Validation of innovative digital microscopes for the diagnosis of schistosomiasis and other helminthiases

Meulah Tcheubousou, B.

7.

General discussion

Adapted and extended from: A review on innovative optical devices for the diagnosis of human soil-transmitted helminthiasis and schistosomiasis: From research and development to commercialisation

Brice Meulah, Michel Bengtson, Lisette Van Lieshout, Cornelis Hendrik Hokke, Andrea Kreidenweiss, Jan-Carel Diehl, Ayola Akim Adegnika, and Temitope Ebenezer Agbana.

Parasitology. 2023;150(2):137-149.

Simple, inexpensive, portable, robust and reliable diagnostics are urgently needed for individual case management, as well as for the assessment of the progress of control strategies for NTDs in endemic settings. This is particularly so for schistosomiasis, soil-transmitted helminths (STHs) and other helminthiases such as filariasis. For these diseases conventional microscopy is still the most commonly used method, despite the fact that there are alternatives available. In the case of schistosomiasis, the haematuria dipstick and the antigen detecting lateral flow tests POC-CCA are the most broadly accepted alternatives for the detection of *S. haematobium* and *S. mansoni*, respectively. For infections with the filaria species: *Wuchereria bancrofti*, the filariasis test strip (FTS) for the detection of circulating antigens in blood can be used to diagnose lymphatic filariasis [1]. However, as the saying goes "no-one-size fits all", each of these tests have their own diagnostic characteristics, which cater to specific contextual needs. For example, the reagent strip test for detecting microhaematuria is only indicative for *S. haematobium* infection and is not parasite-specific [2]. The POC-CCA tests have been validated extensively in settings endemic for *S. mansoni* infection [3-8], while showing variable outcomes in *S. haematobium* endemic setting [9-11]. The more advanced diagnostic alternatives such as the UCP LF-CAA test and PCR are known to be highly accurate, but they are not field friendly and require a relatively high level of training and laboratory infrastructure, especially for PCR. In the case of lymphatic filariasis, the FTS is useful in endemic settings to support ivermectin MDA control programs. However, in settings with unknown co-endemicity with *Loa loa* an additional test is required to guide the treatment due to contraindication of ivermectin for persons with *Loa loa* infection [12]. Conventional microscopy which remains the most commonly used method in endemic settings for schistosomiasis, STH and filariasis is laborious, requires well-trained personnel and infrastructure which are often lacking. Eye defects resulting from long term exposure of the eyes to light of microscopes while in use over a long period of time have been reported [13-15]. Low-cost AI powered digital optical diagnostic device (DODD) platforms have the potential to revolutionise the health systems in LMICs. These devices are relatively easy to use [16] and in combination with digital health applications could be an acceptable alternative to conventional microscopy in field settings, if the diagnostic performance is proven to be equally as good. In this thesis, we carried out validation of the diagnostic performance of DODDs, the Schistoscope and AiDx Assist digital microscope, at different iterations during their development for the diagnosis of schistosomiasis and other helminthiases.

We began by demonstrating the need for microscopy methods in specific field settings (chapter 2). With the limitations of conventional microscopy acknowledged, we validated DODDs which are innovations of microscopes (Schistoscope and AiDx Assist) through research and development to detect and quantify eggs of *Schistosoma* and STHs against a range of diagnostic reference tests in different settings (chapter 3-6). These devices are designed by integrating AI software programs to digital optical hardware systems to automate diagnosis. In Nigeria, a high sensitivity but low specificity as well as low count accuracy (≥100 egg/10mL of urine) of the Schistoscope for the detection of *S. haematobium* eggs compared to conventional microscopy was obtained. This was due to limitations in the Schistoscope's AI framework (chapter 3). The AI framework was further improved for specificity and count accuracy for *S. haematobium* egg detection and validated on a set of microscopy images from the field (chapter

4). A high diagnostic sensitivity and specificity of the Schistoscope was obtained through validation on a large set of clinical samples, consisting of fresh urine and banked filters for *S. haematobium* egg detection using conventional microscopy, real-time PCR and UCP-LF CAA as reference standards in Gabon (chapter 5). A modest performance of the AiDx Assist for the detection and quantification of *S. mansoni* eggs in stool (Kato-Katz (KK) slides) was obtained with high accuracy for detection of *S. haematobium* eggs in urine. The potential of the AiDx Assist to detect STHs was explored. The STHs eggs could be manually identified on images captured with the AiDx Assist (chapter 6). In contrast to the Schistoscope, the AiDx Assist over-estimated *S. haematobium* egg count compared to conventional microscopy. These differential observations are further discussed within this chapter.

Diagnostic sensitivity and specificity of Schistoscope and AiDx Assist for schistosomiasis and soil-transmitted helminths in endemic settings

From the validation studies, the diagnostic performance of the Schistoscope and AiDx Assist at different iterations of their development was reported. With the use of urine samples collected from different endemic settings, the sensitivity and specificity of the Schistoscope for the detection of *S. haematobium* eggs varied depending on the reference test used (chapter 3, 4 and 5 or Table 2). For example, the first field validation of the Schistoscope in Nigeria showed a high sensitivity (85.0 %) but low specificity (48.9%) compared to conventional microscopy for detecting *S. haematobium* eggs in urine. This study importantly revealed that the quality and coverage of the dataset generated and used to train the AI algorithm for the detection of *S. haematobium* eggs was limited in terms of variation of the type of artifacts that could be present in field samples. Overcoming this limitation significantly improved the specificity (77.0 %) of the Schistoscope when validated in Gabon while maintaining a sensitivity (83.1 %) comparable to conventional microscopy (Chapter 4). However, when compared to a more accurate composite reference consisting of UCP-LF CAA and real-time PCR, the sensitivity dropped to 62.9%. This decrease in sensitivity was primarily due to the Schistoscope, similar as conventional microscopy, missing very low infection intensities which were detected by the more sensitive diagnostic procedures such as PCR and CAA detection. This clearly highlights the limitation of microscopy methods in settings approaching elimination of this disease and calls for more sensitive, field compatible tests [17, 18]. Examination of larger urine volumes for negative samples could potentially improve the diagnostic sensitivity. This method normally results in increased work load as well as analysis cost rendering it impractical in resource-limited-settings relying on conventional microscopy. However, with the Schistoscope this approach could be more feasible, given that its design permits the analysis of an unlimited number of slides daily. This implies there is still room to improve the performance of the Schistoscope to match that of conventional microscopy.

Furthermore, the sensitivity (91.9%) and specificity (91.3%) of the AiDx Assist for the detection of *S. haematobium* eggs in urine was comparable to conventional microscopy and was consistent with previous findings [19]. However, for the detection of S. mansoni eggs on Kato-Katz (KK) slides, the sensitivity was modest (56.9 %) with a high specificity (86.8 %). The low

sensitivity is attributed to the fact that majority of the samples missed had light infections (1-99 EPG) and the few eggs available might have been covered by stool components, making detection by AI algorithm challenging (Chapter 6 or Table 2). For the detection *S. haematobium* eggs, the overall sensitivity of the Schistoscope is comparable to that of the AiDx Assist (AI powered mode). However, the specificity is significantly lower. The difference in specificity is due to the fact that the AiDx Assist is designed such that the output of the AI algorithm (positive images) is displayed and confirmed by the operator of the device at the end of each sample analysis. This subsequently improves the specificity of the AiDx Assist as any false positive detection by the AI algorithm is ruled out by the operator. An approach similar to this has been used and reported by Lin et al., for filaria detection [20]. The difference in Lin et al., is that, confirmation of AI detection is done in real time by the user during image capture to improve sensitivity only. And when artifacts are detected by their algorithm, no correction is made by the operator.

Additionally, the Schistoscope performance was validated by analysing banked samples, recording promising results mostly attributed to the high infection intensity and relatively low level of artifacts in the samples used (chapter 5). Regardless, this finding opens up solutions to practical challenges in field settings including access to microscopists for real time analysis of samples for control program evaluation. The option of sampling and storage provides a lot of flexibility for organisation and execution of surveillance program in field settings.

Accuracy and relevance of egg count determination of Schistosoma and soil-transmitted helminths

Egg load serves as a proxy for disease morbidity and so far has been an essential marker in assessing the efficacy of control programs in endemic regions [21-24]. However, there is a debate regarding the relevance of precise egg quantification when dealing with high infection intensities (e.g. ≥50 egg/10mL or ≥400 EPG) as outlined by World Health Organisation (WHO) [25]. The field validation of the fully-automated Schistoscope in Nigeria revealed lower accuracy in quantifying *S. haematobium* eggs in urine samples with ≥100 eggs/10mL compared to conventional microscopy (chapter 3). Subsequent field validation of the improved Schistoscope in Gabon demonstrated better egg count accuracy, although still inferior to conventional microscopy (chapter 5). On the contrary, the fully automated AiDx Assist showed superior accuracy in quantifying *S. haematobium* and *S. mansoni* eggs compared to conventional microscopy based on the semi-automated AiDx Assist counts confirmed by an external quality control performed on digital images. This observation was made particularly for urine and stool samples with high egg counts (≥100 egg/10mL or ≥1000 EPG respectively). The discrepancy in egg count accuracy between the Schistoscope and AiDx Assist observed in the different studies stems from the level of expertise of the microscopist and the AI framework integrated in both devices.

Following the WHO categorization for infection intensity, accurate quantification of eggs for samples with the high infection intensity threshold is less of importance. As a result, these

devices could be programmed to stop counting upon reaching the high infection intensity threshold making the egg differences observed at very high count less of a problem. While this would also reduce the sample analysis time, the data obtained would still be valuable for surveillance in control programs. Despite egg count being considered as a proxy to infection intensity, in clinical settings, detection (positive or negative) of schistosomiasis and STH infections remains the primary interest to physicians in order to decide on whether to treat or not [26-28]. Also, since the treatment of schistosomiasis and STH infections with e.g. praziquantel and albendazole respectively, is independent of infection intensities [26, 27], egg quantification is often unnecessary. In such a case detection of an egg is enough to make decisions about treatment. For such a use case, these devices can as well be programmed to detect and count to a required threshold. This also highlights the importance of stakeholder engagement for the development of such tools [29].

Multiplex diagnosis with the Schistoscope and AiDx Assist

The exploratory potential of DODDs lies in is its ability to be adapted for different parasitic infections. For example, multiple studies have demonstrated or validated DODDs for visualisation or detection of different stool parasites [30-35]. Also, the optical systems of the Schistoscope and AiDx Assist coupled with their computing unit, enables customization for detecting various parasites across different sample types. This makes it possible to develop, train and install new AI algorithm on these devices to detect different disease of interest. These optical systems possess sufficient resolution to effectively resolve parasite signatures e.g. eggs, ranging in size of approximately 20-400 μm in cross-sectional diameter. We demonstrated as a proof-of-concept for use on stool samples, the manual visualisation of Schistosoma and STHs eggs in images of KK slides captured with the Schistoscope [36]. Furthermore, the Schistoscope has been tested for the manual visualisation of hookworm and *S. stercoralis* larvae in stool culture (data unpublished). The AiDx Assist has been validated for the detection of *S. haematobium* and *S. mansoni* eggs in urine and stool respectively in field settings (chapter 6 and Table 2). In addition, during the research period of this thesis we also have tested AiDx Assist for the detection of L. loa microfilaria on Giemsa stained thick blood smear (data unpublished).

In this side study, the AiDx Assist (Semi- and fully-automated mode as described in chapter 6) was validated on 514 thick blood smears for the detection and quantification of microfilaria using conventional microscopy as the reference standard (Table 2). Blood samples were collected from individuals residing in a Lambaréné in Gabon endemic for *Loa loa* and *Mansonella* spp. The positivity rate based on conventional microscopy was found to be 6.2% (Table 1). Table 2 shows the diagnostic performance of the AiDx Assist. Each thick smear was prepared using 5μl of blood, spread over an area of 2x4cm. Although comparable diagnostics performance between the AiDx Assist and conventional microscopy was observed, the sample size in terms of positive cases was limited. Figure 1. shows an image registered with the AiDx Assist showing *Loa loa* microfilaria. A strong correlation was observed between AiDx assist and conventional microscopy for microfilaria quantification ($r=0.37$, 0.92 $P<0.05$). In endemic settings, MDA of ivermectin serves as the primary control measure for onchocerciasis and/or

lymphatic filariasis. However, the deleterious effects observed in individuals co-infected with *Loa loa* during treatment with ivermectin underscore the importance of detecting and quantifying *Loa loa* microfilariae before initiating treatment [37]. Although the AiDx Assist could be beneficial in these MDA programs, practical challenges related to sample collection, processing, and turnaround time limits its real-time application for test-and-treat strategies during community MDA campaigns. In previous studies a digital microscope called the LoaScope was developed and validated for the detection of *Loa loa* microfilaria [12, 38]. This device is designed to detect microfilaria movement directly on fresh blood smears (no staining required) obtained via finger prick, enabling real-time analysis and subsequent treatment decision-making regarding ivermectin administration. Conversely, the AiDx Assist sample preparation approach as well as the approach used by Lin et al, [20] are better suited for use in reference or local laboratory settings where slide fixing and staining are required.

Table 1. Characteristic outcome of the semi-automated, fully-automated AiDx Assist and conventional microscopy for the detection of microfilaria performed on 514 Giemsa thick blood smears.

	Semi-automated AiDx Assist	**Fully-automated AiDx Assist**	**Convectional microscopy**
Positive sample (%)	33 (6.4)	143 (27.8)	32 (6.2)
Median (microfilaria count/5uL)	4	11	8
Range (microfilaria count/5uL)	1-134	1-100	1-147

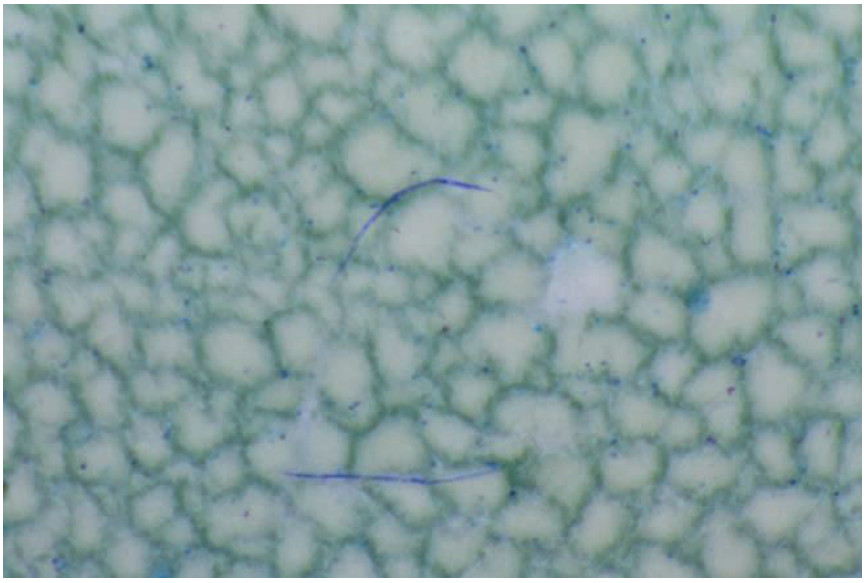

Figure 1. Image captured with the AiDx Assist showing *Loa loa* microfilaria (Image provided by AiDx Medical).

Table 2. summary of diagnostic performance of the Schistoscope and AiDx Assist for schistosomiasis and other helminthiases reported within this thesis.

Device/Validation year/ Chapter #	Setting	Parasite target	Sample type	Device Mode	Reference test	Sensitivity % (95% CI)	Specificity % (95% CI)
Schistoscope/2022/3	Nigeria	*S. haematobium*	Fresh urine	Semi-automated Schistoscope	Conventional microscopy (UF)	80.1 (73.2–86.0)	95.3 (92.4–97.4)
				Fully-automated Schistoscope		87.3 (81.3–92.0)	48.9 (43.3–55.0)
Schistoscope/2022/4	Netherlands	*S. haematobium*	Fresh urine	Fully-automated Schistoscope	Semi-automated Schistoscope	93.75 (-)	93.94 (-)
Schistoscope/2024/5	Gabon	*S. haematobium*	Fresh urine	Fully-automated Schistoscope	Conventional microscopy (UF) real time-PCR/UCP-LF CAA	83.1 (75.5-89.1)	77.0 (70.7-82.5)
						62.9 (55.8-69.6)	78.8 (71.0-85.3)
	Gabon		Banked slides	Fully-automated Schistoscope	Conventional microscopy (UF) real-time PCR/UCP-LF CAA	96.3 (92.6-98.5)	86.8 (80.5-91.6)
						78.0 (72.3-83.0)	90.9 (83.4-95.8)
AiDx Assist/2024/6	Nigeria	*S. mansoni*	Fresh stool	Semi-automated AiDx Assist	Conventional microscopy (KK)	86.8 (80.2-91.9)	81.4 (75.8-86.2)
				Fully-automated AiDx Assist		56.9 (48.4-65.2)	86.8 (80.2-91.9)
		S. haematobium	Fresh urine	Semi-automated AiDx Assist	Conventional microscopy (UF)	94.6 (91.1-97.0)	90.6 (84.4-94.9)
				Fully-automated AiDx Assist		91.9 (87.9-94.9)	91.3 (85.3- 95.4)
		T. trichiura	Fresh stool		-		
		A.lumbricoides	Fresh stool		-		
AiDx Assist/2023/7	Gabon	*Loa loa*	Blood	Semi-automated AiDx Assist	Conventional microscopy	93.8 (79.2-99.2)	99.4 (98.2-99.9)
				Fully-automated AiDx Assist	Conventional microscopy	96.9 (83.8-99.9)	76.8 (72.7-80.5)

UF= urine filtration, KK=Kato-Katz

Therefore, the capabilities of the Schistoscope and the AiDx Assist goes beyond analysing urine or stool samples and could be further explored for blood samples and potentially even be extended to the diagnosis of non-infectious diseases. On the other hand, with these prototypes challenges were encountered in effectively identifying relatively small parasites, such as *Entamoeba coli* cysts in fresh stool smears and *P. falciparum* trophozoites in Giemsa thick blood smears. To address this issue, more robust optical systems are needed. Nevertheless, this versatility renders the Schistoscope and the AiDx Assist customizable and promising for screening multiple parasites in endemic field settings. Other researchers have demonstrated or validated DODDs for the detection of multiple parasitic infections [31, 35, 39-41].

Furthermore, DODDs are currently being exploited by other companies and research groups for diagnostic application on infectious as well as non-infectious disease. For example the group of SpotLab (https://spotlab.ai/) have developed platforms for haematology analysis, differential cell counting in cerebrospinal fluid for meningitis diagnosis in new born, detection and quantification of lung lesions in COVID-19 patients. The group of enaiblers (https://www.enaiblers.com/team) has recently developed a platform to support monitoring of NTD control programs in resource limited settings. Other groups are currently applying DODDs for the detection and characterisation of non-infectious diseases such as cancer [42] and cardiovascular disease (https://medisimaging.com/, https://www.digitaldiagnostics.com/). However, the applicability of these systems in poor resource settings needs to be further explored.

Moving beyond test performance and readiness for LMIC: Economic considerations and accessibility (A Cost-Benefit Analysis and Stakeholder Engagement Perspective)

The Schistoscope and AiDx Assist have shown promising diagnostic performance for schistosomiasis and STH infections across various endemic settings. However, there is still need for a comprehensive cost-benefit analysis to support their implementation and integration into large-scale mapping and impact assessment surveys for national control programs in LIMCs, along with digital health initiatives [43]. This analysis should consider factors concerning the laboratory and case management efficiency gains inherent to the devices' based on the WHO's Target Product Profile (TPP). These factors include the capital cost per device, throughput, cost per test, analysis time per test run, personnel requirements for sample preparation/analysis, quality assurance needs, infrastructure requirements, consumables, repair feasibility, and associated costs compared to conventional microscopy. Also, the impact of DODDs on compliance to MDA programs and the costs associated with unnecessary rounds of annual MDA of praziquantel should be considered. For example, feedback from end-users, including laboratory technicians, community health workers, and final year medical students, regarding both the Schistoscope and AiDx Assist has indicated increased awareness and ease of use with minimal training [16, 44], potentially enhancing capacity building for implementation in LIMCs. Additionally, using the AiDx Assist for on-site analysis of urine samples in communities has led to improved compliance with praziquantel treatment (Personal communication Temitope E. Agbana). This improvement is attributed to dispelling local superstitions surrounding sample

collection for sorcery, due to on-site sample collection and processing, which in turn has augmented participation in surveys and subsequent treatments.

Furthermore, while the WHO TPP does not specify the minimum capital cost per device required for control and elimination of these diseases, several research efforts have developed DODDs ranging in price from US$465 to US$5000 [31-33, 41]. The current prototypes of the Schistoscope and AiDx Assist cost in all approximately US$1000 and US$2000 respectively. This is significantly lower than the AI-based digital microscope developed by Wards et al costing ~US$5000. Although comparable to the Newton Nm1 microscope (commercially available) in terms of price ranging from US$465 to US$1000 [45] depending on the series, the Schistoscope and AiDx Assist offer integrated AI for automated detection, enhancing their diagnostic capabilities. Also, upon mass production of these devices for commercialisation, the price would be expected to decrease.

In field experiences with DODDs

The operation of the Schistoscope encountered several challenges during its deployment, ranging from damages due to transportation to technical malfunctions within the equipment. These challenges included problems with auto-focus, defective membrane detection algorithms, loose screws during long transportation on bumpy roads, and vibrations from nearby instruments such as centrifuge affecting image quality. Additionally, lengthy slide capture and analysis time were noted compared to conventional microscopy which was dependent on the size of the membrane used as well as the infection intensity of the sample. Despite these obstacles, practical solutions were identified, such as implementing robust packaging for transportation, conducting regular maintenance checks, exploring software enhancements to improve efficiency and programming sample analysis to stop when a threshold of parasite count is reached. Addressing these challenges and implementing recommended solutions will be crucial for optimizing the performance and usability of the Schistoscope in field settings, ultimately facilitating more accurate and efficient sample analysis.

Advancements of AI-powered DODDs for human helminthiasis diagnosis: from development to field application

Important advancements have been made in the clinical sector with DODDs for automated malaria parasite detection [46-52]. Comparatively, the automated optical detection of human stool and urine parasites such as schistosomes and STHs was still in its early stages and has now gained momentum. With advancing technology, optical devices could be developed to have a fully functioning computing system, with the potential to accommodate specific features such as imaging sensors, Global Positioning System (GPS) navigation, internet access, data storage, and high processing power, each with added values for different stakeholders in the health system. This would facilitate data generation and distribution making it useful for mapping of *Schistosoma* spp. and other NTDs [53], and impact assessment of MDA programs.

Several DODDs have been developed for the diagnosis of schistosomiasis and STHs [54-59]. While most prototypes require human expertise for image interpretation before conclusive decisions could be reached (semi-automation), the fully-automated devices that's is, with integrated AI models could potentially mitigate the chances of human error and reduce the

need for human training. Complete automation of such devices makes them comparatively easy to use and facilitates multitasking during sample analysis. In this thesis we carried out validation of the AiDx Assist for the detection and quantification of stool parasites. Through digital imaging we also demonstrated the possibility of detecting multiple parasite species in stool (chapter 6). The presence of stool artifacts was found to interfere with AI detection. Some researchers have attempted to customised sample processing method for their devices in an effort to resolve this challenge [30, 60, 61]. However, maintaining the same sample workflow for DODDs as conventional microscopy was observed to not disrupt the workflow of laboratory technicians for processing and may facilitate acceptance in field settings. Also, certain sample processing methods developed by other researchers require high laboratory infrastructure, resenting a constraint on the application of the device in resource-limited settings. Additionally, some of these methods require further validation.

A standardised approach to guide the development of DODD technology for NTDs towards field readiness and application is needed. The proposed modified TRL scale presented in the introduction of this thesis could be a possible solution towards this goal. This scale highlights aspects including small scale and in-field validation of DODDs as well stakeholder engagement in the research and development of these devices to meet required recommendations and context specific needs respectively [62]. Engaging stakeholders provides further information on desired products and context specific needs, that in turn accelerate its readiness, acceptability and enable local productivity.

Conclusion

The development and validation of innovative diagnostic tools such as the Schistoscope and AiDx Assist represent significant strides towards addressing the pressing need for simple, inexpensive, portable, and robust (semi-) automated diagnostic solutions for NTDs like schistosomiasis, STHs and other helminthiases such as filariasis, where conventional diagnostic methods often face limitations. Through preclinical and extensive field validation in comparison with conventional microscopy and other reference tests, the Schistoscope and AiDx Assist have demonstrated promising diagnostic performance in detecting these diseases. While both devices exhibit certain strengths and limitations, particularly in terms of sensitivity, specificity, and parasite quantification, they represent valuable additions to the diagnostic toolkit for NTDs. Moving forward, it is imperative to conduct comprehensive cost-benefit analyses to support the integration of these diagnostic tools into large-scale mapping and impact assessment surveys for national control programs in LMICs. Engaging stakeholders, including developers, end-users, healthcare managers, and policymakers, is essential to ensure that these tools are not only affordable and accessible but also effectively address the specific needs and challenges of endemic regions. Moreover, ongoing research and development efforts should focus on refining the performance and usability of these devices, leveraging advancements in AI and digital imaging technology. Standardized approaches, such as the modified TRL scale proposed in introduction chapter, can guide the development of digital diagnostic technologies for NTDs, facilitating their readiness and application in field settings. Ultimately, the successful implementation of these innovative digital diagnostic tools has the potential to revolutionize

disease surveillance, control, and treatment efforts, ultimately contributing to the control and advancement of NTDs towards elimination in endemic settings.

References

1. Chesnais CB, Vlaminck J, Kunyu-Shako B, Pion SD, Awaca-Uvon NP, Weil GJ, et al. Measurement of Circulating Filarial Antigen Levels in Human Blood with a Point-of-Care Test Strip and a Portable Spectrodensitometer. Am J Trop Med Hyg. 2016;94(6):1324-9.
2. Krauth SJ, Greter H, Stete K, Coulibaly JT, Traoré SI, Ngandolo BNR, et al. All that is blood is not schistosomiasis: experiences with reagent strip testing for urogenital schistosomiasis with special consideration to very-low prevalence settings. Parasites & Vectors. 2015;8(1):584.
3. Greter H, Krauth SJ, Ngandolo BN, Alfaroukh IO, Zinsstag J, Utzinger J. Validation of a Point-of-Care Circulating Cathodic Antigen Urine Cassette Test for Schistosoma mansoni Diagnosis in the Sahel, and Potential Cross-Reaction in Pregnancy. Am J Trop Med Hyg. 2016;94(2):361-4.
4. Mewamba EM, Tiofack AAZ, Kamdem CN, Ngassam RIK, Mbagnia MCT, Nyangiri O, et al. Field assessment in Cameroon of a reader of POC-CCA lateral flow strips for the quantification of Schistosoma mansoni circulating cathodic antigen in urine. PLoS Neglected Tropical Diseases. 2021;15(7):e0009569.
5. Tchuem Tchuenté L-A, Kueté Fouodo CJ, Kamwa Ngassam RI, Sumo L, Dongmo Noumedem C, Kenfack CM, et al. Evaluation of Circulating Cathodic Antigen (CCA) Urine-Tests for Diagnosis of Schistosoma mansoni Infection in Cameroon. PLOS Neglected Tropical Diseases. 2012;6(7):e1758.
6. Casacuberta M, Kinunghi S, Vennervald BJ, Olsen A. Evaluation and optimization of the Circulating Cathodic Antigen (POC-CCA) cassette test for detecting Schistosoma mansoni infection by using image analysis in school children in Mwanza Region, Tanzania. Parasite Epidemiology and Control. 2016;1(2):105-15.
7. Siqueira LMV, Couto FFB, Taboada D, Oliveira ÁAd, Carneiro NFdF, Oliveira E, et al. Performance of POC-CCA® in diagnosis of schistosomiasis mansoni in individuals with low parasite burden. Revista da Sociedade Brasileira de Medicina Tropical. 2016;49.
8. Straily A, Kavere EA, Wanja D, Wiegand RE, Montgomery SP, Mwaki A, et al. Evaluation of the Point-of-Care Circulating Cathodic Antigen Assay for Monitoring Mass Drug Administration in a Schistosoma mansoni Control Program in Western Kenya. Am J Trop Med Hyg. 2021;106(1):303-11.
9. Sanneh B, Joof E, Sanyang AM, Renneker K, Camara Y, Sey AP, et al. Field evaluation of a schistosome circulating cathodic antigen rapid test kit at point-of-care for mapping of schistosomiasis endemic districts in The Gambia. PLOS ONE. 2017;12(8):e0182003.
10. Ochodo EA, Gopalakrishna G, Spek B, Reitsma JB, Van Lieshout L, Polman K, et al. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database of Systematic Reviews. 2015(3).
11. Coulibaly JT, N'gbesso YK, Knopp S, N'guessan NA, Silué KD, van Dam GJ, et al. Accuracy of urine circulating cathodic antigen test for the diagnosis of Schistosoma mansoni in preschool-aged children before and after treatment. PLoS neglected tropical diseases. 2013;7(3):e2109.
12. Kamgno J, Pion SD, Chesnais CB, Bakalar MH, D'Ambrosio MV, Mackenzie CD, et al. A Test-and-Not-Treat Strategy for Onchocerciasis in Loa loa-Endemic Areas. N Engl J Med. 2017;377(21):2044-52.
13. Mbong Ngwese M, Prince Manouana G, Nguema Moure PA, Ramharter M, Esen M, Adégnika AA. Diagnostic Techniques of Soil-Transmitted Helminths: Impact on

Control Measures. Trop Med Infect Dis. 2020;5(2).
14. Söderberg I, Calissendorff B, Elofsson S, Knave B, Nyman K. Investigation of visual strain experienced by microscope operators at an electronics plant. Applied ergonomics. 1983;14(4):297-305.
15. Ngakhushi R, Kaiti R, Bhattarai S, Shrestha G. Prevalence of myopia and binocular vision dysfunctions in microscopists. Int Eye Sci. 2018;18(7):1180-3.
16. Onasanya A, Bengtson M, Agbana T, Oladunni O, van Engelen J, Oladepo O, et al. Towards Inclusive Diagnostics for Neglected Tropical Diseases: User Experience of a New Digital Diagnostic Device in Low-Income Settings. Tropical Medicine and Infectious Disease. 2023;8(3):176.
17. Hoekstra PT, van Dam GJ, van Lieshout L. Context-Specific Procedures for the Diagnosis of Human Schistosomiasis – A Mini Review. Frontiers in Tropical Diseases. 2021;2.
18. Utzinger J, Becker S, Van Lieshout L, Van Dam G, Knopp S. New diagnostic tools in schistosomiasis. Clinical microbiology and infection. 2015;21(6):529-42.
19. Makau-Barasa L, Assefa L, Aderogba M, Bell D, Solomon J, Urude RO, et al. Performance evaluation of the AiDx multi-diagnostic automated microscope for the detection of schistosomiasis in Abuja, Nigeria. Sci Rep. 2023;13(1):14833.
20. Lin L, Dacal E, Díez N, Carmona C, Martin Ramirez A, Barón Argos L, et al. Edge Artificial Intelligence (AI) for real-time automatic quantification of filariasis in mobile microscopy. PLOS Neglected Tropical Diseases. 2024;18(4):e0012117.
21. Wiegand RE, Secor WE, Fleming FM, French MD, King CH, Deol AK, et al. Associations between infection intensity categories and morbidity prevalence in school-age children are much stronger for Schistosoma haematobium than for S. mansoni. PLoS Negl Trop Dis. 2021;15(5):e0009444.
22. Abudho BO, Guyah B, Ondigo BN, Ndombi EM, Ireri E, Carter JM, et al. Evaluation of morbidity in Schistosoma mansoni-positive primary and secondary school children after four years of mass drug administration of praziquantel in western Kenya. Infect Dis Poverty. 2020;9(1):67.
23. World Health Organisation. Soil-transmitted helminth infections fact sheeet World Health Organisation,; 2023 [Available from: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections.
24. Kabatende J, Mugisha M, Ntirenganya L, Barry A, Ruberanziza E, Mbonigaba JB, et al. Prevalence, Intensity, and Correlates of Soil-Transmitted Helminth Infections among School Children after a Decade of Preventive Chemotherapy in Western Rwanda. Pathogens. 2020;9(12).
25. World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee 2002 [Available from: https://apps.who.int/iris/handle/10665/42588.
26. Gray DJ, Ross AG, Li YS, McManus DP. Diagnosis and management of schistosomiasis. Bmj. 2011;342:d2651.
27. Keiser J. Present drugs and future perspectives in treating soil-transmitted helminthiasis. Frontiers in Tropical Diseases. 2023;4:1282725.
28. Van GY, Onasanya A, van Engelen J, Oladepo O, Diehl JC. Improving Access to Diagnostics for Schistosomiasis Case Management in Oyo State, Nigeria: Barriers and Opportunities. Diagnostics (Basel). 2020;10(5).
29. Onasanya AA. Designing for Neglected Tropical Diseases: Co-creating digital diagnostic devices for Low-Resource Settings: Delft University Of Technology 2023.
30. Ayana M, Vlaminck J, Cools P, Ame S, Albonico M, Dana D, et al. Modification and optimization of the FECPAKG2 protocol for the detection and quantification of soil-transmitted helminth eggs in human stool. PLOS Neglected Tropical Diseases. 2018;12:e0006655.
31. Bogoch II, Andrews JR, Speich B, Ame SM, Ali SM, Stothard JR, et al. Quantitative evaluation of a handheld light microscope for field diagnosis of soil-transmitted helminth infection. The American journal of tropical medicine and hygiene. 2014;91(6):1138.

32. Boonyong S, Hunnangkul S, Vijit S, Wattano S, Tantayapirak P, Loymek S, et al. High-throughput detection of parasites and ova in stool using the fully automatic digital feces analyzer, orienter model fa280. Parasites & Vectors. 2024;17(1):13.
33. Dacal E, Bermejo-Peláez D, Lin L, Álamo E, Cuadrado D, Martínez Á, et al. Mobile microscopy and telemedicine platform assisted by deep learning for the quantification of Trichuris trichiura infection. PLoS Negl Trop Dis. 2021;15(9):e0009677.
34. Sangameswaran R. MAIScope: A low-cost portable microscope with built-in vision AI to automate microscopic diagnosis of diseases in remote rural settings. arXiv preprint arXiv:220806114. 2022.
35. Yang A, Bakhtari N, Langdon-Embry L, Redwood E, Grandjean Lapierre S, Rakotomanga P, et al. Kankanet: An artificial neural network-based object detection smartphone application and mobile microscope as a point-of-care diagnostic aid for soil-transmitted helminthiases. PLoS Negl Trop Dis. 2019;13(8):e0007577.
36. Oyibo P, Jujjavarapu S, Meulah B, Agbana T, Braakman I, van Diepen A, et al. Schistoscope: An Automated Microscope with Artificial Intelligence for Detection of Schistosoma haematobium Eggs in Resource-Limited Settings. Micromachines (Basel). 2022;13(5).
37. Kamgno J, Nana-Djeunga HC, Pion SD, Chesnais CB, Klion AD, Mackenzie CD, et al. Operationalization of the test and not treat strategy to accelerate the elimination of onchocerciasis and lymphatic filariasis in Central Africa. International Health. 2018;10(suppl_1):i49-i53.
38. Hasanah A, Nurdian Y. Use of Loa Scope as a Proper Identification and Treatment in Loiasis Disease. 2017.
39. Lundin J, Suutala A, Holmström O, Henriksson S, Valkamo S, Kaingu H, et al. Diagnosis of soil-transmitted helminth infections with digital mobile microscopy and artificial intelligence in a resource-limited setting. PLOS Neglected Tropical Diseases. 2024;18(4):e0012041.
40. Ward P, Dahlberg P, Lagatie O, Larsson J, Tynong A, Vlaminck J, et al. Affordable artificial intelligence-based digital pathology for neglected tropical diseases: A proof-of-concept for the detection of soil-transmitted helminths and Schistosoma mansoni eggs in Kato-Katz stool thick smears. PLoS Neglected Tropical Diseases. 2022;16(6):e0010500.
41. Holmström O, Linder N, Ngasala B, Mårtensson A, Linder E, Lundin M, et al. Point-of-care mobile digital microscopy and deep learning for the detection of soil-transmitted helminths and Schistosoma haematobium. Global health action. 2017;10(sup3):1337325.
42. Sufyan M, Shokat Z, Ashfaq UA. Artificial intelligence in cancer diagnosis and therapy: Current status and future perspective. Computers in Biology and Medicine. 2023;165:107356.
43. Ho J, Ahlers S, Stratman C, Aridor O, Pantanowitz L, Fine J, et al. Can Digital Pathology Result In Cost Savings? A Financial Projection For Digital Pathology Implementation At A Large Integrated Health Care Organization. Journal of pathology informatics. 2014;5:33.
44. Bengtson M, Onasanya A, Oyibo P, Meulah B, Samenjo KT, Braakman I, et al., editors. A usability study of an innovative optical device for the diagnosis of schistosomiasis in Nigeria. 2022 IEEE Global Humanitarian Technology Conference (GHTC); 2022: IEEE.
45. Meulah B, Bengtson M, Van Lieshout L, Hokke CH, Kreidenweiss A, Diehl J-C, et al. A review on innovative optical devices for the diagnosis of human soil-transmitted helminthiasis and schistosomiasis: From research and development to commercialisation. Parasitology. 2022:1-41.
46. Agbana TE, Diehl JC, van Pul F, Khan SM, Patlan V, Verhaegen M, et al. Imaging & identification of malaria parasites using cellphone microscope with a ball lens. PLoS One. 2018;13(10):e0205020.
47. Coulibaly JT, N'Goran EK, Keiser J, Ouattara M, Bogoch II, Andrews JR, et al. Evaluation of Malaria Diagnoses Using a Handheld Light Microscope in a Community-Based Setting in Rural Côte d'Ivoire. The American Journal of Tropical Medicine and Hygiene. 2016;95(4):831-4.
48. de Melo GC, Netto RLA, Mwangi VI, Salazar YEAR, de Souza Sampaio V, Monteiro WM, et al. Performance of a sensitive haemozoin-based malaria

diagnostic test validated for vivax malaria diagnosis in Brazilian Amazon. Malaria Journal. 2021;20(1).
49. Frean J. Improving quantitation of malaria parasite burden with digital image analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;102(11):1062-3.
50. Hashimoto M, Yatsushiro S, Yamamura S, Tanaka M, Sakamoto H, Ido Y, et al. Hydrophilic-treated plastic plates for wide-range analysis of Giemsa-stained red blood cells and automated Plasmodium infection rate counting. Malaria Journal. 2017;16(1).
51. Kaewkamnerd S, Uthaipibull C, Intarapanich A, Pannarut M, Chaotheing S, Tongsima S. An automatic device for detection and classification of malaria parasite species in thick blood film. BMC Bioinformatics. 2012;13(S17).
52. Kumar R, Verma AK, Shrivas S, Thota P, Singh MP, Rajasubramaniam S, et al. First successful field evaluation of new, one-minute haemozoin-based malaria diagnostic device. EClinicalMedicine. 2020;22.
53. Tilahun B, Gashu KD, Mekonnen ZA, Endehabtu BF, Angaw DA. Mapping the role of digital health technologies in the case detection, management, and treatment outcomes of neglected tropical diseases: a scoping review. Trop Med Health. 2021;49(1):17.
54. Agbana TE, Nijman P, Hoeber M, van Grootheest D, van Diepen A, van Lieshout L, et al. Detection of Schistosoma haematobium using lensless imaging and flow cytometry, a proof of principle study. Optical Diagnostics and Sensing XX: Toward Point-of-Care Diagnostics2020.
55. Armstrong M, Harris AR, D'Ambrosio MV, Coulibaly JT, Essien-Baidoo S, Ephraim RKD, et al. Point-of-Care Sample Preparation and Automated Quantitative Detection of Schistosoma haematobium Using Mobile Phone Microscopy. American Journal of Tropical Medicine and Hygiene. 2022;106(5):1442-9.
56. Dacal E, Bermejo-Peláez D, Lin L, Álamo E, Cuadrado D, Martínez Á, et al. Mobile microscopy and telemedicine platform assisted by deep learning for the quantification of Trichuris trichiura infection. PLOS Neglected Tropical Diseases. 2021;15(9):e0009677.
57. Holmstrom O, Linder N, Ngasala B, Martensson A, Linder E, Lundin M, et al. Point-of-care mobile digital microscopy and deep learning for the detection of soil-transmitted helminths and Schistosoma haematobium. Glob Health Action. 2017;10(sup3):1337325.
58. Linder E, Grote A, Varjo S, Linder N, Lebbad M, Lundin M, et al. On-chip imaging of Schistosoma haematobium eggs in urine for diagnosis by computer vision. PLOS Neglected Tropical Diseases. 2013;7(12):e2547.
59. Oyibo P, Jujjavarapu S, Meulah B, Agbana T, Braakman I, van Diepen A, et al. Schistoscope: An Automated Microscope with Artificial Intelligence for Detection of Schistosoma haematobium Eggs in Resource-Limited Settings. Micromachines. 2022;13(5).
60. Sukas S, Van Dorst B, Kryj A, Lagatie O, De Malsche W, Stuyver LJ. Development of a Lab-on-a-Disk Platform with Digital Imaging for Identification and Counting of Parasite Eggs in Human and Animal Stool. Micromachines. 2019;10(12):852.
61. Levecke B, Behnke JM, Ajjampur SS, Albonico M, Ame SM, Charlier J, et al. A comparison of the sensitivity and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. PLoS Neglected Tropical Diseases. 2011;5(6):e1201.
62. Levecke B, Coffeng LE, Hanna C, Pullan RL, Gass KM. Assessment of the required performance and the development of corresponding program decision rules for neglected tropical diseases diagnostic tests: Monitoring and evaluation of soil-transmitted helminthiasis control programs as a case study. PLoS Neglected Tropical Diseases. 2021;15(9):e0009740.