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

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Appendix

Summary

Neglected tropical diseases (NTDs) is a diverse range of infections primarily found in tropical and subtropical regions, and despite their significant impact on health and socio-economic well-being, NTDs have historically received less attention compared to other infectious diseases. The World Health Organization (WHO) has recognized the urgency of addressing NTDs and has developed a roadmap to combat them. Timely diagnosis is crucial for effective management and control of the debilitating life-long disease associated with these infections. Among the major NTDs, schistosomiasis, soil-transmitted helminth (STH) and filariasis are particularly prevalent, causing widespread morbidity with a substantial public health burden, especially in low- and middle-income countries (LMICs). Schistosomiasis, transmitted through contact with contaminated water, can lead to severe urogenital and intestinal complications. Similarly, STH infections, acquired through ingestion of parasite eggs or skin penetration by parasite larvae, contribute to anaemia, malnutrition, and developmental impairments, particularly in children. Lymphatic filariasis and onchocerciasis need proper diagnosis, not only for accurate case management, but also to determine co-infections, specifically with *Loa loa*. Addressing the burden of these diseases requires concerted efforts to enhance diagnosis, treatment, and prevention strategies, ultimately promoting health equity and improving the lives of millions worldwide.

In LMICs, the diagnosis of NTDs such as schistosomiasis, STH and several types of filariasis predominantly relies on conventional microscopy, which involves identifying and quantifying parasite-derived products in urine, stool and blood samples. However, this method is labour-intensive, requires skilled personnel, and often lacks the necessary infrastructure in resource-limited settings. Alternative diagnostic approaches for microscopy include urine-based point-of-care tests (POC-CCA) for detecting circulating cathodic antigens, and up-converting particle lateral flow (UCP-LF) assays for detecting circulating anodic antigens (CAA) in urine and serum for schistosomiasis and blood circulating antigen test for lymphatic filariasis. Nucleic acid amplification tests (NAATs) have also been utilized for detecting parasite-specific nucleic acid sequences in urine and serum for both schistosomiasis and STH infections, even though primarily in research settings in LIMCs. While these alternative methods offer higher accuracy, they are still undergoing validation and standardization for routine clinical use in LMICs. Additionally, they require trained personnel and for most of these tests, a well-equipped laboratory infrastructure posing challenges for widespread implementation in resource-limited settings. Further efforts are needed to validate and standardize these diagnostic techniques to improve their accessibility and utility in field settings.

Innovative digital optical diagnostic devices (DODDs) have been developed to automatically detect parasites in clinical samples, utilizing technical modifications of smartphone optical systems or off-the-shelf optical components. These devices leverage the integrated sensors and processing power of smartphones, as well as simple computer units like Raspberry Pi and Jetson Nano, to run artificial intelligence (AI) algorithms for image analysis. While promising for automated medical diagnosis in endemic settings, these devices are currently insufficiently available for LMICs. Despite their potential, the readiness of DODDs for field

applications in LMICs remains limited. A modified version of the technology readiness level (TRL) scale, incorporating the WHO's Target Product Profile (TPP) and context-specific needs, revealed that the majority of DODDs are not yet ready for field deployment. Meeting the WHO TPP, particularly during field validation stages (TRL 5, 6, and 7), was found to be a significant bottleneck, highlighting the need for further research, development, and validation studies in diverse endemic settings. Two notable AI-powered DODDs, the Schistoscope and AiDx Assist devices, initiatives of the Technical University of Delft, have been developed for diagnosing schistosomiasis, STH infections and other parasitic infections. Both devices feature custom-designed optical bright-field illumination and movement systems, differing primarily in their electronic and computing modules, including the AI framework used. In this thesis preclinical and in-field validations of these devices have been conducted across various iterations of their development in different settings.

The primary objective of this thesis was to, through research and development, validate the diagnostic performance of the Schistoscope and AiDx Assist as morphology (egg and/or larvae)-based detection methods for schistosomiasis and STHs and loiasis through preclinical and in-field studies across different endemic and/or settings. **In Chapter 1**, an overview of the thesis is provided, highlighting the challenges encountered in the research and development leading to the commercialization of DODDs as morphology-based parasite detection methods. **Chapter 2** focuses on demonstrating the context-specific needs for different diagnostic methods, emphasizing the limitations of POC-CCA and haematuria rapid tests in diagnosing schistosomiasis in a setting (community in Tanzania) co-endemic with *S. haematobium* and *S. mansoni* infections, and the need for egg-based detection methods to accurately assess the prevalence of *Schistosoma* spp in a specific endemic setting.

Chapter 3 evaluated the Schistoscope as both a semi-automated and fully-automated digital microscope with AI algorithms, for detecting and quantifying *S. haematobium* eggs in urine in Nigeria, comparing its performance to conventional microscopy. The sensitivity of the semi- and fully -automated mode of the Schistoscope was found to be comparable to that of conventional microscopy. However, the specificity of the fully-automated Schistoscope was significantly inferior to conventional microscopy. The fully-automated Schistoscope also underestimating egg count associated to the AI algorithm, specifically in the high egg count excretion range. In **chapter 4**, the enhancement of the AI algorithm of the Schistoscope through a two-stage automated diagnosis framework for detecting and quantifying *S. haematobium* eggs is described and validated using microscopy images obtained from low-resource settings. Improved performance in terms of sensitivity and specificity was achieved requiring thorough validation in a field setting. **Chapter 5** presented a follow-up validation of the Schistoscope in Lambaréné, Gabon, comparing its performance in detecting and quantifying *S. haematobium* eggs in fresh urine (as well as banked filters) to conventional microscopy and a more sensitive composite reference standard including a urine-based real-time PCR and the UCP-LF-CAA test.

Chapter 6 validated the AiDx Assist digital microscope as a diagnostic tool for *Schistosoma* spp. in urine and stool while exploring its potential for detecting other helminths in stool under field conditions in Nigeria. The AiDx Assist was found to meet the WHO TPP in terms of diagnostic performance for *S. haematobium.* However, for the detection of *S. mansoni* eggs, the

performance was found to be modest and would require further optimisation. **Chapter 7** summarized and discussed the results within this thesis including outcome from our unpublished data of the AiDx Assist performance for the detection and quantification of *Loa loa* microfilaria on 514 Giemsa thick blood smears collected from the field settings. We also highlighted the progress made in the development of DODDs beyond diagnostic performance, considering context-specific needs, and exploring the prospects of DODDs for diagnosing schistosomiasis, STHs and filariasis in endemic settings as well as its usability in diagnosing other infectious and non-infectious diseases.

The Schistoscope and AiDx Assist represent important advancements in NTD diagnosis, offering simple, portable solutions for diseases like schistosomiasis, STHs and filariasis where traditional methods fall short. Through rigorous validation studies, both devices have shown promising performance compared to conventional microscopy. While they have strengths and limitations, their integration into national control programs in LMICs could potentially revolutionize disease surveillance and treatment efforts. Future efforts should focus on conducting thorough cost-benefit analyses to support their integration into large-scale mapping and impact assessment surveys for national control programs in LMICs. Furthermore, continued research and development should focus on improving performance and usability, guided by standardized approaches like the modified TRL scale, facilitating their readiness and deployment in field settings. Ultimately, successful implementation of these tools could greatly impact NTD control and elimination efforts in endemic areas.