Target Product Profile

Soil-Transmitted Helminth Surveillance Diagnostic

Use case: Post-elimination surveillance

Platform: Lateral-flow test (multiplex)

Biomarker: Soil-transmitted helminth species-specific antibodies
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Executive Summary

Neglected tropical diseases (NTD) affect the poorest populations. Several NTDs, including soil-transmitted helminths (STH), are controlled by preventive chemotherapy (PC) in the form of periodic mass drug administration (MDA). In areas with insufficient sanitation, schistosomes and STH are transmitted by eggs excreted in human stool and/or urine that contaminate the environment. Globally, over 2 billion individuals are infected with at least one, though often more than one, of the following STH species: *Ascaris lumbricoides; Trichuris trichiura;* and the hookworm species, *Ancylostoma duodenale* and *Necator americanus.*

Control programs based on MDA have the following four designated stages: mapping disease distribution, impact monitoring of MDA interventions, stopping decisions for MDA, and post-elimination surveillance. Current diagnostics, including the Kato-Katz technique, are thought to be sufficient for mapping disease distribution (Appendix A: Common diagnostic tools for soil-transmitted helminths). As the most commonly used method for STH 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 diminishes its utility in later disease control stages. To support STH control programs to continue to move towards 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 accurate post-elimination surveillance. 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 and differentiates STH antibodies. Important to note, there are limited guidelines for post-elimination surveillance of NTDs, especially STH, as few if any locations have reached this goal. Attributes were informed based on current knowledge and would benefit from further refinement as new guidelines are created.
## Overview of Target Product Profile

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Context (use case)</td>
<td>More sensitive than current microscopic methods, field deployable, rapid diagnostic test to monitor elimination.</td>
<td>More sensitive than current microscopic methods, field deployable, rapid diagnostic test to monitor elimination.</td>
</tr>
<tr>
<td>1.1 Clinical and/or surveillance need (value proposition)</td>
<td>Post-elimination surveillance after stopping mass drug administration (MDA).</td>
<td>Post-elimination surveillance after stopping mass drug administration.</td>
</tr>
<tr>
<td>1.2 Intended use (use case)</td>
<td>Primary school children 6 to 14 years old.</td>
<td>Primary school children 6 to 14 years old and other high-risk populations including preschool-aged children, women of reproductive age, and adults exposed to soil-transmitted helminth (STH) infections.</td>
</tr>
<tr>
<td>1.3 Target populations</td>
<td>Countries where MDA for STH has recently stopped.</td>
<td>Countries where MDA for STH has recently stopped.</td>
</tr>
<tr>
<td>1.5 Location of use (infrastructure level)</td>
<td>Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.</td>
<td>Tier 2 facility, school setting at the community level, minimal or no infrastructure requirements.</td>
</tr>
<tr>
<td>1.6 Target user</td>
<td>Surveillance teams made up of technicians from regional level, such as community health workers, with minimal training.</td>
<td>Surveillance teams made up of technicians from regional level, such as community health workers, with minimal training.</td>
</tr>
<tr>
<td>1.7 Fit with clinical workflow/ linkage to action (process map)</td>
<td>Identify recrudescence of infection by estimating community-wide seroprevalence.</td>
<td>Identify recrudescence of infection by estimating community-wide seroprevalence.</td>
</tr>
<tr>
<td>1.8 Desired stability, storage, and cold chain requirements</td>
<td>45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.</td>
<td>45°C, 40% to 88% relative humidity, withstand daily temperature fluctuations from 25°C to 40°C, no cold chain required.</td>
</tr>
<tr>
<td>2. Design</td>
<td>STH species-specific antibody.</td>
<td>STH species-specific antibody.</td>
</tr>
<tr>
<td>2.1 Analyte (diagnostic marker)</td>
<td>Serum or finger stick blood &lt; 100 μL.</td>
<td>Finger stick blood &lt; 10 μL.</td>
</tr>
<tr>
<td>2.2 Sample type and volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attribute</td>
<td>Acceptable</td>
<td>Ideal</td>
</tr>
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<td>------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.3 Sample preparation</td>
<td>Minimal collection or processing steps</td>
<td>None.</td>
</tr>
<tr>
<td>2.4 Sample transport stability</td>
<td>2 hours at ambient temperature, or time necessary to collect and analyze specimen.</td>
<td>6 hours at ambient temperature, or time necessary to collect and analyze specimen.</td>
</tr>
<tr>
<td>2.5 Waste management (hazardous materials/chemicals)</td>
<td>Minimal or no hazardous materials, per World Health Organization (WHO) and country standards.</td>
<td>Minimal or no hazardous materials, per WHO and country standards.</td>
</tr>
<tr>
<td>2.6 Nature of result</td>
<td>Qualitative.</td>
<td>Qualitative.</td>
</tr>
<tr>
<td>2.7 Time to result</td>
<td>Same-day result, &lt; 24 hours.</td>
<td>Same-day result, &lt; 15 minutes.</td>
</tr>
<tr>
<td>2.8 Throughput</td>
<td>&gt; 50 samples per user per day.</td>
<td>&gt; 100 samples per user per day.</td>
</tr>
<tr>
<td>2.9 Instrumentation format and complexity level</td>
<td>Field-based, rapid diagnostic test, few timed steps, no technically difficult techniques, CLIA-waived.</td>
<td>Field-based, rapid diagnostic test, no more than 1 timed step, automatic result reading, no technically difficult techniques, CLIA-waived</td>
</tr>
<tr>
<td>2.10 Infrastructure requirements</td>
<td>Minimal, consistent with Tier 2 facility.</td>
<td>None.</td>
</tr>
<tr>
<td>2.11 Test-specific training requirements</td>
<td>Minimal, consistent with Tier 2 facility.</td>
<td>None.</td>
</tr>
<tr>
<td>2.12 Instrumentation size and weight</td>
<td>Small, easily deployable in the field.</td>
<td>No instrument.</td>
</tr>
<tr>
<td>2.13 Ancillary supplies</td>
<td>Minimal supplies to ensure optimal test performance, packaged as a kit.</td>
<td>None.</td>
</tr>
<tr>
<td>2.14 Mean time between failure</td>
<td>Minimal for instrument, not applicable for single-use test.</td>
<td>No failures.</td>
</tr>
<tr>
<td>2.15 Quality control</td>
<td>Positive and negative control.</td>
<td>Positive and negative control.</td>
</tr>
<tr>
<td>2.16 Calibration</td>
<td>No run-to-run calibration required, instrument calibration not required in field.</td>
<td>None.</td>
</tr>
<tr>
<td>2.17 Product shelf life</td>
<td>12-month shelf life.</td>
<td>36-month shelf life; packaging should include thermal indicator</td>
</tr>
<tr>
<td>3. Performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 Analytical limit of detection</td>
<td>Concentration of antibody corresponding to recent infection.</td>
<td>Concentration of antibody corresponding to recent infection.</td>
</tr>
<tr>
<td>Attribute</td>
<td>Acceptable</td>
<td>Ideal</td>
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<td>-----------------------------------</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>3.2 Analytical specificity</td>
<td>Detect and distinguish specific antibodies against <em>Ascaris lumbricoides</em> (roundworm), <em>Trichuris trichiura</em> (whipworm), <em>Necator americanus</em> and <em>Ancylostoma duodenale</em> (hookworm); no cross-reaction with other parasites.</td>
<td>Detect and distinguish specific antibodies against <em>Ascaris lumbricoides</em> (roundworm), <em>Trichuris trichiura</em> (whipworm), <em>Necator americanus</em> and <em>Ancylostoma duodenale</em> (hookworm), and <em>Strongyloides stercoralis</em> (threadworm); no cross-reaction with other parasites.</td>
</tr>
<tr>
<td>3.3 Clinical sensitivity</td>
<td>&gt; 75%</td>
<td>&gt; 90%</td>
</tr>
<tr>
<td>3.4 Clinical specificity</td>
<td>&gt; 95%</td>
<td>&gt; 99%</td>
</tr>
<tr>
<td>3.5 Reproducibility and robustness</td>
<td>Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.</td>
<td>Replicate determinations of weak positive and weak negative samples classify the same ≥ 95% of the time.</td>
</tr>
<tr>
<td>3.6 Comparative reference method</td>
<td>Kato-Katz (multiple slides and/or multiple days).</td>
<td>An appropriate composite reference standard.</td>
</tr>
<tr>
<td>4. Commercialization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1 Desired end-user price</td>
<td>To be determined.</td>
<td>To be determined.</td>
</tr>
<tr>
<td>4.2 Channels to market</td>
<td>To be determined.</td>
<td>To be determined.</td>
</tr>
<tr>
<td>4.3 Supply, service, and support</td>
<td>To be determined.</td>
<td>To be determined.</td>
</tr>
<tr>
<td>4.4 Product registration path and WHO prequalification</td>
<td>Not required for surveillance tests.</td>
<td>Not required for surveillance tests.</td>
</tr>
</tbody>
</table>
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 monitor elimination.

Ideal: More sensitive than current microscopic methods, field deployable, rapid diagnostic test to monitor elimination.

Neglected tropical diseases (NTD) affect the poorest populations. Several NTDs, including soil-transmitted helminths (STH), are controlled by preventive chemotherapy (PC) in the form of periodic mass drug administration (MDA). In areas with insufficient sanitation, STH and schistosomes are transmitted by eggs excreted in human stool and/or urine that contaminate soil and water sources. For STH as well as schistosomiasis control, the school infrastructure is essential to administer MDA, as school-aged children have the greatest burden of infection and morbidity. Globally, over 2 billion individuals are infected with at least one, though often more than one, of the following STH species: *Ascaris lumbricoides*; *Trichuris trichiura*; and the hookworm species, *Ancylostoma duodenale* and *Necator americanus*. 1,2

Control programs based on MDA have the following four designated stages: mapping disease distribution, impact monitoring of MDA interventions, stopping decisions for MDA, and post-elimination surveillance. 3 Based on stakeholder opinions solicited at the STH Diagnostics Meeting (hosted by the Task Force for Global Health, Decatur, GA, August 2013), current diagnostics, including the Kato-Katz technique, are thought to be sufficient for mapping disease distribution (Appendix A: Common diagnostic tools for soil-transmitted helminths). However, as disease prevalence decreases through effective control strategies, a more-sensitive diagnostic will be necessary to inform control programs. 5

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 STH 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 STH is endemic in some very remote areas, a test that can function in the field with very minimal infrastructure is necessary. Surveillance activities may continue in the school setting even after MDA has stopped. Therefore, an acceptable test should be relatively simple and ready to use, requiring minimal training.
1.2 **Intended use (use case)**

**Acceptable:** Post-elimination surveillance after stopping MDA.

**Ideal:** Post-elimination surveillance after stopping MDA.

The purpose of this diagnostic is use during post-elimination surveillance to rapidly identify if recrudescence of infection occurs. 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 support elimination efforts. The target population may 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. Current guidelines and information suggest a need for a surveillance test which would not require individual patient follow-up. 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.

1.3 **Target populations**

**Acceptable:** Primary school children 6 to 14 years old.

**Ideal:** Primary school children 6 to 14 years old and other high-risk populations including pre-school-aged children, women of reproductive age, and adults exposed to STH infections.

STH 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 STH infections and schistosomiasis because the infections occur:

- 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 World Health Organization (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. Current guidelines are unclear if target populations for MDA would differ from target populations for post-MDA surveillance. We assume cohorts aged 6 to 14 years from sentinel primary schools will continue to be tested to assess their parasitological status. WHO guidelines suggest monitoring new cohorts of 6-year-olds may provide evidence of transmission levels within the community. The ideal
target population reflects the extended target population that may need to be targeted with MDA to achieve STH infection elimination.  

1.4 **Target countries/geographic coverage**

**Acceptable:** Countries where MDA for STH has recently stopped.

**Ideal:** Countries where MDA for STH has recently stopped.

Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China, and East Asia.

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 STH is endemic may have minimal or no infrastructure.

*Figure 1: The spectrum of point-of-care testing for target product profiles.*

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.

Surveillance activities may continue in the school setting even after MDA has stopped. The target user of the diagnostic would be surveillance teams composed of central or regional technicians, possibly community health workers. Therefore, an ideal test would be relatively simple and ready to use, requiring minimal training.

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

Acceptable: Identify recrudescence of infection by estimating community-wide seroprevalence.

Ideal: Identify recrudescence of infection by estimating community-wide seroprevalence.

While goals for other NTDs are elimination of infection, it is recognized that elimination of STH transmission and infection requires not only MDA but also improvements in water, sanitation, and hygiene. As a result, WHO recommendations and strategic plan for controlling STH infections focus on eliminating the morbidity of STH as a public health problem, which requires a decrease in the number of individuals with moderate- or high-intensity infections.

Treatment for STH is most often empiric rather than by a test-and-treat paradigm. Periodic treatment for STH 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. After MDA is stopped, frequent monitoring of infection exposure in the form of seroprevalence will be important to ensure recrudescence of infection has not occurred. Recrudescence would necessitate reengagement of the control program in the community. Currently there are no globally accepted criteria for post-MDA surveillance, but comparisons to other control programs (such as lymphatic filariasis) may be of value.
Based on field observation in Kenya, an example process map was generated depicting the current work flow of a helminth surveillance project (Appendix B: Process map of helminth surveillance project in Kenya). Most stakeholders involved in helminth surveillance commented on the logistical challenges of using stool as a specimen. Post-elimination surveillance would use some form of blood as the specimen, varying the ideal work flow. Immediate actions still would not be 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 have suggested that there are temperature fluctuations in the areas this test would serve, with results showing roughly 25°–40°C variations on a daily basis. This variability is unavoidable without cold chain support.

2. Design

2.1 Analyte (diagnostic marker)

Acceptable: STH species-specific antibody.

Ideal: STH species-specific antibody.

Antibodies specific to *A. lumbricoides*, *A. duodenale*, *N. americanus*, and *T. trichiura* will be used. Previous research will be utilized to identify promising targets.

Additional data are currently not available.

2.2 Sample type and volume

Acceptable: Serum or finger stick blood < 100 μL.

Ideal: Finger stick blood < 10 μL.

Due to the life cycle of STH parasites in the host, stool has been the necessary sample for diagnosis. Interviews with stakeholders involved in helminth surveillance mentioned that finger stick blood is an acceptable sample type. If efforts are made to integrate multiple NTD surveillance programs, using one common sample type is preferred, and blood will be the ideal sample type.

From a participant burden perspective, it has been recommended that blood sampling > 100 μL is typically unreasonable as it would require venipuncture sampling (Schistosomiasis and STH Diagnostics Meeting, August 2013). In the context of a large survey of children where the majority are expected to be uninfected and treatment is delivered irrespective of infection status, the risks and discomfort associated with venipuncture sampling may outweigh the benefit afforded to them, potentially making this method of sample collection unreasonable. If multiple assays could be evaluated from a single blood draw, either in a multiplex test or parallel tests, the risks of venipuncture sampling may be outweighed by the benefits. Ten μL is a realistic volume of finger stick blood used for rapid diagnostic test assays and is, therefore, suggested as an ideal volume.¹⁴

2.3 Sample preparation

Acceptable: Minimal collection or processing steps.

Ideal: None.
The more-commonly used diagnostics, such as the Kato-Katz technique, require significant sample preparation using stool. 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 additional steps to meet limit of detection (LOD) requirements, and these necessary sample processing steps may be acceptable to reach better diagnostic performance.

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. The sample stability should allow flexibility in the work flow in a point-of-care (POC) setting. Acceptable sample stability would allow for a reasonable time for sample preparation and analysis (~2 hours) or the time necessary to transport the specimen from the collection to the 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. Sample transport is not applicable for finger stick blood.

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.

WHO recommendations and strategic plan for controlling STH infections focus on eliminating the morbidity of STH as a public health problem, which requires a decrease in the number of individuals with moderate- or high-intensity infections. Morbidity due to helminths 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.

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.\textsuperscript{18} Additionally, while WHO-endorsed MDA guidelines are based on prevalence estimates (qualitative result), the WHO targets for STH control are based on intensity of infection (quantitative result).\textsuperscript{19}

For the use case of post-elimination surveillance, a qualitative test based on antibody detection would be sufficient to determine if recrudescence of infection has occurred.

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 work flow 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 work flow 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 work flow 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.\textsuperscript{4} 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.\textsuperscript{4} Recent discussions with stakeholders involved in helminth surveillance programs also specified that they expect a
throughput of 50 samples/day when using the Kato-Katz technique. Therefore, a throughput of 50 samples/user/day is acceptable, while 100 samples/user/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, CLIA-waived.

Ideal: Field-based, rapid diagnostic test, no more than 1 timed step, automatic result reading, no technically difficult techniques, CLIA-waived.

The test would ideally be in a rapid diagnostic format, particularly when using surveillance teams in the community. In this case, the test should be POC and field deployable, which experts also have stated is the ideal format.

The level of complexity should be consistent with the site where it is used (POC in the community) and the end-user (surveillance lab 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 lab technician.

Using the US Food and Drug Administration’s (FDA’s) 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.”

2.10 Infrastructure requirements

Acceptable: Minimal, consistent with Tier 2 facility.

Ideal: None.

STH 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. Ideally, the test would 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 currently not 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. If supplies are necessary to ensure optimal sensitivity, such as specimen concentration, or quality control, such as verification cartridges, this may be acceptable. If other supplies are needed, it is acceptable if they are provided as a kit. Ideally, no instruments or other supplies are required.

Additional data are currently not available for this attribute.

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 currently not 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.
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 currently not 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 exposure to extreme conditions.

3.  Performance

3.1  Analytical limit of detection

Acceptable: Concentration of antibody corresponding to recent infection.

Ideal: Concentration of antibody corresponding to recent infection.

The unit of measurement of current direct microscopy tests is the number of parasite eggs per gram of stool, which is a proxy for number of worms infecting the individual (see 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, immunologic status of the host, as well as fecal sampling method and daily fluctuations in egg excretion.

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 LOD is acceptable. However, if true infection detection is desired, an analytical LOD of one worm pair is necessary. Adult STH worms can live from 1–7 years depending on the species, causing the persistence of even a single egg-laying worm pair to constitute a risk for future transmission. Greater sensitivity is a priority when prevalence levels and infection intensities are low, such as when stopping MDA. A diagnostic test to identify recrudescence of infection would need to detect the lowest unit of infection. However, antibodies are markers of
exposure rather than infection. Understanding how immune responses correlate with infection over time will be integral to using seroprevalence as a marker of recrudescence.

Acceptable performance depends on the correlation between analytical LOD 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:** Detect and distinguish specific antibodies against *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* and *Ancylostoma duodenale* (hookworm); no cross-reaction with other parasites.

**Ideal:** Detect and distinguish specific antibodies against *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* and *Ancylostoma duodenale* (hookworm), and *Strongyloides stercoralis* (threadworm); no cross-reaction with other parasites.

*Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Necator americanus* and *Ancylostoma duodenale* (hookworm) are the major soil-transmitted helminths which are targeted for control through MDA programs. Detection and differentiation of these parasites is important for monitoring control programs.

The current tool, Kato-Katz, has problems detecting hookworm eggs due to their rapid degradation in fresh stool samples, so robust detection of hookworm infection is necessary for a new diagnostic. Additionally, detection of *Strongyloides stercoralis* is ideal due to its largely underestimated burden of disease, increasing interest and concern of stakeholders, and unique diagnostic challenges.

It is critical that the tests do not cross-react with other parasite infections.

### 3.3 Clinical sensitivity

**Acceptable:** > 75%.

**Ideal:** > 95%.

Technology platforms may have upper limits in terms of attainable sensitivity. The attainable and, therefore, acceptable clinical sensitivity for a test detecting antibodies may be 75%, depending on additional equipment such as a reader.

Additional data are currently not 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 MDA is stopped, the level of false positives should be minimal.
Acceptable clinical specificity for post-elimination surveillance should be > 95%, and ideally > 99% to minimize false positives at low prevalence.

Additional data are currently not available for this attribute.

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.28

Additional data are currently not available for this attribute.

3.6 Comparative reference method

Acceptable: Kato-Katz (multiple slides and/or multiple days).

Ideal: An appropriate composite reference standard (CRS).

It has been mentioned that microscopy will always be the cornerstone of STH identification.29 Direct microscopy, such as the Kato-Katz technique, has significant limitations as a diagnostic for STH, however, it is the most commonly used reference method. Studies have been conducted to quantify the shortcomings of the Kato-Katz test or optimize it such as taking multiple samples over multiple days to improve sensitivity.30 While impractical to base aggressive elimination programs on such a technique, 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 1). Though there is no universally accepted method to adjust for the lack of a perfect gold standard,31 one option is to develop a CRS.32 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.33,34 Future evaluations of new diagnostics for helminths may benefit from the use of a CRS as the reference test.

McCarthy et al. note that a priority for helminth diagnostics is to “improve and validate tools for quantifying intensity of infection (worm burden), including both biomarkers (coproantigen) and molecular methods (PCR-based)”11 An ideal component of a CRS may be qPCR, based on stakeholder opinions solicited at the STH Diagnostics Meeting (hosted by the Task Force for Global Health, Decatur, GA, August 2013). It was chosen because of its high sensitivity and the ability to understand not only the prevalence but also transmission dynamics of STH and potentially intensity of infection. A possible CRS
may include the following: multiple microscopy techniques performed on one stool sample, multiple microscopy techniques performed on stool samples collected over multiple consecutive days, or 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.

Moving from a measure of active infection to a measure of recent infection will also present challenges. A second immunoassay developed in parallel may be useful as part of a reference standard. More research is needed for this attribute.

Table 1: A sample of studies using methods to compensate for an imperfect gold standard test for helminth infection.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study purpose</th>
<th>Helminth species</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisoffi, Z.; 2014; <em>PLoS NTD</em>36</td>
<td>Evaluating the diagnostic accuracy of the 5 serologic assays for detecting <em>S. stercoralis</em> infection</td>
<td><em>S. stercoralis</em></td>
<td>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)</td>
</tr>
<tr>
<td>Carvalho, G.L.X.; 2012; <em>Mem Inst Oswaldo Cruz</em>37</td>
<td>Compare the diagnostic accuracy of TF-Test with results from 4 other copromicroscopic techniques</td>
<td><em>S. mansoni</em>, <em>A. lumbricoides</em>, hookworm, <em>S. stercoralis</em></td>
<td>Combined results from all 5 copromicroscopic techniques</td>
</tr>
<tr>
<td>Glinz, D.; 2010; <em>PLoS NTD</em>38</td>
<td>Determine the diagnostic accuracy of 4 copromicroscopic techniques</td>
<td><em>S. mansoni</em>, <em>A. lumbricoides</em>, hookworm, <em>T. trichiura</em></td>
<td>Combined results of all 4 methods and at all time points investigated</td>
</tr>
<tr>
<td>Verani, J.; 2011; <em>AJTMH</em>39</td>
<td>Cross-sectional evaluation of <em>S. mansoni</em> prevalence in pre-school age children compared to school age children in Kenya</td>
<td><em>S. mansoni</em></td>
<td>Stool positive (Kato-Katz, duplicate slides on 3 consecutive samples) or schistosome adult worm protein-specific ELISA positive</td>
</tr>
<tr>
<td>Utzinger, J.; 2008; <em>Trans R Soc Trop Med Hyg</em>40</td>
<td>Evaluate FLOTAC as new technique to diagnosis hookworm infection</td>
<td>Hookworm</td>
<td>Combined results from Kato-Katz, FLOTAC, and ether concentration technique</td>
</tr>
<tr>
<td>Study</td>
<td>Study purpose</td>
<td>Helminth species</td>
<td>Reference Standard</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>------------------------------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Knopp, S.; 2008; <em>PLoS NTD</em></td>
<td>Elucidate the effect of repeated stool sampling and the use of different</td>
<td><em>T. trichiura</em>, hookworm, <em>A. lumbricoides</em>,</td>
<td>Mathematical model by Marti et al.<em>43</em></td>
</tr>
<tr>
<td></td>
<td>diagnostic methods for STH</td>
<td><em>S. stercoralis</em></td>
<td></td>
</tr>
<tr>
<td>Marti, H.; 1993; <em>J Clin Epi</em></td>
<td>To obtain adjusted estimates of prevalence and sensitivity using a</td>
<td><em>E. histolytica</em>, <em>G. lamblia</em>, <em>T. trichiura</em>,</td>
<td>Mathematical model using multiple stool samples to</td>
</tr>
<tr>
<td></td>
<td>mathematical model</td>
<td><em>A. lumbricoides</em>, hookworms,</td>
<td>estimate false-negative rates and obtain estimates of</td>
</tr>
<tr>
<td>Nikolay, B.; 2014; *Int J</td>
<td>Robust global assessment (meta-analysis) of relative performance of available</td>
<td><em>A. lumbricoides</em>, hookworm, <em>T. trichiura</em></td>
<td>prevalence and sensitivity</td>
</tr>
<tr>
<td>Parasitol*</td>
<td>diagnostic tools for STH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goncalves, A.Q.;</td>
<td>Comparing repeatability, concordance, and accuracy of 2 spontaneous</td>
<td><em>G. lamblia</em>, <em>E. histolytica</em>, <em>Blastocystis</em></td>
<td>Bayesian latent class model</td>
</tr>
<tr>
<td>2014; <em>Acta Tropica</em></td>
<td>sedimentation techniques</td>
<td><em>spp.</em>, <em>A. lumbricoides</em>, hookworm, *T.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>trichiura</em>, <em>C. hepaticum</em></td>
<td></td>
</tr>
<tr>
<td>Parasitol*</td>
<td>approach in the absence of a “gold standard”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Booth, M.; 2003; <em>Parasitology</em></td>
<td>Estimate single- and dual-species infections based on raw egg count data and</td>
<td><em>S. mansoni</em>, hookworm</td>
<td>Bayesian latent class model</td>
</tr>
<tr>
<td></td>
<td>after latent class analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steinmann, P.; 2008; *Am J</td>
<td>Evaluate the prevalence of multiparasitism in China examining multiple stool</td>
<td>8 helminth and 7 protozoa species</td>
<td>1) Combined results of all diagnostic methods</td>
</tr>
<tr>
<td>Trop Med Hyg*</td>
<td>samples with 4 copromicroscopic techniques</td>
<td></td>
<td>2) Mathematical model by Marti et al.*43 using</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>multiple Kato-Katz measures</td>
</tr>
</tbody>
</table>

### 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–$12, depending on school or community-based sampling.\(^4^9\) Whether this is the desired end-user price is unclear.

Additional data are currently not available for this attribute.

**4.2 Channels to market**

**Acceptable:** To be determined.

**Ideal:** To be determined.

No data are currently available.

**4.3 Supply, service, and support**

**Acceptable:** To be determined.

**Ideal:** To be determined.

No data are 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 be revisited as it would be case management.\(^1^2\)
Appendices

Appendix A: Common diagnostic tools for soil transmitted helminths

<table>
<thead>
<tr>
<th></th>
<th>Kato-Katz (K-K)</th>
<th>MINI-FLOTAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technology</strong></td>
<td>Microscopy</td>
<td>Microscopy</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>World Health Organization (WHO)-recommended technique that uses filtered stool of precise sample volume for microscopic egg detection</td>
<td>Modified, field-applicable FLOTAC approach that does not require centrifugation</td>
</tr>
<tr>
<td><strong>Infrastructure required</strong></td>
<td>Laboratory</td>
<td>Laboratory</td>
</tr>
<tr>
<td><strong>User</strong></td>
<td>Well-trained microscopist (half-day training)</td>
<td>Well-trained microscopist</td>
</tr>
<tr>
<td><strong>Diagnostic target</strong></td>
<td>Helminth egg</td>
<td>Helminth egg</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>Fresh stool</td>
<td>Fresh or preserved stool</td>
</tr>
<tr>
<td><strong>Sample volume</strong></td>
<td>41.7 mg</td>
<td>8 g</td>
</tr>
<tr>
<td><strong>Sample preparation</strong></td>
<td>Manual</td>
<td>Manual</td>
</tr>
<tr>
<td><strong>Level of detection</strong></td>
<td>24 eggs per gram (epg)</td>
<td>10 epg</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hookworm</strong></td>
<td>Low intensity(^{a})</td>
<td>High intensity(^{a})</td>
</tr>
<tr>
<td>1 K-K slide: 41.2%</td>
<td>1 K-K slide: 72.1%</td>
<td></td>
</tr>
<tr>
<td>2 K-K slides: 52.6%</td>
<td>2 K-K slides: 74.0%</td>
<td></td>
</tr>
<tr>
<td><strong>T. trichiura</strong></td>
<td>Low intensity(^{a})</td>
<td>High intensity(^{a})</td>
</tr>
<tr>
<td>1 K-K slide: 69.0%</td>
<td>1 K-K slide: 93.4%</td>
<td></td>
</tr>
<tr>
<td>2 K-K slides: 79.8%</td>
<td>2 K-K slides: 95.3%</td>
<td></td>
</tr>
<tr>
<td><strong>A. lumbricoides</strong></td>
<td>Low intensity(^{a})</td>
<td>High intensity(^{a})</td>
</tr>
<tr>
<td>1 K-K slide: 48.8%</td>
<td>1 K-K slide: 95.8%</td>
<td></td>
</tr>
<tr>
<td>2 K-K slides: 55.2%</td>
<td>2 K-K slides: 97.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Result type</strong></td>
<td>Quantitative (epg)</td>
<td>Quantitative (epg)</td>
</tr>
<tr>
<td><strong>Time to results(^{b})</strong></td>
<td>~30–60 minutes</td>
<td>Approximately 10 minutes</td>
</tr>
<tr>
<td>(20–40 min clearing time required)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hardware/ancillary supplies</strong></td>
<td>Microscope, K-K kit</td>
<td>Mini-FLOTAC kit, flotation solutions, microscope</td>
</tr>
<tr>
<td><strong>Commercially available</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Stability, storage, and cold chain requirements</strong></td>
<td>No cold chain required</td>
<td>No cold chain required</td>
</tr>
<tr>
<td><strong>End-user price (USD per test)</strong></td>
<td>$0.03–$0.04(^{c}) (cost represents 2009 USD)</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Manufacturer</strong></td>
<td>Helm-Test Kit made by Labmaster Ltd, Belo Horizonte, MG Brazil;(^{37}) also provided by WHO (per 1998 WHO Geneva Supply Services document online)</td>
<td>Created and patented by Professor Giuseppe Cringoli (University of Naples, Italy)</td>
</tr>
</tbody>
</table>

\(^{a}\) High intensity was defined as ≥ 2,500 eggs per gram of faeces (epg), ≥ 400 epg, and ≥ 165 epg average infection intensity for *Ascaris lumbricoides*, *Trichuris trichiura*, and *hookworm*, respectively.

\(^{b}\) Includes only stool sample processing time at the testing site. Does not include sample collection time.

\(^{c}\) Price is an average of all supplies required for one stool test, for example the K-K 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


