Abstract

Global efforts to address schistosomiasis and soil-transmitted helminthiases (STH) include deworming programs for school-aged children that are made possible by large-scale drug donations. Decisions on these mass drug administration (MDA) programs currently rely on microscopic examination of clinical specimens to determine the presence of parasite eggs. However, microscopy-based methods are not sensitive to the low-intensity infections that characterize populations that have undergone MDA. Thus, there has been increasing recognition within the schistosomiasis and STH communities of the need for improved diagnostic tools to support late-stage control program decisions, such as when to stop or reduce MDA. Failure to adequately address the need for new diagnostics could jeopardize achievement of the 2020 London Declaration goals. In this report, we assess diagnostic needs and landscape potential solutions and determine appropriate strategies to improve diagnostic testing to support control and elimination programs. Based upon literature reviews and previous input from experts in the schistosomiasis and STH communities, we prioritized two diagnostic use cases for further exploration: to inform MDA-stopping decisions and post-MDA surveillance. To this end, PATH has refined target product profiles (TPPs) for schistosomiasis and STH diagnostics that are applicable to these use cases. We evaluated the limitations of current diagnostic methods with regards to these use cases and identified candidate biomarkers and diagnostics with potential application as new tools. Based on this analysis, there is a need to develop antigen-detecting rapid diagnostic tests (RDTs) with simplified, field-deployable sample preparation for schistosomiasis. Additionally, there is a need for diagnostic tests that are more sensitive than the current methods for STH, which may include either a field-deployable molecular test or a simple, low-cost, rapid antigen-detecting test.

Introduction

The World Health Organization (WHO) has issued a roadmap for control, elimination, or eradication of 17 neglected tropical diseases (NTDs) by 2020 [1]. An international consortium of public and private sector partners have signed the London Declaration on NTDs, pledging
specific efforts against ten NTDs [2] including schistosomiasis and soil-transmitted helminthiases (STH), which are both caused by parasitic worms.

Schistosomiasis is a debilitating disease resulting from infection by trematode worms of the genus Schistosoma [3,4]. The species that cause most of the morbidity are Schistosoma haematobium (Africa), S. mansoni (Africa and South America), and S. japonicum (Asia).

STH diseases result from intestinal infections of nematode worms, including the large roundworm Ascaris lumbricoides, the whipworm Trichuris trichiura, and two hookworm species, Ancylostoma duodenale and Necator americanus.

Details of the natural history and life cycles of schistosomes and STH have been described in numerous publications.[5–7] Schistosoma spp. spend part of their life cycle in cognate planorbid snail intermediate hosts. Tools to monitor the prevalence of infection in snails are therefore important; however, we have confined our scope to human diagnostics.

Estimates of morbidity and mortality and guidelines for control programs on determining regional prevalence and determining treatment are comprehensively described in WHO publications for both diseases [8]. The WHO goal for both schistosomiasis and STH is disease control and reduction of morbidity, to be achieved by a national coverage rate of 75% of the targeted communities with praziquantel (PZQ) [3] and mebendazole and albendazole for STH. For schistosomiasis, regional elimination is now also a goal [9].

The public sector and private pharmaceutical companies have contributed significantly to schistosomiasis and STH control programs through large-scale donations of drugs used for treatment. Monitoring the effectiveness of these efforts relies on the accuracy of WHO-approved diagnostic methods, which currently consist of microscopic examinations of stool or urine samples for parasite eggs. However, better diagnostic tools will be needed to inform decisions to reduce or stop mass drug administration (MDA) as treatments are scaled up and prevalence begins to decline. In the absence of more sensitive and robust diagnostic tools, disease prevalence estimates which are used to inform decisions regarding use of MDA will remain limited in their accuracy. Incorrect decisions that result in the over- or underuse of MDA ultimately comes with costs to donors, control programs, and affected populations.

In this paper, we review diagnostic needs for schistosomiasis and STH control programs, assess the potential of new tests, and determine appropriate strategies for improving diagnostic testing to support the goals for these two diseases laid out in the WHO 2020 roadmap [1].

**Methods**

Between August and December 2014, we gathered input from representative international stakeholders, including disease experts, laboratory and field researchers, test developers, program implementers, mathematical modelers, policymakers, and donors. In parallel with stakeholder analysis, we conducted a review of publicly available literature and manufacturer websites to assess diagnostic needs, identify potential solutions, and determine an appropriate strategy for diagnostic testing to support disease elimination efforts. We used an exploratory search strategy for both websites and public databases including PUBMED and MEDLINE, starting with known entry points (reviews, key stakeholders, etc.) and allowing those interactions to inform the next cycle of search terms and contacts in a cascade search. We judged the search to be complete when saturation was reached. Key publications are noted in the Top Five Papers box.

**Findings**

**Current diagnostic tests**

The current gold standard method for determining the presence of Schistosoma parasite eggs is microscopic examination of stool and urine samples [9]. The Kato-Katz method [10] is the
most common preparation for copromicroscopy (stool microscopy), and uroscopy (urine microscopy) is also used, after filtration of urine. However, neither method is sensitive to the low-intensity infections that characterize populations treated with PZQ. A rapid diagnostic test (RDT) for the schistosome excretory/secretory (ES) circulating cathodic antigen (CCA) has received considerable attention recently and is now recommended as an alternative to Kato–Katz for *S. mansoni*, but it is insensitive to *S. haematobium*, and validation has not been demonstrated for other species [11–20].

For STH, the gold standard diagnostic test is also Kato–Katz copromicroscopy, which is also used to distinguish helminth eggs for each of the four STH species. Helminth eggs are not excreted in urine, so uroscopy is not used for STH. Another copromicroscopy method, the mini-FLOTAC, has been recommended recently for use in STH surveillance by WHO and may improve specimen preservation and the ease of slide reading, although it requires specialized test materials for sample collection and analysis, adding to cost and complexity [21]. A recent study [22] compared these copromicroscopy tests for detecting STH using Bayesian latent class analysis in the absence of perfect reference standard. Their data suggested that the most commonly used double slide Kato–Katz method had a sensitivity of 74%–97% at high STH infection intensity, with a reduction in its sensitivity to 53%–80% in low intensity settings. This is similar to the sensitivity of the mini-FLOTAC method. The FLOTAC has the highest sensitivity in both infection intensities (69%–86% and 97%–99% for low and high intensities, respectively). Although the sensitivity of copromicroscopy methods is sufficient for informing early stages of control, it is inadequate when infections are reduced to low levels by MDA.

**Use cases for improved tests: reducing or stopping MDA and post-elimination surveillance**

Control programs based on MDA have four designated stages: mapping disease prevalence, monitoring the impact of MDA interventions, making decisions to reduce or stop MDA, and performing surveillance after elimination has been certified [23]. The current microscopy-based diagnostic tools for schistosomiasis and STH are suitable for addressing testing needs for the first and second stages by using methods that count the number of parasite eggs excreted in urine or stool. The main strengths of this type of tool are extensive validation and familiarity worldwide. For schistosomiasis, the egg counts are also a better proxy for disease (as opposed to infection), since the primary cause of morbidity is the damaging effects of the eggs migrating through tissue—further bolstering the use of microscopy (and other morbidity markers) in the first and second stages of control programs [24]. Because the operational requirements of microscopy-based testing are relatively modest, the technique can be used at lower levels of the health system (Fig 1). A major limitation is insufficient sensitivity for detecting infections that have a low burden of parasites, which diminishes usefulness in later disease control stages. While the presence of eggs in excreta is a good proxy for morbidity at high worm burdens, it is not the best proxy for future transmission risk with lower worm burdens [25]. For example, recent work has shown that reliance on egg counting to infer efficacy of PZQ dosing in intestinal schistosomiasis has resulted in systematic under-dosing and concomitant failure to clear all worms from a pediatric Ugandan population [26]. In addition, although the Kato–Katz technique is generally considered a low-cost method, a “true cost” analysis (including all personnel required and the time it takes to administer the program) suggests the overall cost is much higher than previously thought—about US$2.00 per child for a single Kato–Katz per child, the least sensitive embodiment. In comparison, the current CCA test costs about US$2.20 (assuming US$1.75 for the test and US$0.50 program costs) [13].
Control programs for schistosomiasis and STH need accurate surveillance for the third and fourth control program stages to inform decisions about reducing and stopping MDA and to conduct postelimination surveillance once elimination is certified. In both of these use cases, the diagnostic tools must be more sensitive than the current microscopic methods, which can miss eggs because of low parasite burden after MDA and because of naturally occurring variability in samples, even from the same individual. As more countries move toward reducing prevalence and reaching elimination goals, the ability to identify and target reservoirs of infection that persist or reemerge could expedite global elimination goals and facilitate the subsequent winding down and conclusion of massive, costly drug donation and disease control programs. The emphasis in the late stages should be on tools that detect infection, even in the absence of disease.

Developing new diagnostic tools

Basic considerations. In developing new diagnostic tools, researchers must make decisions about biomarkers and platforms—the type of test that will support detection of the

Fig 1. The steps required for gold standard microscopy in deworming programs. In the typical surveillance testing performed to assess the prevalence of helminth infection and the impact of deworming programs, stool samples (or sometimes urine for schistosomiasis) are collected and transported to a nearby laboratory space for microscopic analysis and follow-on reporting. There are numerous factors affecting each step of the process that contribute to making this analysis less than optimal.

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biomarker while meeting operational requirements of the control program. Biomarker properties that target specificity, biological uniqueness, and abundance and persistence in accessible (and acceptable) sample types need to be considered (Table 1). Many biomarkers may be applied to multiple platforms (e.g., antigens can be applied to both enzyme-linked immunosorbent assays [ELISA] and immunochromatographic RDTs), but if a biomarker is not unique to the parasite under investigation, or if people are unwilling to collect the types of specimen required, no platform will make it useful. In regard to biomarkers, tests can be direct or indirect. In general, direct tests (interrogating the presence of the parasite) are more appropriate for informing MDA as prevalence and infection intensity fall, because they detect actual infection. Indirect tests (interrogating the host response) are more appropriate in postelimination use cases because they detect exposure, which should be absent postelimination [27].

Table 1 provides an overview of STH and Schistosoma diagnostic landscapes.

| Biomarker                        | Surveillance Measure | Description of Putative Biomarker Candidates | Sample Type | Format                        | Examples in STH or SCH Diagnostics                                                                 | Stages in Product Development                                                                 | Identified Use Cases                                                                 |
|----------------------------------|----------------------|---------------------------------------------|-------------|-------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------|
| Parasite                         | Infection            | • Eggs                                       | Stool, Urine | Microscopic exam               | • Kato-Katz                                                                                     | Developed, commercial products available and in common use                                    | Mapping, Impact monitoring                     |
| Parasite proteins                | Infection            | • STH: ES; somatic proteins • SCH: circulating anodic antigen (CAA), CCA; soluble egg antigens (SEAs); ploycomb group protein (PcG), RAD23 | Stool, Blood, Stool, Blood, stool, saliva | Antigen detection immunoassay | • STH: Research and development; no commercial diagnostic product currently available • SCH: Commercial product either available (CCA-RDT) or in development (CAA-RDT, CAA-UCP, RDT) | • SCH: Commercial product either available (CCA-RDT) or in development (CAA-RDT, CAA-UCP, RDT) | Mapping, Impact monitoring, MDA-reduction decision, Post-MDA surveillance |
| Parasite nucleic acid            | Infection            | • STH, SCH: rRNA genes, internal transcribed spacer (ITS); mRNA (cox1); high copy number, noncoding repeat DNA sequence • SCH: Dra1 tandem repeat; miRNA223 | Blood, stool, saliva | Lab- or field-based molecular tests | • Conventional polymerase chain reaction (PCR) • Real-time PCR (multiplex of multiparallel) • Isothermal amplification assay | • “Homebrew” PCR assays available for research use only • Limited field demonstrations | Mapping, Impact monitoring, MDA-reduction decision, Post-MDA surveillance |
| Antibodies against parasite antigens | Exposure            | • STH: α-Es, α-L3 • SCH: α-SmCTF, α-AWA, α-SEA, α-CEF6, α-SbgA | Blood        | Antibody detection immunoassay | • STH: Research and development; No commercial product available • SCH: New products in development (α-SmCTF RDT) | • STH: Research and development; No commercial product available • SCH: New products in development (α-SmCTF RDT) | Post-MDA surveillance |

Table 1. Overview of STH and Schistosomiasis (SCH) diagnostic landscapes.

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New target product profiles (TPPs) for tests to detect various biomarkers in specific use cases for both schistosomiasis and STH have recently been developed and are publicly available at http://sites.path.org/dx/ntd. TPPs have been constructed for lateral flow tests for antigen biomarkers and for nucleic acid amplification tests (NAATs)—both intended to inform decisions to adjust or stop MDA. A TPP was also developed for lateral flow tests for antibody biomarkers, for the use case of postelimination surveillance. The TPPs define acceptable and ideal requirements for test characteristics such as intended use, stability, specimen type, throughput, and performance, including clinical sensitivity and specificity. The ASSURED (Affordable,
Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, Delivered to those who need it) criteria [28] were considered at all times during TPP development to align test characteristics with their intended use within low resource settings.

Control programs for both schistosomiasis and STH have historically focused monitoring efforts on school age children. Decisions are made about regional MDA based on prevalence and treatment efficacy surveys on sentinel schools. As prevalence drops and elimination is approached, the population sample should be expanded to include younger children and adults to ensure accurate tracking of infection and morbidity and precision in the administration of treatment [26]. The expansion of the sample frame and declining prevalence may also change the mode of testing from the current paradigm of community diagnosis though the sentinel schools, toward individual diagnosis. All of these factors influence the TPPs of new diagnostic products for attributes like robustness, throughput, training of the user, and ancillary equipment. The applicability of the ASSURED criteria will also change with the changing requirements. An excellent discussion of the topics in this paragraph on schistosomiasis in Africa and Arabia has been published previously [29].

Ultimately, the optimal design of a diagnostic tool will be decided by field user feedback and operations research with actual products or prototypes. Thus, TPPs are living documents that require updates as new data is gathered and user needs change.

**Schistosomiasis.** Ideally, a new diagnostic test for schistosomiasis will offer improved accuracy and operational characteristics sufficient to justify the transition costs to control programs from current microscopic methods [7]. Low limit of detection (LOD) requirements for the new test can be addressed by improving the signal-to-noise (S/N) ratio in several ways, including preanalytical concentration of analyte from a large sample volume, preanalytical enzymatic amplification of analyte or use of high S/N labels (fluorophores or up-converting phosphors), and the use of instruments to reduce noise and eliminate bias [30–44].

Key opinion leaders from the Schistosomiasis Consortium for Operational Research and Evaluation (www.score.uga.edu) have concluded that a sensitive RDT for a circulating antigen constitutively produced by a fecund adult worm pair is the most promising tool for development. Researchers at Leiden University Medical Center (LUMC) have already identified suitable antigens [32, 43, 45–48]; and an RDT for one of them, CCA, was successfully commercialized and is becoming widely adopted. However, CCA has only been shown to be useful for *S. mansoni* [11, 14–20] and also has a potential for cross-reaction with other parasite antigens and some human cancers [49,50].

Diagnostics using CAA have also been developed and evaluated extensively, including field demonstrations [32–33, 40–41]. CAA is a polysaccharide waste product of adult worm pairs that is present in several accessible sample types (blood and urine), very stable, and genus specific (one product can guide MDA in all endemic countries, regardless of the geographic distribution of *Schistosoma* species). This work has established CAA as the preferred analytical target. The current, most sensitive embodiment of the LUMC CAA RDT uses a preanalytical concentration step, up-converting phosphor particles, and a reader instrument in a lateral flow strip format. While this combination has demonstrated excellent performance with a LOD for the most sensitive version corresponding to a single worm pair [33], it does not yet meet TPP optimal operational requirements in terms of ease of use and throughput, primarily due to the format of the preconcentration step. Translation of the preconcentration step to a method that is more field-deployable and is better matched to the workflow of the RDT will be required to address field surveillance use cases.

Another advantage of the antigens identified by LUMC is that the antigens have been characterized with an experimental primate model that allows correlation to actual adult worm burden and, therefore, good estimation of how serum or urine concentration of the antigen
relates to the parameter of most interest to elimination programs (low number of fecund worm pairs) [51]. Correlation of the antigen concentration to egg counts when the fecund worm burden is low is difficult, because the LOD of Kato-Katz is too high to serve as a good reference in this worm burden regime. This is especially true after MDA when some of the worms may not be producing eggs, but are still viable, and may recover to lay eggs again. The ability of these excretory antigens to signal the presence of worms even when the primary source of morbidity (eggs) has been abolished makes them uniquely suited to elimination use cases; although, they can also be used to stratify morbidity risks at higher worm burdens [26].

Other diagnostic tests that may eventually prove valuable adjuncts to CAA testing in the postelimination use cases include antibody tests and new molecular diagnostic tests targeting Schistosoma DNA. Several antibody tests have been described and with further development or and/or validation could have postelimination application [52–54]. Molecular tests, which use a preanalytical enzymatic amplification to achieve sensitivity, appear to have high potential but have not as yet been developed into commercial human diagnostic products or adapted for point-of-care (POC) use. PCR for a variety of targets has been investigated the most [35, 55–69], but several isothermal methods have also been described [44, 70–71]. Advantages and limitations of molecular methods for schistosomiasis are similar to those for STH, discussed below, with the exception that stool may not be the only suitable sample. The ongoing emergence of new POC molecular platforms and the potential for multiplexing with other NTDs suggests that molecular tests will become increasingly important. They are also the most likely candidates to adequately address detection of infection in the snail intermediate hosts, as adult worms (and their antigens) are not found in the snails.

**STH.** The situation for STH is very different from that for schistosomiasis, because there is no validated STH antigen that acts as a biomarker for developing a new diagnostic test. Based on their current stage of development, including the availability of well-characterized genomic biomarkers and assay protocols that have undergone initial verification, NAATs for STH are promising [72–82]. PCR-based approaches have demonstrated improved performance over microscopy for detection of STH species and allowed simultaneous analysis of multiple STH targets in parallel or through multiplexing [83–85]. However, as with molecular methods for other infectious diseases, the use of real-time PCR assays for STH are limited to research or use by a limited number of country programs with the necessary infrastructure and adequate resources such as expensive and specialized equipment, reagents, and trained personnel. Nonetheless, the potential sensitivity improvements over microscopy, the availability of characterized genomic targets, and the potential to add additional targets such as those for other important enteric pathogens make real-time PCR an attractive option for STH detection. Currently, there is no molecular assay for STH diagnosis that has been adapted into a commercialized product, and the STH research community continues to advance numerous lab-developed assays toward a single-reference standard protocol for STH detection.

For new tests to be viable alternatives for current methods, they must have performance characteristics that make them suitable replacements. This includes the ability to detect and distinguish each of the four high-priority STH species—which is important for selection of drugs to use in MDA programs—and to quantitate the intensity of infection. Further research may be required to understand the correlation of DNA levels with numbers of live worms or eggs and the impact that DNA from nonviable worms may have on assay interpretation, including prevalence and intensity estimates. From an assay design standpoint, further research is needed to ensure assays can provide robust, reliable quantification of STH genomic targets and to determine how quantitative NAAT results will be developed into guidelines as an alternative indicator to counts of helminth eggs for assessing infection intensity. Once STH molecular assays have been validated, protocols will likely require further refinement to adapt
lab-developed methods for use in commercial tests, including those intended for use with field-deployable molecular platforms that may be needed to ensure access for all STH programs.

Stakeholders have indicated a preference for a low-cost and sensitive RDT for assessing parasite antigens, as it could potentially be performed immediately onsite during sample collection and reduces requirements for specialized training associated with current microscopy-based methods. To date, however, there are limited data validating STH antigens for use in such tests, particularly for a complete set of biomarkers that would allow detection of all four major STH species either alone or in concert. Results of a limited number of early research studies have identified candidate antigen biomarkers such as the ES proteins that may be of use in detection of different STH species. Research [86–89] must ensure that new antigen markers offer adequate specificity and do not cross-react with analogous proteins in other related helminth species found in the same environment. Although lab-based platforms such as ELISAs would likely accommodate multiplex immunoassays if suitable biomarkers are identified, stakeholders expressed a preference for a multiplex test that is field-deployable. New POC immunoassay platforms are emerging that may accommodate the multiplexing needed for pan-STH detection, including some newer RDT formats that may not require the use of a separate reader. Further validation of emerging biomarkers is needed.

Development of improved specimen collection tools is also an important pathway to new diagnostic tests. Stool is currently the only validated sample due to the biology of the STH infection. Control programs would prefer blood or urine samples for diagnostic testing, but that will require further research to identify and assess the feasibility of alternative STH biomarkers in other matrices such as blood or urine. If stool remains the only feasible sample for STH diagnosis, additional research is needed to improve specimen collection methods, handling, and processing techniques. These include evaluation of alternative stool collection methods, such as Bio-wipes [90] or rectal swabs [91], that may be more acceptable to users.

Tables that detail the comprehensive landscape of biomarkers and technologies for both schistosomiasis and STH are attached as electronic supplementary information (S1–S4). Key learnings are summarized in the Key Learning Points box.

**Conclusions and recommendations**

The lack of appropriate diagnostic tools to support late-stage decisions for MDA of schistosomiasis and STH remains a serious gap in disease control and elimination programs. microscopic methods to count parasite eggs will remain the WHO-recommended method for schistosomiasis and STH surveillance until evidence is generated that clearly demonstrates how diagnostic tools measuring alternative indicators—infestation and exposure—will significantly improve decision-making by control programs. Currently, key scientific advisors to WHO continue to prefer microscopy due, in part, to its perceived low cost; demonstrated efficacy in high-prevalence, high-worm burden settings; and longstanding use by control programs.

If performance requirements can be met, our review suggests that field-deployable, highly sensitive, and specific RDTs for unique parasite antigens are the preferred solution for supporting decisions to adjust community MDA programs based on cost and familiarity with such a format. Postelimination surveillance may be supported by similar POC tests for antibody biomarkers. Preferably, these new diagnostics should cost programs no more that approximately US$2/child to purchase and use—more expensive tests may not be adopted due to concerns about extra costs relative to microscopic techniques.
Schistosomiasis

Our findings lead to several conclusions and recommendations specific to the development of new diagnostic tests for schistosomiasis. First, the analyte should be CAA. The CAA test is preferred because of the advanced state of CAA characterization, current availability of commercialized platforms, and strong advocacy among key stakeholders. If other tests are developed, prioritized biomarkers would include an antibody. Molecular markers may become more important as POC platforms become more practical and available. Second, RDTs based on lateral flow immunochromatographic strips using highly sensitive labels and a cognate reader to increase S/N ratios will provide adequate sensitivity for low-intensity infections while retaining desired ease of use. Finally, field-deployable POC CAA preconcentration methods are needed for the lowest worm burden use cases (elimination certification and post-MDA surveillance).

STH

For STH later-stage control programs, no single-test option is currently validated for use as an alternative to the current Kato-Katz method. Molecular tests for parasite nucleic acid markers show the most promise to meet technical performance requirements for next-generation assays. Both lab-based and field-deployable molecular test options need to be considered. Stakeholders have indicated a preference for immunoassay RDTs that assess specific antigens or antibodies for a complete set of STH biomarkers, because this will provide a relatively low-cost, simple, and familiar format, assuming that the technical performance requirements can be met. However, because the availability and validation of these biomarkers is still limited, this represents a long-term and high risk solution.

Investments are needed now to ensure continued progress so that new tools become available. In this early stage, a portfolio-based approach will help to reduce risk while advancing test development across a variety of formats and platforms. Because copromicroscopy methods remain critical for surveillance, investments should also be considered for developing new sample collection, transport, or processing tools or for improving access to current technologies that could enhance performance and usability of current diagnostic tests.

Top Five Papers

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 2014;7:37.

2. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol 2009 Apr;25(4):151–6.

3. Solomon AW, Engels D, Bailey RL, Blake IM, Brooker S, Chen JX, 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.

4. World Health Organization. Schistosomiasis: progress report 2001–2011, strategic plan 2012–2020.

5. Weerakoon KG, Gobert GN, Cai P, McManus DP. Advances in the Diagnosis of Human Schistosomiasis. Clin Microbiol Rev 28(4):939–967. 2015.
Key Learning Points

- As control and elimination programs realign to meet London Declaration goals, the requirements for diagnostics change.
- Better performing, more easily deployed diagnostic tools may support decisions on appropriate allocation of scarce drug resources.
- The current gold-standard microscopy techniques are operationally challenging and do not perform adequately, especially in lower prevalence areas.
- ES parasite antigen tests—if available—can provide the right combination of performance and deployability.
- Molecular testing provides excellent performance, but operational challenges must be overcome.

Supporting Information

S1 Table. Biomarker landscape for schistosomiasis.
(DOCX)

S2 Table. Diagnostic landscape for schistosomiasis.
(DOCX)

S3 Table. Methods for detecting STH.
(DOCX)

S4 Table. Biomarkers and diagnostic technologies for detecting STH.
(DOCX)

References

1. World Health Organization. Accelerating work to overcome the global impact of neglected tropical diseases—A roadmap to implementation. Geneva: WHO; 2012.
2. Uniting to Combat NTDs (2012) The London Declaration on Neglected Tropical Diseases. Uniting to Combat NTDs Available: http://unitingtocombatntds.org/resource/london-declaration.
3. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014; 383: 2253–2264. doi: 10.1016/S0140-6736(13)61949-2 PMID: 2469483
4. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006; 368: 1106–1118. doi: 10.1016/S0140-6736(06)69440-3 PMID: 16997665
5. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet. 2006; 367: 1521–1532. doi: 10.1016/S0140-6736(06) 68653-4 PMID: 16679166
6. Knopp S, Steinmann P, Keiser J, Utzinger J. Nematode infections: soil-transmitted helminths and trichinellosis. Infect Dis Clin North Am. 2012; 26: 341–358. doi: 10.1016/j.idc.2012.02.006 PMID: 22632643
7. Weerakoon KG, Gobert GN, Cai P, McManus DP. Advances in the Diagnosis of Human Schistosomiasis. Clin Microbiol Rev. 2015; 28: 939–967. doi: 10.1128/CMR.00137-14 PMID: 26224883
8. World Health Organization (2002) Prevention and control of schistosomiasis and soil-transmitted helminthiasis: Report of a WHO expert committee. World Health Organization Available: http://www.who.int/iris/handle/10665/42588. Accessed: 1 December 2014.
9. World Health Organization (2013) Schistosomiasis: progress report 2001–2011, strategic plan 2012–2020. World Health Organization Available: http://apps.who.int/iris/handle/10665/78074. Accessed: 1 December 2014.

10. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo. 1972; 14: 397–400. PMID: 4675644

11. Ashton RA, Stewart BT, Petty N, Lado M, Finn T, et al. Accuracy of circulating cathodic antigen tests for rapid mapping of Schistosoma mansoni and S. haematobium infections in Southern Sudan. Trop Med Int Health. 2011; 16: 1099–1103. doi: 10.1111/j.1365-3156.2011.02815.x PMID: 21692957

12. Colley DG, Binder S, Campbell C, King CH, Tchuem Tchuente LA, et al. A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of Schistosoma mansoni. Am J Trop Med Hyg. 2013; 88: 426–432. doi: 10.4269/ajtmh.12-0639 PMID: 23339189

13. Adriko M, Standley CJ, Tinkitina B, Tukahebwa EM, Fenwick A, et al. Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for Schistosoma mansoni in different transmission settings within Bugiri District, Uganda. Acta Trop. 2014; 136: 50–57. doi: 10.1016/j.actatropica.2014.04.001 PMID: 24727052

14. Coulibały JT, N’goran EK, Utzinger J, Doenhoff MJ, Dawson EM. A new rapid diagnostic test for detection of anti-Schistosoma mansoni and anti-Schistosoma haematobium antibodies. Parasit Vectors. 2013; 6: 29. doi: 10.1186/1756-3305-6-29 PMID: 23360734

15. Legesse M, Erko B. Field-based evaluation of a reagent strip test for diagnosis of schistosomiasis mansoni by detecting circulating cathodic antigen (CCA) in urine in low endemic area in Ethiopia. Parasite. 2008; 15: 151–155. PMID: 18642508

16. Ochodo EA, Gopalakrishna G, Spek B, Reitsma JB, van LL, et al. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database of Systematic Reviews. 2015; 1: 1–292.

17. Shane HL, Verani JR, Abudho B, Montgomery SP, Blackstock AJ, et al. Evaluation of urine CCA assays for detection of Schistosoma mansoni infection in Western Kenya. PLoS Negl Trop Dis. 2011; 5: e951. doi: 10.1371/journal.pntd.0000951 PMID: 21283613

18. Standley CJ, Lwambo NJ, Lange CN, Kariuki HC, Adriko M, et al. Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shorelines communities of Lake Victoria. Parasit Vectors. 2010; 3: 7. doi: 10.1186/1756-3305-3-7 PMID: 20181101

19. Stothard JR, Kabaterine NB, Tukahebwa EM, Kazibwe F, Rollinson D, et al. Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta Trop. 2006; 97: 219–228. doi: 10.1016/j.actatropica.2005.11.004 PMID: 16386231

20. Stothard JR. Improving control of African schistosomiasis: towards effective use of rapid diagnostic tests within an appropriate disease surveillance model. Trans R Soc Trop Med Hyg. 2009; 103: 325–332. doi: 10.1016/trstmh.2008.12.012 PMID: 19171359

21. World Health Organization. Assessing the epidemiology of soil-transmitted helminths during a transmission assessment survey in the Global programme to Eliminate Lymphatic Filariasis. Geneva, Switzerland: World Health Organization; 2015. Available: http://apps.who.int/iris/bitstream/10665/153240/1/978924908384_eng.pdf.

22. 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: 765–774. doi: 10.1016/j.ijpara.2014.05.009 PMID: 24992655

23. Soloman AW, Engels D, Bailey RL, Blake IM, Brooker S, 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: e1746. doi: 10.1371/journal.pntd.0001746 PMID: 22860146

24. Webster JP, Koukounari A, Lambertson PH, Stothard JR, Fenwick A. Evaluation and application of potential schistosome-associated morbidity markers within large-scale mass chemotherapy programmes. Parasitology. 2009; 136: 1789–1799. doi: 10.1017/S0031182009006350 PMID: 19523252

25. 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: 778–782. PMID: 1801353

26. Bustinduy AL, Waterhouse D, de Sousa-Figueiredo JC, et al. Population Pharmacokinetics and Pharmacodynamics of Praziquantel in Ugandan Children with Intestinal Schistosomiasis: Higher Dosages Are Required for Maximal Efficacy. mBio. 2016; 7(4):e00227–16. doi: 10.1128/mBio.00227-16 PMID: 27507822

27. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol. 2009; 25: 151–156. doi: 10.1016/j.pt.2009.01.004 PMID: 19269899
28. Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. Nat Rev Microbiol. 2004; 2: 231–240. doi: 10.1038/nrmicro841 PMID: 15083158

29. Stothard JR, Stanton MC, Bustinduy AL, Sousa-Figueiredo JC, van Dam GJ, et al. Diagnostics for schistosomiasis in Africa and Arabia: a review of present options in control and future needs for elimination. Parasitology. 2014; 141: 1947–1961. doi: 10.1017/S0031182014001152 PMID: 25158604

30. Cai YC, Xu JF, Steinmann P, Chen SH, Chu YH, et al. Field comparison of circulating antibody assays versus circulating antigen assays for the detection of schistosomiasis japonica in endemic areas of China. Parasit Vectors. 2014; 7: 138. doi: 10.1186/1756-3305-7-138 PMID: 24684924

31. Carvalho do Espirito-Santo MC, Pinto PL, Gargioni C, varado-Mora MV, Pagliusi C, V, et al. Detection of Schistosoma mansoni antibodies in a low-endemic area using indirect immunofluorescence and circumoval precipitin test. Am J Trop Med Hyg. 2014; 90: 1146–1152. doi: 10.4269/ajtmh.13-0746 PMID: 24639303

32. Corstjens PL, van LL, Zuiderwijk M, Kornelis D, Tanke HJ, et al. Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum. J Clin Microbiol. 2008; 46: 171–176. doi: 10.1128/JCM.00877-07 PMID: 17942645

33. Corstjens PL, do Dood CJ, Kornelis D, Fat EM, Wilson RA, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. Parasitology. 2014; 141: 1841–1855. doi: 10.1017/S0031182014000626 PMID: 24932595

34. Deelder AM, de JN, Boerman OC, Fillie YE, Hilberath GW, et al. Sensitive determination of circulating anodic antigen in Schistosoma mansoni infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. Am J Trop Med Hyg. 1989; 40: 268–272. PMID: 2494898

35. Gomes LI, Enk MJ, Rabello A. Diagnosing schistosomiasis: where are we? Rev Soc Bras Med Trop. 2014; 47: 3–11. doi: 10.1590/0037-8682-0231-2013 PMID: 24553804

36. Kane RA, Stothard JR, Rollinson D, Evraerts J, et al. Detection and quantification of schistosome DNA in freshwater snails using either fluorescent probes in real-time PCR or oligochromatographic dipstick assays targeting the ribosomal intergenic spacer. Acta Trop. 2013; 128: 241–249. doi: 10.1016/j.actatropica.2011.10.019 PMID: 22100540

37. Knopp S, Salim N, Schindler T, Karagiannis Voules DA, Rothen J, 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: 535–545. doi: 10.4269/ajtmh.13-0268 PMID: 24445211

38. Tarp B, Black FT, Petersen E. The immunofluorescence antibody test (IFAT) for the diagnosis of schistosomiasis used in a non-endemic area. Trop Med Int Health. 2000; 5: 185–191. PMID: 10747281

39. Tjon Kon Fat E, Abrams W, Niedbala R, Corstjens PL. Lateral flow sandwich assay utilizing upconverting phosphor (UCP) reporters Methods in Cell Biology. 2012; 112: 203–234.

40. van Dam GJ, de Dood CJ, Lewis M, Deelder AM, van LL, et al. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. Exp Parasitol. 2013; 135: 274–282. doi: 10.1016/j.exppara.2013.06.017 PMID: 23859995

41. van Dam GJ, Xu J, Bergquist R, de Dood CJ, Utzinger J, et al. An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of Schistosoma japonicum in a low-endemic area, People's Republic of China. Acta Trop. 2015; 141: 190–197. doi: 10.1016/j.actatropica.2014.08.004 PMID: 25128703

42. van Dam GJ, Odermatt P, Acosta L, Bergquist R, de Dood CJ, et al. Evaluation of banked urine samples for the detection of circulating anodic and cathodic antigens in Schistosoma mekongi and S. japonicum infections: a proof-of-concept study. Acta Trop. 2015; 141: 190–197. doi: 10.1016/j.actatropica.2014.08.004 PMID: 25225158

43. 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: 69–80. PMID: 10996122

44. Xu J, Rong R, Zhang HQ, Shi CJ, Zhu XQ, et al. Sensitive and rapid detection of Schistosoma japonicum DNA by loop-mediated isothermal amplification (LAMP). Int J Parasitol. 2010; 40: 327–331. doi: 10.1016/j.ijpara.2009.08.010 PMID: 19735662

45. Deelder AM, de JN, Fillie YE, Kornelis D, Helaha D, et al. Quantitative determination of circulating antigens in human schistosomiasis mansoni using an indirect hemagglutination assay. Am J Trop Med Hyg. 1989; 40: 50–54. PMID: 2492777

46. Polman K, Diakafte MM, Engels D, Nahimana S, van Dam GJ, et al. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. Trop Med Int Health. 2000; 5: 534–537. PMID: 10995094
47. 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: 557–564. PMID: 8691363

48. van Dam GJ, Wichers JH, Ferreira TM, Ghati D, van AA, et al. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. J Clin Microbiol. 2004; 42: 5458–5461. doi: 10.1128/JCM.42.12.5458-5461.2004 PMID: 15583265

49. van Dam GJ, Bergwerff AA, Thomas-Oates JE, Rotmans JP, Kamerling JT, et al. The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke Schistosoma mansoni have Lewis x as repeating unit. Eur J Biochem. 1994; 225: 467–482. PMID: 7925469

50. van Dam GJ, Claas FH, Yazdanbakhsh M, Kruize YC, van Keulen AC, et al. Schistosoma mansoni excretory circulating cathodic antigen shares Lewis-x epitopes with a human granulocyte surface antigen and evokes host antibodies mediating complement-dependent lysis of granulocytes. Blood. 1996; 88: 4246–4251. PMID: 8943680

51. Alan WR, van Dam GJ, Kariuki TM, Farah IO, Deelder AM, et al. The detection limits for estimates of infection intensity in schistosomiasis mansoni established by a study in non-human primates. Int J Parasitol. 2006; 36: 1241–1244. doi: 10.1016/j.ijpara.2006.07.002 PMID: 16930605

52. Demerdash Z, Mohamed S, Hendawy M, Rabia I, Attia M, et al. Monoclonal antibody-based dipstick assay: a reliable field applicable technique for diagnosis of Schistosoma mansoni infection using human serum and urine samples. Korean J Parasitol. 2013; 51: 93–98. doi: 10.3347/kjp.2013.51.1.93 PMID: 23467705

53. Moendeg KJ, Angeles JM, Goto Y, Leonar do LR, Kirinoki M, et al. Development and optimization of cocktail-ELISA for a unified surveillance of zoonotic schistosomiasis in multiple host species. Parasitol Res. 2015; 114: 1225–1228. doi: 10.1007/s00436-015-4312-7 PMID: 25595656

54. Smith HJ, Doenhoff M, Alten C, Bailey WJ, M, et al. Comparison of Schistosoma mansoni soluble cercarial antigens and soluble egg antigens for serodiagnosing schistosome infections. PLoS Negl Trop Dis. 2012; 6: e1815. doi: 10.1371/journal.pntd.0001815 PMID: 23029577

55. Cnops L, Tannich E, Polman K, Clerinx J, Van EM. Schistosoma real-time PCR as diagnostic tool for international travellers and migrants. Trop Med Int Health. 2012; 17: 1208–1216. doi: 10.1111/j.1365-3162.2012.03060.x PMID: 22882536

56. Enk MJ, Oliveira E Silva, Rodrigues NB. Diagnostic accuracy and applicability of a PCR system for the detection of Schistosoma mansoni DNA in human urine samples from an endemic area. PLoS One. 2012; 7: e38947. doi: 10.1371/journal.pone.0038947 PMID: 22701733

57. Espirito-Santo MC, varado-Mora MV, as-Neto E, Botelho-Lima LS, Moreira JP, et al. Evaluation of real-time PCR assay to detect Schistosoma mansoni infections in a low endemic setting. BMC Infect Dis. 2014; 14: 558. doi: 10.1186/s12879-014-0558-4 PMID: 25339851

58. Gomes LI, Marques LH, Enk MJ, Coelho PM, Rabello A. Further evaluation of an updated PCR assay for the detection of Schistosoma mansoni DNA in human stool samples. Mem Inst Oswaldo Cruz. 2009; 104: 1194–1196. PMID: 20140385

59. Gomes LI, Dos Santos Marques LH, Enk MJ, de Oliveira MC, Coelho PM, et al. Development and evaluation of a sensitive PCR-ELISA system for detection of schistosomiasis infection in feces. PLoS Negl Trop Dis. 2010; 4: e664. doi: 10.1371/journal.pntd.0000664 PMID: 20421918

60. Kato-Hayashi N, Leonardo LR, Arevalo NL, Tagum MN, Apin J, et al. Detection of active schistosome infection by cell-free circulating DNA of Schistosoma japonicum in highly endemic areas in Sorsogon Province, the Philippines. Acta Trop. 2015; 141: 178–183. doi: 10.1016/j.actatropica.2014.06.003 PMID: 24836919

61. Lier T, Simonsen GS, Haasheim H, Hjelmveit SO, Vennervald BJ, et al. Novel real-time PCR for detection of Schistosoma japonicum in stool. Southeast Asian J Trop Med Public Health. 2006; 37: 257–264. PMID: 17124983

62. Lier T, Simonsen GS, Wang T, Lu D, Haukland H, et al. Real-time polymerase chain reaction for detection of low-intensity Schistosoma japonicum infections in China. Am J Trop Med Hyg. 2009; 81: 428–432. PMID: 19706908

63. Lodh N, Mwansa JC, Mutengo MM, Shiff CJ. Diagnosis of Schistosoma mansoni without the stool: comparison of three diagnostic tests to detect Schistosoma [corrected] mansoni infection from filtered urine in Zambia. Am J Trop Med Hyg. 2013; 89: 46–50. doi: 10.4269/ajtmh.13-0104 PMID: 23716406

64. Oliveira LM, Santos HL, Goncalves MM, Barreto MG, Peralta JM. Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity Schistosoma mansoni infection. Diagn Microbiol Infect Dis. 2010; 68: 416–421. doi: 10.1016/j.diagmicrobio.2010.07.016 PMID: 20884153

65. Pontes LA, as-Neto E, Rabello A. Detection by polymerase chain reaction of Schistosoma mansoni DNA in human serum and feces. Am J Trop Med Hyg. 2002; 66: 157–162. PMID: 12135287
66. Sandoval N, Siles-Lucas M, Perez-Arellano JL, Carranza C, Puente S, et al. A new PCR-based approach for the specific amplification of DNA from different Schistosoma species applicable to human urine samples. Parasitology. 2006; 133:581–587. doi: 10.1017/S003118200600898 PMID: 16834820

67. ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, et al. Multiplex real-time PCR for the detection and quantification of Schistosoma mansoni and S. haematobium infection in stool samples collected in northern Senegal. Trans R Soc Trop Med Hyg. 2008; 102: 179–185. doi: 10.1016/j.trstmh.2007.10.011 PMID: 18177680

68. Wang C, Chen L, Yin X, Hua W, Hou M, et al. Application of DNA-based diagnostics in detection of schistosomal DNA in early infection and after drug treatment. Parasit Vectors. 2011; 4: 164. doi: 10.1186/1756-3305-4-164 PMID: 21864384

69. Wichmann D, Panning M, Quack T, Kramme S, Burchard GD, et al. Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. PLoS Negl Trop Dis. 2009; 3: e422. doi: 10.1371/journal.pntd.0000422 PMID: 19381285

70. Fernandez-Soto P, Gandasegui AJ, Sanchez HA, Lopez AJ, Vicente SB, et al. A loop-mediated isothermal amplification (LAMP) assay for early detection of Schistosoma mansoni in stool samples: a diagnostic approach in a murine model. PLoS Negl Trop Dis. 2014; 8: e3126. doi: 10.1371/journal.pntd.0003126 PMID: 25187956

71. Rosser A, Rollinson D, Forrest M, Webster BL. Isothermal Recombinase Polymerase amplification (RPA) of Schistosoma haematobium DNA and oligochromatographic lateral flow detection. Parasit Vectors. 2015; 8: 446. doi: 10.1186/s13071-015-1055-3 PMID: 26338510

72. Basuni M, Muh J, Othman N, Verweij JJ, Ahmad M, et al. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. Am J Trop Med Hyg. 2011; 84: 338–343. doi: 10.4269/ajtmh.2011.0499 PMID: 21292911

73. Cutillas C, Galiejo R, De RM, Tewes B, Ubeda JM, et al. Trichuris suis and Trichuris trichiura are different nematode species. Acta Trop. 2009; 111: 299–307. doi: 10.1016/j.actatropica.2009.05.011 PMID: 19467214

74. de Grujiter JM, van LL, Gasser RB, Verweij JJ, Brienen EA, et al. Polymerase chain reaction-based differential diagnosis of Ancylostoma duodenale and Necator americanus infections in humans in northern Ghana. Trop Med Int Health. 2005; 10: 574–580. doi: 10.1111/j.1365-3156.2005.01440.x PMID: 15941421

75. Gasser RB, Stewart LE, Speare R. Genetic markers in ribosomal DNA for hookworm identification. Acta Trop. 1996; 62: 15–21. PMID: 8971275

76. Gordon CA, Gray DJ, Gobert GN, McManus DP. DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. Mol Cell Probes. 2011; 25: 143–152. doi: 10.1016/j.mcp.2011.05.002 PMID: 21651977

77. Jonker FA, Calis JC, Phiri K, Brienen EA, Khoffi H, et al. Real-time PCR demonstrates Ancylostoma duodenale is a key factor in the etiology of severe anemia and iron deficiency in Malawian pre-school children. PLoS Negl Trop Dis. 2012; 6: e1555. doi: 10.1371/journal.pntd.0001555 PMID: 22514750

78. Liu GH, Zhou W, Nisbet AJ, Xu MJ, Zhou DH, et al. Characterization of Trichuris trichiura from humans and T. suis from pigs in China using internal transcribed spacers of nuclear ribosomal DNA. J Helminthol. 2014; 88: 64–68. doi: 10.1017/S0022149X13000740 PMID: 23113971

79. Liu J, Gratz J, Amour C, Kibiki G, Becker S, et al. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. J Clin Microbiol. 2013; 51: 472–480. doi: 10.1128/JCM.02658-12 PMID: 23175269

80. Monti JR, Chilton NB, Qian BZ, Gasser RB. Specific amplification of Necator americanus or Ancylostoma duodenale DNA by PCR using markers in ITS-1 rDNA, and its implications. Mol Cell Probes. 1998; 12: 71–78. PMID: 9633041

81. Sato M, Sanguankiat S, Yoonuan T, Pongvongs T, Keomoungkhoum M, et al. Copro-molecular identification of infections with hookworm eggs in rural Lao PDR. Trans R Soc Trop Med Hyg. 2010; 104: 617–622. doi: 10.1016/j.trstmh.2010.06.006 PMID: 20673938

82. Verweij JJ, Brienen EA, Ziem J, Yelfarli L, Polderman AM, et al. Simultaneous detection and quantification of Ancylostoma duodenale, Necator americanus, and Oesophagostomum bifurcum in faecal samples using multiplex real-time PCR. Am J Trop Med Hyg. 2007; 77: 685–690. PMID: 17979072

83. Llewellyn S, Inpankaew T, Nery SV, Gray DJ, Verweij JJ, et al. Application of a Multiplex Quantitative PCR to Assess Prevalence and Intensity Of Intestinal Parasite Infections in a Controlled Clinical Trial. PLoS Negl Trop Dis. 2016; 10: e0004380. doi: 10.1371/journal.pntd.0004380 PMID: 26820626

84. Cimino RO, Jeur J, Juarez M, Cajal PS, Vargas P, et al. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. Parasit Vectors. 2015; 8: 380. doi: 10.1186/s13071-015-0994-z PMID: 26183074
85. Easton AV, Oliveira RG, O’Connell EM, Kepha S, Mwandawiro CS, et al. Multi-parallel qPCR provides increased sensitivity and diagnostic breadth for gastrointestinal parasites of humans: field-based inferences on the impact of mass deworming. Parasit Vectors. 2016; 9: 38. doi: 10.1186/s13071-016-1314-y PMID: 26813411

86. Bungiro RD Jr., Cappello M. Detection of excretory/secretory coproantigens in experimental hookworm infection. Am J Trop Med Hyg. 2005; 73: 915–920. PMID: 16282303

87. Elsemore DA, Geng J, Flynn LA, Crawford M; Idexx Laboratories, Inc. and Divergence, Inc. Methods, devices, kits and compositions for detecting roundworm, whipworm, and hookworm. United States Patent 7951547 B2. 2009 May 18.

88. Elsemore DA, Flynn LA, Crawford M; Idexx Laboratories, Inc. and Divergence, Inc. Methods, devices, kits and compositions for detecting whipworm. United States Patent 7993861 B2. 2009 May 18.

89. Elsemore DA, Flynn LA, Crawford M; Idexx Laboratories, Inc. and Divergence, Inc. Methods, devices, kits and compositions for detecting roundworm. United States Patent 7993862 B2. 2009 May 18.

90. Mans J, van Zyl WB, Taylor MB, Page NA, Sobsey MD, et al. Applicability of Bio-wipes for the collection of human faecal specimens for detection and characterisation of enteric viruses. Trop Med Int Health. 2014; 19: 293–300. doi: 10.1111/tmi.12251 PMID: 24372706

91. Budding AE, Grasman ME, Eck A, Bogaards JA, Vandenburggrauls CM, et al. Rectal swabs for analysis of the intestinal microbiota. PLoS One. 2014; 9: e101344. doi: 10.1371/journal.pone.0101344 PMID: 25020051