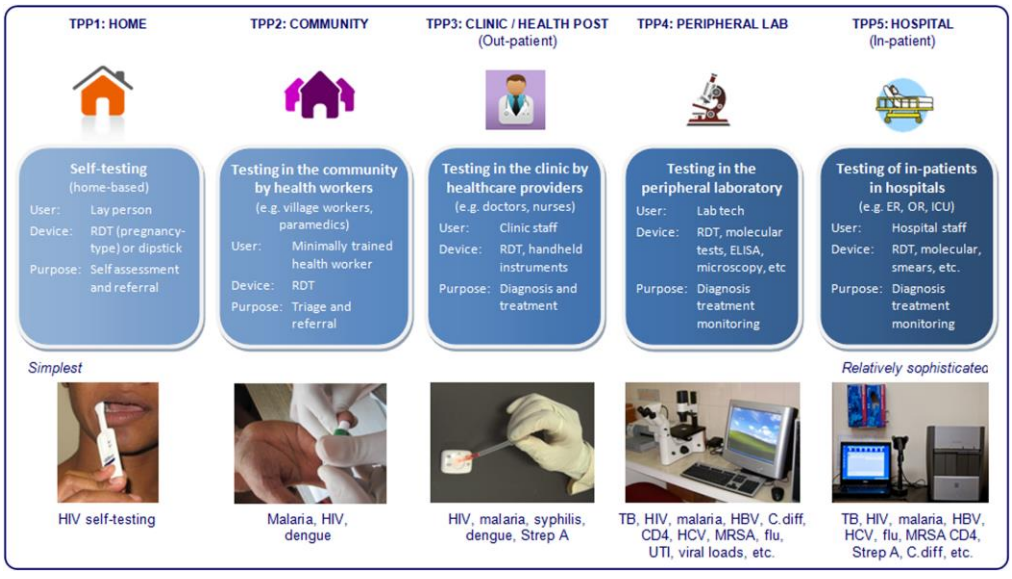
1.5 Location of use (infrastructure level)

Acceptable: Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.

Ideal: Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.

WHO guidelines emphasize school-based surveillance, and school settings in areas where schistosomiasis is endemic may have minimal or no infrastructure.

Figure 1: The spectrum of point-of-care testing for target product profiles.[9]



1.6 Target user

Acceptable: Surveillance teams made up of technicians from the regional level, such as community health workers, with minimal training.

Ideal: Surveillance teams made up of technicians from the regional level, such as community health workers, with minimal training.

It is anticipated that surveillance activities would be performed alongside control programs, which distribute praziquantel in the school setting through moderately trained school teachers or community health workers.[5] The target user of the diagnostic would be surveillance teams composed of central or regional technicians, possibly community health workers.[3] Therefore, an ideal test would be relatively simple and ready to use, requiring minimal training.

Stakeholder interviews and field observations provided additional data on the acceptable target user. The current tool, microscopy, requires an experienced microscopist to reliably generate accurate data. The availability of experienced microscopists at the national and regional level varies greatly from country to country. A diagnostic tool that required a less skilled technician would be useful. However, some stakeholders noted the importance of having the diagnostic test performed by a trained medical or laboratory technician rather than an unskilled worker.

1.7 Fit with clinical workflow/linkage to action (process map)

Acceptable: Inform schistosomiasis control programs by estimating community-wide prevalence.

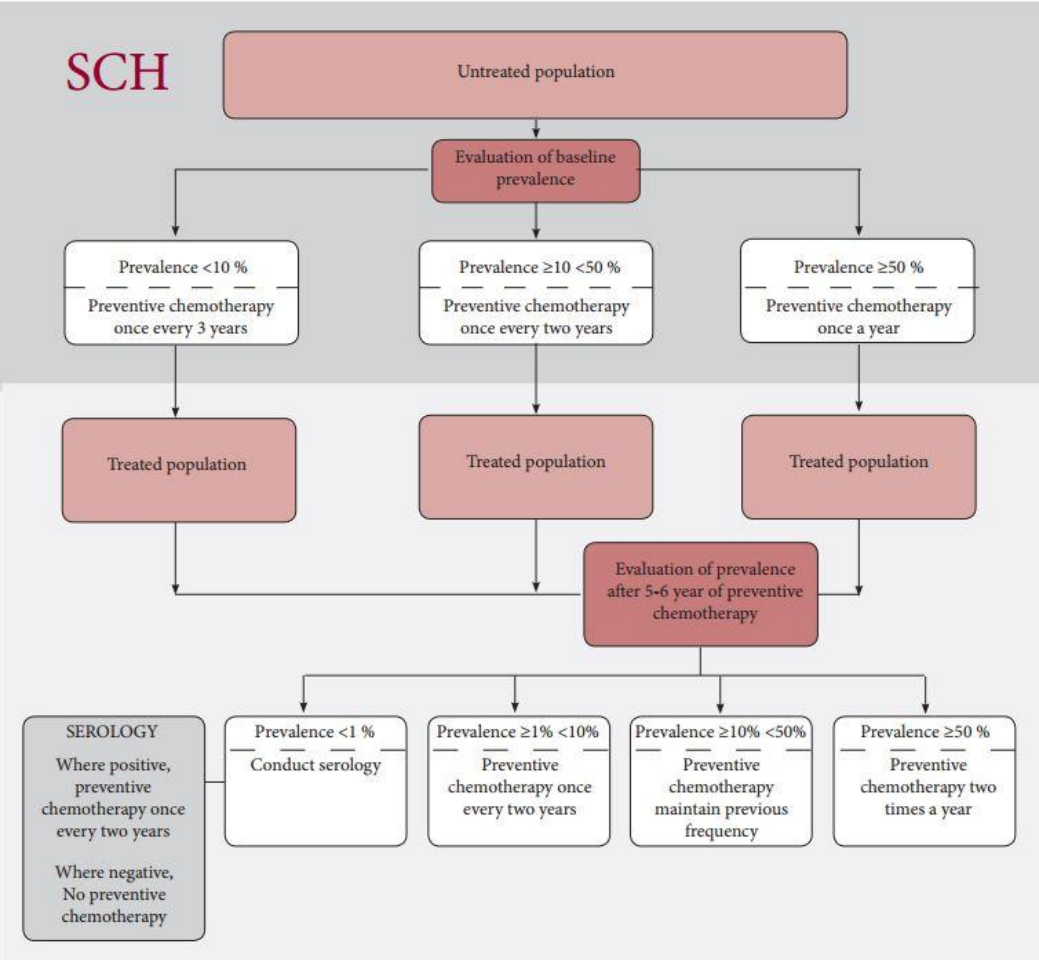
Ideal: Inform schistosomiasis control programs by estimating community-wide prevalence.

The following points are of interest to the structure and outcomes of a schistosomiasis control program:[5]

- Praziquantel treatment can cure infection in a single dose; however, reinfection from local water sources is common.
- Occasionally praziquantel is not 100% effective, and remaining worms may alter egg excretion, muddling measurements of treatment efficacy and prevalence.
- Praziquantel treatment is not considered an elimination strategy, but contributes to reduced morbidity.
- The PHASE approach to schistosomiasis control includes preventive chemotherapy, health education, access to clean water, sanitation improvements, and environmental vector control.[8]

Treatment for schistosomiasis is most often empiric, rather than by a test-and-treat paradigm. Periodic treatment for schistosomiasis is performed at the population level with the frequency of treatment determined by the community-wide prevalence estimate. Decisions to reduce MDA use are also based on the estimated prevalence of infection at time intervals following MDA.[3] Additionally, while the stopping decision may be based on diagnostic prevalence, other factors, such as vector populations and coverage surveys, also contribute. Guidelines on MDA stopping and post-elimination surveillance are currently in development, though comparisons to other control programs (such as lymphatic filariasis) may be of value. [10]

Figure 2: Frequency of mass drug administration based on prevalence estimates and prior community-based treatment. [3]



Based on field observation in Kenya, an example process map was generated depicting the workflow of a helminth surveillance project (see Appendix B). Another field observation in the Philippines identified variation in where the specimen was produced. In the Philippines, collection cups are distributed to the students the day before to take home and bring back to school the next day with specimen. This variation was based on the cultural sensitivity of the community to giving stool specimens. Additionally, based on stakeholder interviews there was variation in whether specimens were processed at the school or at another location, such as a nearby health clinic or laboratory. Immediate actions were not associated with individual results; however, stakeholder interviews noted the importance of providing quick feedback to the community to continue community engagement and high participation.

1.8 Desired stability, storage, and cold chain requirements

Acceptable: 45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.

Ideal: 45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.

Internal PATH data has suggested that there are temperature fluctuations in the areas this test would serve, with results showing roughly 25°C to 40°C variations on a daily basis. This variability is unavoidable without cold chain support.

2. Design

2.1 Analyte (diagnostic marker)

Acceptable: Circulating anodic antigen (CAA).

Ideal: CAA.

The target analyte, CAA, is an adult worm gut-associated circulating antigen produced in all major human-disease causing species of *Schistosoma*, and several animal-disease causing species as well. These antigens are released into the circulation of the infected host at regular time intervals from the gut of adult schistosomes, and present oligosaccharide epitopes capable of inducing high affinity and very specific antibodies.[11] CAA levels in serum and urine generally correlate well with worm burden (as determined by egg output); however the actual quantities produced by the worms and detected in the host circulation or excreta may depend on many factors, including host and parasite species, clearance rates, or duration and intensity of infection.[12] CAA is extremely stable and unique (no biological background). It is also very specific to the genus, especially when the sample is pre-analytically precipitated by tri-chloroacteic acid.[13]

2.2 Sample type and volume

Acceptable: Clean-catch urine (~4 mL) or finger stick blood < 100 µL.

Ideal: Urine (~4 mL) or finger stick blood < 10 µL.

The CAA antigen has been extensively studied in urine and serum samples, demonstrating less fluctuation in the day-to-day variations in the detection of CAA compared to fecal egg counts.[14]

The need to detect CAA with increasing sensitivity may require a greater volume of sample to be collected and processed. This has implications on the sample type used, as greater volumes of more invasive sample types may not be feasible.

Due to volume constraints and participant burden considerations, urine samples are currently considered the better sample choice. Urine samples are commonly obtained through a patient-controlled "clean catch" of the sample to avoid contamination of the sample by the early urine flow. This introduces an additional sampling burden on the patient, and compliance is variable; therefore, it would be ideal if the test is not impacted by potentially confounding substances in the early urine stream. Based on field experience and stakeholder feedback, 4 mL of urine is an easily obtained volume. Collecting greater volumes of urine may be possible to increase diagnostic sensitivity, more so than for other sample types.

From a participant burden perspective, it has been recommended that blood sampling > 100 µL is typically unreasonable as it would require venipuncture sampling (Schistosomiasis Diagnostics Meeting, August 2013). In the context of a large survey of children where the majority are expected to be

uninfected and will not receive treatment, the risks and discomfort associated with venipuncture sampling would outweigh the potential benefit afforded to them, making this method of sample collection unreasonable. Ten μL is a realistic volume of finger stick blood used for rapid diagnostic test assays and is therefore suggested as an ideal volume.[15] However, it is unclear whether it is feasible to detect CAA with finger stick blood even though detection of CAA in serum is well studied.

Interviews with stakeholders involved in helminth surveillance mentioned that both urine and finger stick blood were acceptable sample types. If efforts are made to integrate multiple NTD surveillance programs, using one common sample type is ideal. Currently, lymphatic filariasis surveillance programs collect finger stick blood.

2.3 Sample preparation

Acceptable: Minimal collection or processing steps.

Ideal: None.

The more commonly used diagnostics, such as urine filtration or the Kato-Katz technique, require significant sample preparation using urine or stool.[16] An improvement over this diagnostic would be acceptable. Since the location of use is community-based school settings, minimal to no sample preparation is ideal. However, technical constraints may require concentration steps to meet limit of detection requirements and these necessary sample processing steps may be acceptable to reach higher limits of detection.

2.4 Sample transport stability

Acceptable: ≥ 2 hours at ambient temperature, or time necessary to collect and analyze specimen.

Ideal: ≥ 6 hours at ambient temperature, or time necessary to collect and analyze specimen.

A limitation of the current diagnostic, the Kato-Katz technique, is the rapid degradation of some helminth eggs in the stool sample.[17] The sample stability should allow flexibility in the workflow in a point-of-care setting. Acceptable sample stability would allow for a reasonable time for sample preparation/analysis (~2 hours), or the time necessary to transport the specimen from collection to analysis site. An ideal time would allow for stability of the sample for most of the day (~6 hours) prior to analysis, which could facilitate batching.

2.5 Waste management (hazardous materials/chemicals)

Acceptable: Minimal or no hazardous materials, per WHO and country standards.

Ideal: Minimal or no hazardous materials, per WHO and country standards.

The test should not contain hazardous reagents per WHO and in-country safety, environmental, and transport requirements. Any hazardous waste in the form of biologic specimens should be contained on the diagnostic device and disposed of appropriately.

2.6 Nature of result

Acceptable: Qualitative.

Ideal: Qualitative and quantitative.

The goal of helminth control programs is to reduce morbidity due to helminths, which is not related to the presence or absence of infection (qualitative result) but rather the intensity of infection (quantitative result), determined by the number of worms infecting the human host (worm burden). While greater morbidity is due to higher worm burdens, the precise number of worms necessary to cause morbidity may vary from person to person. A study looking at how prevalence of infection may relate to prevalence of morbidity found that population risk of morbidity increases non-linearly with prevalence of infection. Until the prevalence of infection is around 60%, the predicted morbidity is thought to be low, but after 60% the predicted morbidity increases rapidly.[18]

Determining the number of worms infecting a host is done by direct assessment at post-mortem examination or an indirect assessment by counting worms expelled after drug treatment. Quantifying the number of eggs excreted by the adult female worms and shed by the human host in stool is more feasible and as a result, the accepted method to determine prevalence and intensity of infection.[19] CAA levels in serum and urine generally correlate well with worm burden (as determined by egg output); however, the actual quantities produced by the worms and detected in the host circulation or excreta may depend on many factors, including host and parasite species, clearance rates, or duration and intensity of infection.[12]

Additionally, WHO-endorsed MDA guidelines are based on prevalence estimates (qualitative result); however, WHO targets for schistosomiasis control are based on intensity of infection (quantitative result).[20] For the use case of MDA reduction or stopping decision, the accepted nature of result is qualitative, though the ideal nature of result is also quantitative.

Table 3: Categories of infection intensity.[3]

Parasite	Light-intensity infections[b]	Moderate-intensity infections[b]	Heavy-intensity infections[b]
A. lumbricoides	1–4 999 epg	5 000–49 999 epg	≥50 000 epg
T. trichiura	1–999 epg	1 000–9 999 epg	≥10 000 epg
Hookworms	1–1 999 epg	2 000–3 999 epg	≥4 000 epg
S. mansoni	1–99 epg	100–399 epg	≥400 epg
S. haematobium	1–50 eggs/10 ml of urine		>50 eggs/10 ml of urine (or visible haematuria)

[b] epg = eggs per gram of feces.

Table 4: Goals, interventions, and targets for schistosomiasis control and elimination [20]

Group	Countries eligible for control of morbidity	Countries eligible for elimination as a public-health problem	Countries eligible for elimination (interruption of transmission)	VERIFICATION	Countries that have achieved elimination
Goal	Control morbidity	Eliminate schistosomiasis as a public-health problem	Eliminate schistosomiasis (nterrupt transmission)		Implement post-elimination surveillance
Recommended interventions	Preventive chemotherapy Complementary public-health interventions where possible	Adjusted preventive chemotherapy Complementary public-health interventions strongly recommended	Intensified preventive chemotherapy in residual areas of transmission Complementary public-health interventions considered essential		Surveillance to detect and respond to resurgence of transmission and to prevent reintroduction
Targets	100% geographical coverage and ≥75% national coverage Prevalence of heavy-intensity infection <5% across sentinel sites	Prevalence of heavy-intensity infection <1% in all sentinel sites	Reduce incidence of infection to zero		Incidence of infection remains zero (no cases)
Estimated time to progress from one group to the next	Up to 5–10 years from joining the group	Up to 3–6 years from joining the group	Up to 5 years from joining the group		To continue until all countries have interrupted transmission

2.7 Time to result

Acceptable: Same-day result, < 24 hours.

Ideal: Same-day result, < 15 minutes.

Results should be same-day to expedite surveillance team workflow and travel, but could take hours if throughput is still reasonable. Since this test is primarily focused on surveillance rather than clinical case management, time to result is not necessarily bound to the logistics of the clinical intervention.

The important related criteria is the overall throughput, where it is presumed that time to result should fit into the surveillance team's workflow such that they are able to meet the daily testing goals. Therefore, while contributions to time to result related to direct labor of the test administrator (hands-on time) are important, contributions based on wait times for results to develop are less important. Additionally, test batching may help reach hands-on and wait time goals while maintaining throughput needs. Ideally, however, there is some value to the workflow in obtaining results quickly, which is reflected in the ideal case.

Interviews with stakeholders involved in helminth surveillance programs mentioned the importance of having results quickly, regardless of clinical management. The results are more likely to reach the communities in a timely manner if rapid tests are used, and the more immediately the result is generated, the easier it is to provide to the participant. It was considered ethically necessary to provide results to the

communities, as well as to the participants. Additionally, returning results quickly was important for continued community engagement.

2.8 Throughput

Acceptable: > 50 samples per user per day.

Ideal: > 100 samples per user per day.

WHO recommendations for monitoring and evaluation of helminth control programs suggest a sentinel site method.[3] Sentinel sites composed of schools should be in each ecological zone and proportional to the number of school-age children in that zone. Roughly one sentinel site per 200,000 to 300,000 targeted children and a cluster sampling of approximately 50 children per school is suggested. The number of sentinel sites evaluated per day may depend on their distance from each other. The recommendation states that the surveillance team "should be able to collect and analyze specimens from at least 50 children in a sentinel site in one or two days," though possibly two sentinel sites per day may be ideal.[3] Recent discussions with stakeholders involved in helminth surveillance programs also specified that they expect a throughput of 50 samples per day when using the Kato-Katz technique. Therefore, a throughput of 50 samples per user per day is acceptable, while 100 samples per user per day may be ideal.

A balance would need to be found between available personnel resources, number of surveillance sites, and level of throughput. A semi-batch strategy, where samples are prepared quickly and then analyzed in parallel, may be recommended to reach higher throughput needs and potentially allow for resource savings.

2.9 Instrumentation format and complexity level

Acceptable: Field-based, rapid diagnostic test, few timed steps, no technically difficult techniques, Clinical Laboratory Improvement Amendments (CLIA)-waived.

Ideal: Field-based, rapid diagnostic test, no more than one timed step, automatic result reading, no technically difficult techniques, CLIA-waived.

Data from stakeholder interviews support the need for a simple, field-based rapid diagnostic test. Stakeholders noted logistical challenges of conducting surveillance in remote communities. Namely, transporting supplies and specimens is labor and cost-intensive. This test format is further supported in the literature.[2]

The level of complexity should be consistent with the site where it is used (point-of-care in the community) and the end user (surveillance technician, community health worker). It should consist of only a few timed steps, ideally one, and not require technical steps such as precision pipetting. Results would ideally be automatic and simple to interpret. Any necessary training should be very minimal for a surveillance technician. Using the US Food and Drug Administration's (FDA) categories for complexity of diagnostic tests as a reference, the assay should be CLIA-waived.

> "The FDA categorizes diagnostic tests by their complexity—from the least to the most complex: waived tests, moderate complexity tests, and high complexity tests. Diagnostic tests are

categorized as waived based on the premise that they are simple to use, and there is little chance the test will provide wrong information or cause harm if it is done incorrectly. Tests that are cleared by the FDA for home or over-the-counter use are automatically assigned a waived categorization."[21]

2.10 Infrastructure requirements

Acceptable: Minimal, consistent with Tier 2 facility.

Ideal: None.

Schistosoma infections are due to a lack of efficient sanitation infrastructure. Lack of efficient sanitation infrastructure is often seen in areas where there is lack of general health infrastructure. Therefore, if the product format is a field-deployable rapid diagnostic test, minimal infrastructure requirements must be needed. The test should not require any external power sources, only a self-contained portable source if necessary. There is no guarantee of usable water in the field environments where this would be used; therefore, the test should not have water requirements.

2.11 Test-specific training requirements

Acceptable: Minimal, consistent with Tier 2 facility.

Ideal: None.

Based on the target user and location of use, any necessary test-specific training needs to be minimal and not technical in nature.

2.12 Instrumentation size and weight

Acceptable: Small, easily deployable in the field.

Ideal: No instrument.

For a field-deployable test, the instrument must be small enough to be carried into potentially remote communities. Ideally, there would be no additional instrumentation or equipment.

Additional data are not currently available for this attribute.

2.13 Ancillary supplies

Acceptable: Minimal supplies to ensure optimal test performance, packaged as a kit.

Ideal: None.

A testing platform that is field-deployable requires that ancillary supplies must be minimal. To attain an optimal sensitivity or a semi-quantitative result, the addition of a reader may be acceptable. Other supplies that may be acceptable are for specimen concentration or quality control checks, such as verification cartridges. If other supplies are needed, it is acceptable that they are provided as a kit. Ideally, no instruments or other supplies are required.

2.14 Mean time between failures

Acceptable: Minimal for instrument, not applicable for single use test.

Ideal: No failures.

An acceptable time frame for failures would be minimal, though an instrument that has no failures would be ideal.

Additional data are not currently available for this attribute.

2.15 Quality control

Acceptable: Positive and negative control.

Ideal: Positive and negative control.

The manufacturer should maintain appropriate industry quality standards. Positive and negative controls are necessary for each test or batch of tests.

Additional data are not currently available for this attribute.

2.16 Calibration

Acceptable: No run-to-run calibration required, instrument calibration not required in field.

Ideal: None.

Ideally, no calibration would be required, particularly in a field scenario. If required for a portable field instrument, the interval between calibrations should be sufficiently long to not burden surveillance teams.

Additional data are not currently available for this attribute.

2.17 Product shelf life

Acceptable: 12-month shelf life.

Ideal: 36-month shelf life, packaging should include thermal indicator.

Based on PATH experience, it is suggested that a shelf life less than 12 months is insufficient as the time from manufacturing to delivering a test to the user in country is often a minimum of 12 months. It is suggested that a shelf life of 1 year is acceptable, and as many as 3 years would be closer to ideal. Additionally, it would be ideal for the test or kit to have an on-board temperature indicator to alert to extreme conditions exposure.

3. Performance

3.1 Analytical limit of detection

Acceptable: Concentration of CAA corresponding to the number of worm pairs equivalent to the desired clinical sensitivity.

Ideal: Concentration of CAA corresponding to a single worm pair ~1 pg/mL in serum, or ~0.1 pg/mL in urine.

The unit of measurement of current direct microscopy tests is the number of parasite eggs per gram (epg) of stool, which is a proxy for number of worms infecting the individual (see Section 2.6: Nature of result). Though the correlation between egg counts and worm burden is acceptable, several factors cause variability in this correlation such as density-dependent fecundity and recent deworming treatment,[19,22] immunologic status of the host,[23] as well as fecal sampling method and daily fluctuations in egg excretion.[24,25] Additionally, the positive correlation between CAA and egg counts is affected by the same variability as the correlation between egg counts and worm burden. It has been suggested that CAA is a better proxy for worm burden than egg counts.[26,27] A direct comparison of CAA levels and worm burden in primate models where direct measurement of worms is possible supports this assertion.[28]

A single worm pair is the smallest discrete unit of infection. When diagnosing a symptomatic case, or determining prevalence in high-transmission regions, a higher limit of detection is acceptable. However, if true *infection detection* is desired, an analytical limit of detection of one worm pair is necessary. Worm pairs live on average 3 to 5 years and as long as 30 years in some cases. With a theoretical reproductive potential of 600 billion, the persistence of even a single egg-laying worm pair constitutes a significant risk for future transmission.[29] Greater sensitivity is a priority when prevalence levels and infection intensities are low, such as when deciding to reduce or stop MDA. The ideal case would detect the lowest unit of infection.

Acceptable performance depends on the correlation between analytical limit of detection and clinical sensitivity, which would be specific to the test design. Acceptable levels, therefore, would achieve the desired clinical sensitivity.

3.2 Analytical specificity

Acceptable: Detects *S. mansoni* and *S. haematobium*, does not detect other helminths.

Ideal: Detects *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and/or *S. guineensis*, does not detect other helminths.

Table 5: Geographic distribution of Schistosoma species.[20]

	Species	Geographical distribution
Intestinal schistosomiasis	*mansoni*	Africa, the Middle East, the Caribbean, Brazil, Venezuela, Suriname
	japonicum	China, Indonesia, the Philippines
	mekongi	Several districts of Cambodia and the Lao People's Democratic Republic
	guineensis and related *S. intercalatum*	Rain forest areas of central Africa
Urogenital schistosomiasis	*haematobium*	Africa, the Middle East

According to WHO, it is estimated that at least 90% of those requiring treatment live in Africa. The most prevalent and clinically important species are *S. mansoni* and *S. haematobium*, with *S. japonicum* and others contributing to < 10% of the disease burden. Based on this information, acceptable diagnostics would be specific for *S. mansoni* and *S. haematobium*, and an ideal test would also detect *S. japonicum*, *S, mekongi*, *S. intercalatum*, and/or *S. guineensis*. Also of note is that many of the regions where *S. japonicum* is endemic are in China, where elimination programs are already well under way and there is less relative need for intervention. Additionally, it is critical that the tests do not cross-react with other helminth infections.

3.3 Clinical sensitivity

Acceptable: > 70%.

Ideal: > 95%.

The clinical sensitivity required will be related to prevalence levels. For the MDA monitoring scenario, 70% sensitivity may be sufficient as early MDA rounds will have higher prevalence. However, as the stopping decision approaches and prevalence is significantly reduced, presumably at or below 1%,[8] sensitivity would need to be the highest attainable with a lateral flow test. Priority at the stopping decision is to identify as many cases as possible since there will be few, and each positive is of increased importance.

Additional data are not currently available for this attribute.

3.4 Clinical specificity

Acceptable: > 95%.

Ideal: > 99%.

Clinical specificity becomes increasingly important as prevalence is reduced. At high prevalence, a 5% false-positive level is not a barrier, but as the stopping decision is approached, the level of false positives should be a minimal fraction of the prevalence level. It is presumed that at the stopping decision, with very low numbers of positives, all positives detected would be investigated and retested with the same or an alternative testing method. However, to avoid overburdening the surveillance program, samples requiring follow-up should be kept to a minimum.

Acceptable clinical specificity for the MDA monitoring and stopping-decision scenario should be at least > 95%, and ideally > 99% to minimize false positives at low prevalence.

3.5 Reproducibility and robustness

Acceptable: Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.

Ideal: Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.

As a preliminary target, replicate determinations of weak positive and weak negative samples (close to the presumptive cutoff) should classify the same ≥ 95% of the time.[30]

Additional data are not currently available for this attribute.

3.6 Comparative reference method

Acceptable: Kato-Katz (multiple slides and multiple days) and/or urine filtration/egg counting.

Ideal: An appropriate composite reference standard (CRS).

Direct microscopy such as Kato-Katz and/or urine filtration (egg counting) have significant limitations as diagnostics for schistosomiasis, however, they are the most commonly used reference method. Studies have been conducted to quantify the shortcomings of these tests, or optimize them such as taking multiple samples over multiple days to improve sensitivity.[31] While impractical to base aggressive elimination programs on such techniques, egg-counting data are necessary as part of any reference standard until new tests are fully validated.

Numerous studies have noted the absence of a "gold standard" for the detection of helminths. Studies evaluating Kato-Katz, as well as newer diagnostic technologies, have used a range of techniques to compensate including combining multiple tests as a reference and using mathematical models such as latent class analysis (see Table 6). Though there is no universally accepted method to adjust for the lack of a perfect gold standard,[32] one option is to develop a CRS.[33] A CRS combines more than one imperfect diagnostic test with the goal of increasing diagnostic accuracy (compared to truth—the true presence of infection). An important consideration is that the index test under evaluation is not included in the CRS, as this leads to biased diagnostic accuracy estimates.[34,35] Future evaluations of new diagnostics for helminths may benefit from the use of a CRS as the reference test. Possible CRS may include: multiple microscopy techniques performed on one stool sample, multiple microscopy techniques performed on stool samples collected over multiple consecutive days, and a microscopy technique and PCR technique performed on the same stool samples. Mathematical models may also be utilized to further explore the diagnostic accuracy of new tests under evaluation.

Table 6: A sample of studies using methods to compensate for an imperfect gold standard test for helminth infection.

Study	Study purpose	Helminth species	Reference standard
Knopp, S.; *Am. J. Trop. Med. Hyg.*; 2014[36]	Evaluate the diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR	Hookworm, *S. stercoralis*	3 methods: direct comparison, composite reference, Bayesian method
Bisoffi, Z.; 2014; *PLoS NTD*[37]	Evaluate the diagnostic accuracy of the 5 serologic assays for detecting *S. stercoralis* infection	*S. stercoralis*	Stool positive (formol-ether concentration, Baermann, or agar/charcoal culture, 3 samples) or at least 3 positive results out of 5 serologic tests (3 non-commercial tests and 2 commercial tests)

Study	Study purpose	Helminth species	Reference standard
Carvalho, G.L.X.; 2012; *Mem Inst Oswaldo Cruz* [38]	Compare the diagnostic accuracy of TF-Test with results from 4 other copromicroscopic techniques	*S. mansoni, A. lumbricoides,* hookworm, *S. stercoralis*	Combined results from all 5 copromicroscopic techniques
Glinz, D.; 2010; *PLoS NTD* [39]	Determine the diagnostic accuracy of 4 copromicroscopic techniques	*S. mansoni, A. lumbricoides,* hookworm, *T. trichiura*	Combined results of all 4 methods and at all time-points investigated
Verani, J.; 2011; *AJTMH* [40]	Cross-sectional evaluation of *S. mansoni* prevalence in pre-school age children compared to school age children in Kenya	*S. mansoni*	Stool positive (Kato-Katz, duplicate slides on 3 consecutive samples) or schistosome adult worm protein-specific ELISA positive
Utzinger, J.; 2008; *Trans R Soc Trop Med Hyg* [41]	Evaluate FLOTAC as new technique to diagnosis hookworm infection	Hookworm	Combined results from Kato-Katz, FLOTAC, and ether concentration technique
Goodman, D.; 2007; *Am J Trop Med Hyg* [42]	Compare multiple methods for the detection of STH eggs in infants	*T. trichiura,* hookworm, *A. lumbricoides*	Combined results from all copromicroscopic methods evaluated
Knopp, S.; 2008; *PLoS NTD* [43]	Elucidate the effect of repeated stool sampling and the use of different diagnostic methods for STH	*T. trichiura,* hookworm, *A. lumbricoides, S. stercorali*	Mathematical model by Marti et al.[44]
Marti, H.; 1993; *J Clin Epi* [44]	Obtain adjusted estimates of prevalence and sensitivity using a mathematical model	*E. histolytica, G. lamblia, T. trichiura, A. lumbricoides,* hookworms	Mathematical model using multiple stool samples to estimate false negative rates and obtain estimates of prevalence and sensitivity
Nikolay, B.; 2014; Int J Parasitol [45]	Conduct robust global assessment (meta-analysis) of relative performance of available diagnosis tools for STH	*A. lumbricoides,* hookworm, *T. trichiura*	Bayesian latent class model
Goncalves, A.Q.; 2014; *Acta Tropica* [46]	Compare repeatability, concordance, and accuracy of 2 spontaneous sedimentation techniques	*G. lamblia, E. histolytica, Blastocystis spp., A. lumbricoides,* hookworm, *T. trichiura, C. hepaticum*	Bayesian latent class model
Tarafder, M.R.; 2010; *Int J Parasitol* [47]	Estimate the sensitivity and specificity of Kato-Katz using a Bayesian approach in the absence of a 'gold standard'	*A. lumbricoides,* hookworm, *T. trichiura*	Bayesian latent class model

Study	Study purpose	Helminth species	Reference standard
Booth, M.; 2003; *Parasitology* [48]	Estimate single and dual-species infections base on raw egg count data and after latent class analysis	*S. mansoni,* hookworm	Bayesian latent class model
Steinmann, P.; 2008; *Am J Trop Med Hyg* [49]	Evaluate the prevalence of multiparasitism in China examining multiple stool samples with 4 copromicroscopic techniques	8 helminth and 7 protozoa species	1) Combined results of all diagnostic methods 2) Mathematical model by Marti et al.[44] using multiple Kato-Katz measures

4. Commercialization

Research on the commercialization attributes is ongoing. Further detail will be added as it is available.

4.1 Desired end-user price

Acceptable: To be determined.

Ideal: To be determined.

The cost per child tested using a single Kato-Katz test was $10 to $12, depending on school or community-based sampling.[50]

Additional data are not currently available for this attribute.

4.2 Channels to market

Acceptable: To be determined.

Ideal: To be determined.

No data currently available.

4.3 Supply, service, and support

Acceptable: To be determined.

Ideal: To be determined.

No data currently available.

4.4 Product registration path and WHO prequalification

Acceptable: Not required for surveillance tests.

Ideal: Not required for surveillance tests.

Note: If WHO wants to use the test in low-risk areas where there is < 20% prevalence in school children to do "case by case treatment," then this would need to revisited as it would be case management.[51]

Appendices

Appendix A: Common diagnostic tools for schistosomiasis

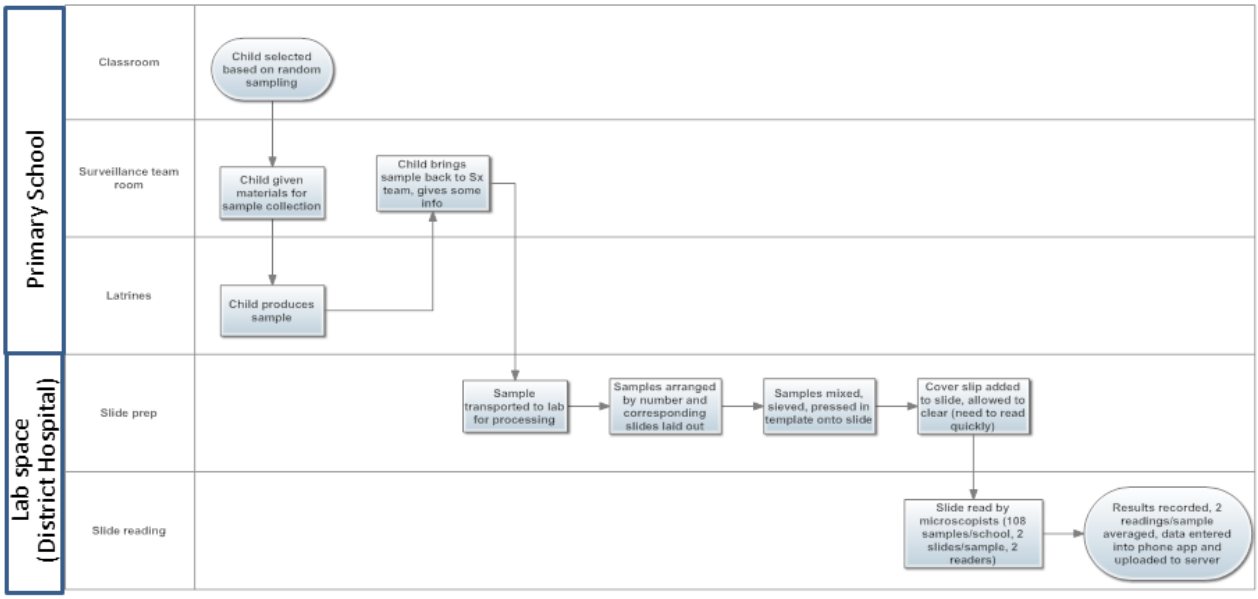
	Urine filtration	Kato-Katz
Technology	Microscopy	Microscopy
Description	Urine sample is fixed, stained, and filtered using a low-pressure vacuum pump, then examined via microscopy[52]	WHO-recommended technique that uses filtered stool of precise sample volume for microscopic egg detection[53]
Infrastructure required	Laboratory	Laboratory
User	Well-trained microscopist	Well-trained microscopist (half day training)[54]
Diagnostic target	Helminth egg	Helminth egg
Species detected	*S. haematobium*	*S. mansoni, S. japonicum, S. haematobium*
Sample type	Urine (collected 10am-2pm, last few drops of micturition to capture greatest egg burden)[55]	Fresh stool
Sample volume	10mL	41.7mg
Sample preparation	Manual	Manual
Limit of detection	Unknown	24 epg (eggs per gram) [39]
Sensitivity reference[a]	Dazo 1974[52]	Gold standard[b]
Result type	Quantitative (egg count)	Quantitative (epg)
Time to results (not including sample collection time)	12 to 24 hours (to allow for staining)[52]	~30 to 60 minutes (20 to 40 minutes clearing time required)[54]
Hardware / ancillary supplies	Aseptic filtration system, filter papers, vacuum pump, microscope	Microscope, K-K kit[39]
Commercially available	Yes	Yes
Stability, storage, and cold chain requirements	No cold chain required	No cold chain required
End user price (US$ per test)	Variable, depends on filtration system, microscope	$0.03 to $0.04[c] (cost represents 2009 US$)[54]
Manufacturer	Multiple depending on supplies	Helm-Test Kit made by Labmaster Ltd, Belo Horizonte, MG Brazil[38]; also provided by WHO (per 1998 WHO Geneva Supply Services document online)

[a] Paper cited includes latest test performance data.

[b] The Kato-Katz method is typically used as the gold standard. WHO advises two Kato-Katz slides from a single stool to diagnose schistosomiasis;[53] See Enk 2008[56] for data on relationship between number of Kato-Katz slides and disease prevalence.

[c] Price is an average of all supplies required for one stool test; for example, the Kato-Katz kit, microscope (based on assumed life expectancy and use), gloves, etc. It does not include staff salaries or infrastructure costs.

Appendix B: Process map of helminth surveillance (Sx) project in Kenya



1) Child selected in classroom based on random number generation
2) Child sent to classroom where Sx team is set up, given materials for sample collection
3) Child sent to latrine to make sample
4) Child brings sample back to Sx team
   - Child gives some data and sample to Sx team
5) Samples taken from school to clinic lab space
6) Samples sorted and materials arranged
7) Sample mixed, sieved, pressed in template on slide
8) Coverslip added to sample on slide, pressed, allowed to clear at least 10 min (not too long though)
9) Slide read by microscopist, 2 slides/sample, 216 slides per school
10) Results written down by microscopist
11) Written down data entered into phone app, saved on server

References

1. Lustigman S, Prichard RK, Gazzinelli A, et al. A research agenda for helminth diseases of humans: the problem of helminthiases. *PLoS Negl Trop Dis.* 2012;6(4):e1582

2. Solomon AW, Engels D, Bailey RL, et al. A diagnostics platform for the integrated mapping, monitoring, and surveillance of neglected tropical diseases: rationale and target product profiles. *PLoS Negl Trop Dis.* 2012;6(7):e1746

3. World Health Organization. *Helminth control in school-age children: A guide for managers of control programmes, Second Edition.* 2011.

4. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol.* 2009;25(4):151–156.

5. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. *Lancet.* 2014;383(9936):2253–2264.

6. World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: Report of a WHO expert committee. 2002. Available at: http://apps.who.int/iris/bitstream/10665/42588/1/WHO_TRS_912.pdf.http://www.who.int/iris/handle/10665/42588. Accessed December 1, 2014.

7. Solomon AW, Engels D, Bailey RL, et al. A diagnostics platform for the integrated mapping, monitoring, and surveillance of neglected tropical diseases: rationale and target product profiles. *PLoS Negl Trop Dis.* 2012;6(7):e1746

8. World Health Organization. *Schistosomiasis: progress report 2001-2011, strategic plan 2012-2020.* 2013.

9. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Med.* 2012;9(9):e1001306

10. World Health Organization. *Report of an informal consultation on schistosomiasis control, Geneva, Switzerland, 30 March-1 April 2011.* 2011.

11. van LL, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. *Acta Trop.* 2000;77(1):69–80.

12. Van Dam GJ, Bogitsh BJ, van Zeyl RJ, Rotmans JP, Deelder AM. Schistosoma mansoni: in vitro and in vivo excretion of CAA and CCA by developing schistosomula and adult worms. *J Parasitol.* 1996;82(4):557–564.

13. Polman K, Diakhate MM, Engels D, et al. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. *Trop Med Int Health.* 2000;5(8):534–537.

14. Polman K, Engels D, Fathers L, Deelder AM, Gryseels B. Day-to-day fluctuation of schistosome circulating antigen levels in serum and urine of humans infected with Schistosoma mansoni in Burundi. *Am J Trop Med Hyg.* 1998;59(1):150–154.

15. Luchavez J, Lintag ME, Coll-Black M, Baik F, Bell D. An assessment of various blood collection and transfer methods used for malaria rapid diagnostic tests. *Malar J.* 2007;6:149

16. Utzinger J, N'goran EK, Caffrey CR, Keiser J. From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis. *Acta Trop.* 2011;120 Suppl 1:S121–S137.

17. Dacombe RJ, Crampin AC, Floyd S, et al. Time delays between patient and laboratory selectively affect accuracy of helminth diagnosis. *Trans R Soc Trop Med Hyg.* 2007;101(2):140–145.

18. Guyatt HL, Bundy DA. Estimating prevalence of community morbidity due to intestinal helminths: prevalence of infection as an indicator of the prevalence of disease. *Trans R Soc Trop Med Hyg.* 1991;85(6):778–782.

19. Anderson RM, Schad GA. Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. *Trans R Soc Trop Med Hyg.* 1985;79(6):812–825.

20. World Health Organization. Sustaining the drive to overcome the global impact of neglected tropical diseases: Second WHO report on neglected tropical diseases. 2013. Available at: http://www.who.int/iris/bitstream/10665/77950/1/9789241564540_eng.pdf.http://www.who.int/neglected_diseases/9789241564540/en/. Accessed December 1, 2014.

21. U.S.Food and Drug Administration. CLIA Categorizations. 2014. Available at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm393229.htm. Accessed December 1, 2014.

22. Kotze AC, Kopp SR. The potential impact of density dependent fecundity on the use of the faecal egg count reduction test for detecting drug resistance in human hookworms. *PLoS Negl Trop Dis.* 2008;2(10):e297

23. Muok EM, Simiyu EW, Ochola EA, et al. Association between CD4+ T-lymphocyte counts and fecal excretion of Schistosoma mansoni eggs in patients coinfected with S. mansoni and human immunodeficiency virus before and after initiation of antiretroviral therapy. *Am J Trop Med Hyg.* 2013;89(1):42–45.

24. Hall A, Anwar KS, Tomkins A, Rahman L. The distribution of Ascaris lumbricoides in human hosts: a study of 1765 people in Bangladesh. *Trans R Soc Trop Med Hyg.* 1999;93(5):503–510.

25. Hall A, Holland C. Geographical variation in Ascaris lumbricoides fecundity and its implications for helminth control. *Parasitol Today.* 2000;16(12):540–544.

26. Leutscher PD, van Dam GT, Reimert CM, Ramarakoto CE, Deelder AM, Ornbjerg N. Eosinophil cationic protein, soluble egg antigen, circulating anodic antigen, and egg excretion in male urogenital schistosomiasis. *Am J Trop Med Hyg.* 2008;79(3):422–426.

27. De CD, Sacko M, Vercruysse J, et al. Comparison of the circulating anodic antigen detection assay and urine filtration to diagnose Schistosoma haematobium infections in Mali. *Trans R Soc Trop Med Hyg.* 1995;89(4):395–397.

28. Alan WR, Van Dam GJ, Kariuki TM, Farah IO, Deelder AM, Coulson PS. The detection limits for estimates of infection intensity in schistosomiasis mansoni established by a study in non-human primates. *Int J Parasitol.* 2006;36(12):1241–1244.

29. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet.* 2006;368(9541):1106–1118.

30. U.S.Food and Drug Administration. Recommendations: Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices. 2008. Available at: http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm070890.pdf.http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079632.htm. Accessed December 1, 2014.

31. de Vlas SJ, Gryseels B, van Oortmarssen GJ, Polderman AM, Habbema JD. A pocket chart to estimate true Schistosoma mansoni prevalences. *Parasitol Today.* 1993;9(8):305–307.

32. Reitsma JB, Rutjes AW, Khan KS, Coomarasamy A, Bossuyt PM. A review of solutions for diagnostic accuracy studies with an imperfect or missing reference standard. *J Clin Epidemiol.* 2009;62(8):797–806.

33. Alonzo TA, Pepe MS. Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Stat Med.* 1999;18(22):2987–3003.

34. Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med.* 2004;140(3):189–202.

35. Whiting PF, Rutjes AW, Westwood ME, Mallett S. A systematic review classifies sources of bias and variation in diagnostic test accuracy studies. *J Clin Epidemiol.* 2013;66(10):1093–1104.

36. Knopp S, Salim N, Schindler T, et al. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and Strongyloides stercoralis infections in Tanzania. *Am J Trop Med Hyg.* 2014;90(3):535–545.

37. Bisoffi Z, Buonfrate D, Sequi M, et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS Negl Trop Dis.* 2014;8(1):e2640

38. Carvalho GL, Moreira LE, Pena JL, Marinho CC, Bahia MT, hado-Coelho GL. A comparative study of the TF-Test(R), Kato-Katz, Hoffman-Pons-Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis. *Mem Inst Oswaldo Cruz.* 2012;107(1):80–84.

39. Glinz D, Silue KD, Knopp S, et al. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for Schistosoma mansoni and soil-transmitted helminths. *PLoS Negl Trop Dis.* 2010;4(7):e754

40. Verani JR, Abudho B, Montgomery SP, et al. Schistosomiasis among young children in Usoma, Kenya. *Am J Trop Med Hyg.* 2011;84(5):787–791.

41. Utzinger J, Rinaldi L, Lohourignon LK, et al. FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Trans R Soc Trop Med Hyg.* 2008;102(1):84–90.

42. Goodman D, Haji HJ, Bickle QD, et al. A comparison of methods for detecting the eggs of Ascaris, Trichuris, and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants. *Am J Trop Med Hyg.* 2007;76(4):725–731.

43. Knopp S, Mgeni AF, Khamis IS, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis.* 2008;2(11):e331

44. Marti H, Koella JC. Multiple stool examinations for ova and parasites and rate of false-negative results. *J Clin Microbiol.* 1993;31(11):3044–3045.

45. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. *Int J Parasitol.* 2014;44(11):765–774.

46. Goncalves AQ, Abellana R, Pereira-da-Silva HD, et al. Comparison of the performance of two spontaneous sedimentation techniques for the diagnosis of human intestinal parasites in the absence of a gold standard. *Acta Trop.* 2014;131:63–70.

47. Tarafder MR, Carabin H, Joseph L, Balolong E Jr, Olveda R, McGARVEY ST. Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, Ascaris lumbricoides and Trichuris trichiura infections in humans in the absence of a 'gold standard'. *Int J Parasitol.* 2010;40(4):399–404.

48. Booth M, Vounatsou P, N'goran EK, Tanner M, Utzinger J. The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing Schistosoma mansoni and hookworm co-infections in rural Cote d'Ivoire. *Parasitology.* 2003;127(Pt 6):525–531.

49. Steinmann P, Du ZW, Wang LB, et al. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg.* 2008;78(5):760–769.

50. Assefa LM, Crellen T, Kepha S, et al. Diagnostic accuracy and cost-effectiveness of alternative methods for detection of soil-transmitted helminths in a post-treatment setting in western Kenya. *PLoS Negl Trop Dis.* 2014;8(5):e2843

51. World Health Organization. Soil-transmitted helminthiases: Eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and strategic plan 2011-2020. 2012. Available at: http://apps.who.int/iris/bitstream/10665/44804/1/9789241503129_eng.pdf.http://www.who.int/iris/handle/10665/44804. Accessed December 1, 2014.

52. Dazo BC, Biles JE. Two new field techniques for detection and counting of Schistosoma haematobium eggs in urine samples, with an evaluation of both methods. *Bull World Health Organ.* 1974;51(4):399–408.

53. Lamberton PH, Kabatereine NB, Oguttu DW, Fenwick A, Webster JP. Sensitivity and specificity of multiple Kato-Katz thick smears and a circulating cathodic antigen test for Schistosoma mansoni diagnosis pre- and post-repeated-praziquantel treatment. *PLoS Negl Trop Dis.* 2014;8(9):e3139

54. Speich B, Knopp S, Mohammed KA, et al. Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. *Parasit Vectors.* 2010;3:71

55. van LL, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. *Acta Trop.* 2000;77(1):69–80.

56. Enk MJ, Lima AC, Drummond SC, Schall VT, Coelho PM. The effect of the number of stool samples on the observed prevalence and the infection intensity with Schistosoma mansoni among a population in an area of low transmission. *Acta Trop.* 2008;108(2-3):222–228.