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

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Validation of AiDx Assist device for automated detection of *Schistosoma* eggs in stool and urine samples in Nigeria

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Abstract

Schistosomiasis is a public health concern and there is need for reliable, field compatible diagnostic methods in endemic settings. The AiDx Assist, an artificial intelligence (AI)-based automated microscope, has shown promising results for the detection of *Schistosoma haematobium* eggs in urine. It has been further developed for the detection of *Schistosoma mansoni* eggs in stool. In this study, we evaluated the performance of the AiDx Assist for the detection of *S. mansoni* eggs in stool as well as further validating the performance of the AiDx Assist for the detection of *S. haematobium* eggs in urine. Additionally, the potential of AiDx Assist for the detection of other helminths in stool was explored.

In total, 405 participants from an area endemic for both *S. mansoni* and *S. haematobium* provided stool and urine samples which were subjected to AiDx Assist (semi- and fully-automated), while conventional microscopy was used as the diagnostic reference. Only samples with complete test results were included in the final analysis, resulting in 375 stool and 398 urine, of which 38.4% and 65.3% showed *Schistosoma* eggs by conventional microscopy. The collected images of stool samples were retrospectively examined for other helminth eggs via manual analysis.

For the detection of *S. mansoni* eggs, the sensitivity of the semi-automated AiDx Assist (86.8%) was significantly higher compared to the fully-automated AiDx Assist (56.9%) while the specificity was comparable, being 81.4% and 86.8%, respectively. Retrospectively, eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were visualized. For the examination of urine samples, a comparable sensitivity in the detection of *S. haematobium* eggs was seen between the semi- and the fully automated mode of the AiDx Assist, showing 94.6% and 91.9%, respectively. Also the specificity was comparable, with 90.6% and 91.3% respectively.

The AiDx Assist meets the World Health Organization Target Product Profile criteria in terms of diagnostic accuracy for the detection of *S. haematobium* eggs in urine, while performing modestly for the detection of *S. mansoni* eggs in stool. With some further improvements, it has the potential to become a valuable diagnostic tool for screening multiple helminth parasites in stool and urine.

Introduction

Schistosomiasis is a neglected tropical disease and a public health concern in endemic settings [1]. Conventional microscopy is the reference method for the diagnosis of schistosomiasis. It involves the detection and quantification of *Schistosoma* eggs in stool or urine [WHO 2]. The need for trained experts to perform this method limits its wide application in resource limited settings. Furthermore, the high demands for microscopy expertise not met by the number of trained microscopists in endemic settings highlights the need for high throughput methods [3, 4]. Automating microscopy method such that the dependency on trained experts is reduced could be a matching solution.

Several automated microscopes with embedded artificial intelligence (AI) algorithms have been developed for detecting *S. mansoni* or *S. haematobium* eggs [5-10]. To the best of our knowledge, none of these microscopes have been validated as a single system for the detection and quantification of both *S. mansoni* and *S. haematobium* eggs in stool and urine respectively, within a field setting.

The AiDx Assist is a low-cost and compact automated microscope with integrated AI. It is relatively easy to use without the need for high-level training compared to conventional microscopy [11] and has been validated for the diagnosis of *S. haematobium* infection in rural endemic settings in two modes: semi-automated and fully-automated mode [5]. In the semi-automated mode, the AI algorithm is disabled, and parasite count is detected and counted by an expert based on visual examination of images registered by the device. Operations in the fully-automated mode however, include automated parasite detection and counting by the integrated AI algorithm. The design of the AiDx Assist makes it possible to develop and customize for the detection of different parasites in the same or different sample types.

The AiDx Assist has been shown to be a promising diagnostic tool for urogenital schistosomiasis [5] and could also have great potential for future and timely diagnosis of intestinal schistosomiasis. Since the first evaluation for the detection of *S. haematobium* eggs in Nigeria, the device has been further developed for the detection of *S. mansoni* eggs on Kato-Katz (KK) slides and now requires validation in an endemic setting. In the current study, we carried out a validation of the AiDx Assist demonstrating its performance in detecting *S. mansoni* and *S. haematobium* eggs in stool and urine samples collected in Nigeria in a setting endemic for both *Schistosoma* species. We also explored the potential of the AiDx Assist to detect other helminth parasites in stool samples.

Methods

Study design

This cross-sectional study was conducted in local communities of the Federal Capital Territory, Abuja, Nigeria with known endemicity for *S. mansoni* and *S. haematobium* infections. The number of egg positive samples needed to achieve an assumed sensitivity and specificity of 90% using conventional microscopy as the reference was calculated to be approximately 130 [12]. A school-based approach was employed for sampling participants across 5 communities

based on the schistosomiasis prevalence data (approximately 40%) obtained from the database of the Neglected Tropical Disease Division of the Federal Ministry of Health. To attain 130 positive samples, sampling of 325 participants was aimed. Participants age 5 or older were eligible to take part in the study.

Ethics statement

Ethical approval for this study was obtained from the Federal Capital Territory (FCT, Nigeria), Health Research Ethics Committee (FCT, HREC). Before collecting samples, a written consent was obtained from adults and from the parents or legal guardians of children who wanted to take part, which was confirmed by their signatures. To safeguard the confidentiality and anonymity of the results, distinct codes were assigned to each of the samples. Following sample collection, mass treatment with praziquantel was administered to all communities according to local guidelines by the NTD unit of the public health department.

Sample processing

Each participant was given two sterile containers to provide a stool and urine sample at designated collection sites. These samples were then transported in appropriate boxes within two hours of collection to the laboratory. From the stool sample, KK slides were prepared [13]: 41.7mg of sieved stool was transferred to a microscopy slide using a template and then covered with cellophane, which has been dipped in malachite green overnight. Some light pressure was applied to the slide, in order to spread out the smear, and examination started after 10 minutes. For the urine sample, microscopy slides were prepared by urine filtration (UF). Briefly, 10 ml of homogenised urine was pressed through a 13 mm membrane (pore size 30 µm; Whatmann International Ltd) using a syringe and a filter holder and transferred onto a glass slide. The slides were examined using the semi- and fully-automated mode of the AiDx Assist and conventional microscopy (Figure 1).

Slide examination by the AiDx Assist and conventional microscopy

Each KK and UF slide was analysed with the AiDx Assist (Figure 2) in the semi-automated and the fully-automated mode, as previously described [5]. In the semi-automated mode, the images registered by the AiDx Assist were visually examined by an expert for the presence of *Schistosoma* eggs and counted. In the fully-automated mode, the artificial intelligence algorithm was enabled to automatically detect and count *Schistosoma* eggs in the images. The output of the AI algorithm was displayed and confirmed by the operator of the device at the end of each sample analysis. Results from the AiDx Assist was exported in an Excel compatible format. The same slides were subsequently analysed by conventional microscopy (10/40(×) objective on a Leica microsystems DM 300 microscope). Two independent microscopy readings were done and recorded. The average of the eggs counted between the two readings was considered for each sample. Quality control was performed retrospectively on images captured with the AiDx assist as well as for the presence of other stool parasites on KK slides on a selected sample with high infection intensity based on conventional microscopy.

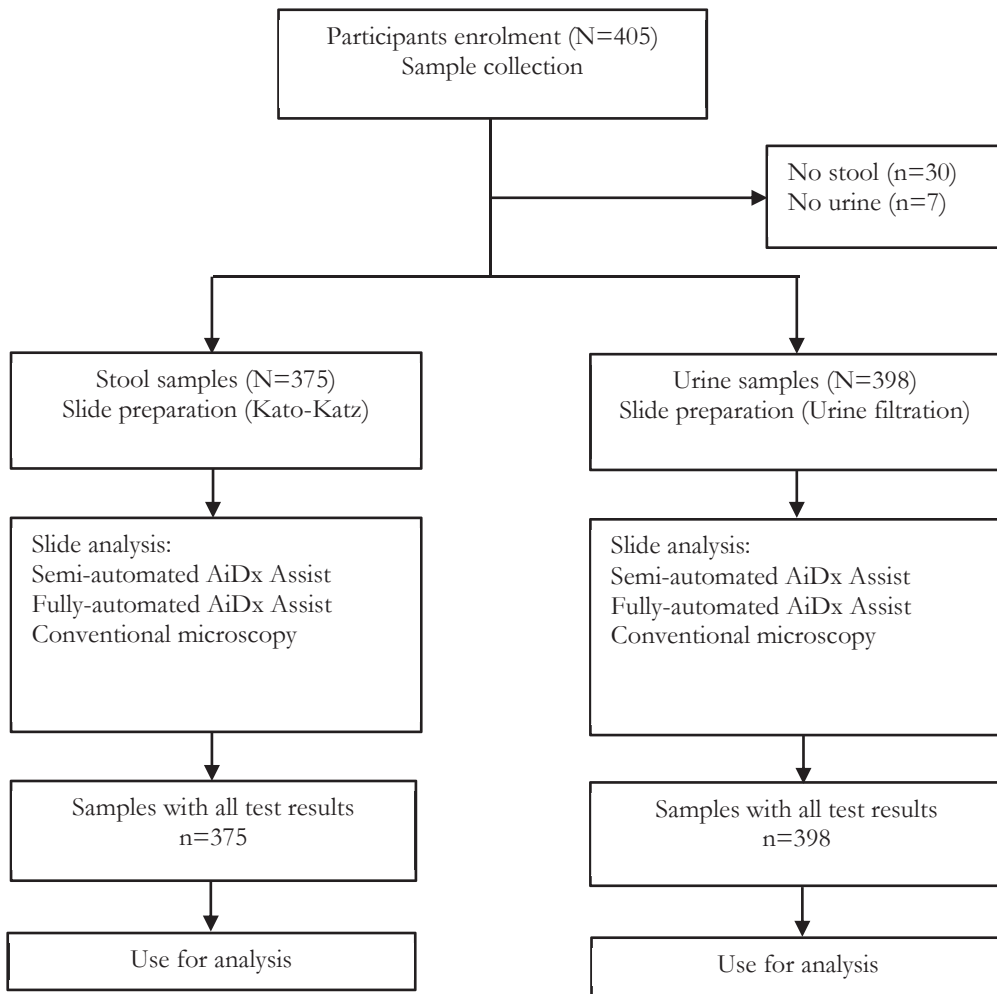


Figure 1. Flow chart of stool and urine sample collection, processing and analyses by the AiDx Assist and conventional microscopy.

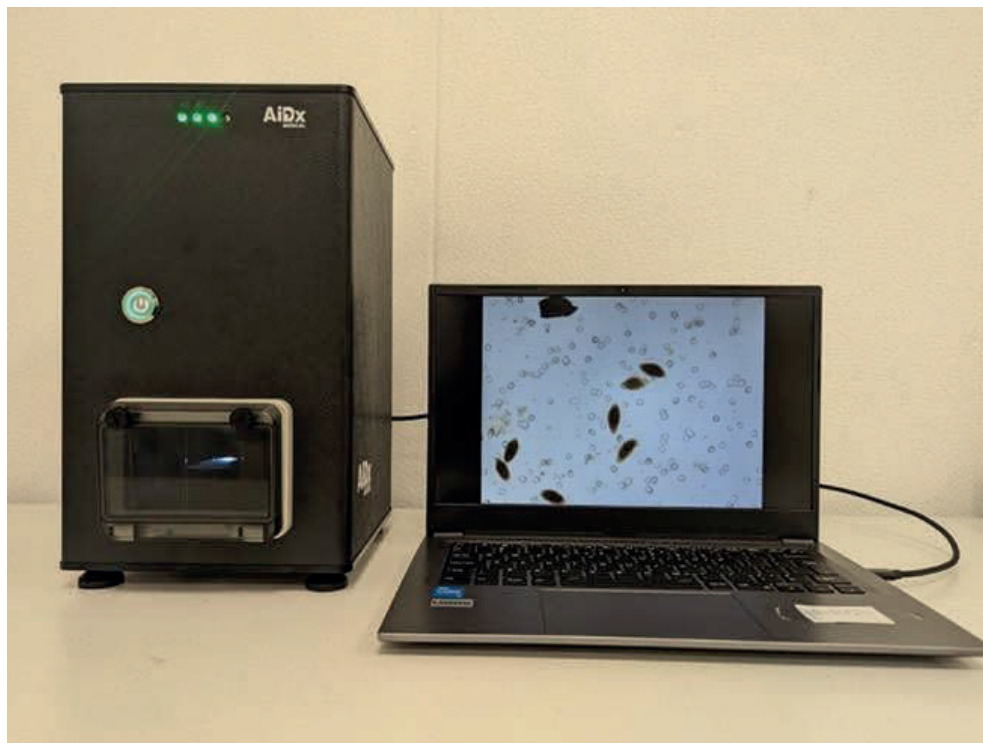


Figure 2. AiDx Assist digital microscope (104).

Statistical analysis

The number of eggs counted for all methods from the KK and UF slides were expressed in eggs per gram (EPG) of stool and eggs per 10 ml of urine, respectively. The percentage positive by AiDx Assist (semi- and fully-automated mode) and conventional microscopy was determined. The sensitivity and specificity of the AiDx Assist (semi- and fully-automated) was assessed using conventional microscopy as a reference. Cohen's Kappa (κ) statistics was computed to assess the qualitative agreement between methods. Spearman's correlation (r) was used to assess the pairwise strength of association between eggs counted by the different methods. All statistical analyses were performed with IBM Statistical Package for Social Sciences version 25 (SPSS Inc., Chicago, United States of America) and graphs were generated using GraphPad Prism version 9.0.1 for Windows (GraphPad Software, San Diego, California USA).

Results

*AiDx Assist performance for detection of *S. mansoni* eggs on Kato-Katz slides*

A total of 375 stool samples had results for all diagnostic methods and were therefore included in the final analysis (Figure 1). Table 1 shows the proportion of positive per method for *S. mansoni* infection. The semi-automated AiDx Assist found the highest proportion of positive (44.8%) followed by conventional microscopy (38.4%) and then the fully-automated AiDx Assist (25.9%). Different median egg counts were observed for semi-automated (96 EPG),

fully-automated AiDx Assist (48 EPG) and conventional microscopy (72 EPG). In addition to *S. mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura* eggs were manually detected on digital images captured with the AiDx Assist following retrospective image analysis (Figure 3a, b & c respectively).

Table 1. Characteristic outcomes of the semi-automated and fully-automated AiDx Assist in comparison to conventional microscopy for *Schistosoma* egg detection

Tests	<i>S. mansoni</i> (N=375)			<i>S. haematobium</i> (N=398)		
	Semi-automated AiDx-assist	Fully automated AiDx-assist	Conventional microscopy	Semi-automated AiDx-assist	Fully automated AiDx-assist	Conventional microscopy
Positive samples (%)	168 (44.8%)	97 (25.9%)	144 (38.4%)	259 (65.1%)	251 (63.1%)	260 (65.3%)
Low intensity	85 (50.6%)	74 (76.3%)	99 (68.8%)	170 (65.6%)	173 (68.9%)	179 (68.8%)
Moderate intensity	46 (27.4%)	20 (20.6%)	39 (27.0%)	-	-	-
High intensity	37 (22.0%)	3 (3.1%)	6 (4.2%)	89 (34.4%)	78 (31.1%)	81 (31.2%)
Range	24-2304 EPG	24-792 EPG	24- 576 EPG	1-1483 eggs/10ml	1-1169 eggs/10ml	1-181 eggs/10ml
Median	96 EPG	48 EPG	72 EPG	18 eggs/10ml	19 eggs/10ml	16 eggs/10ml

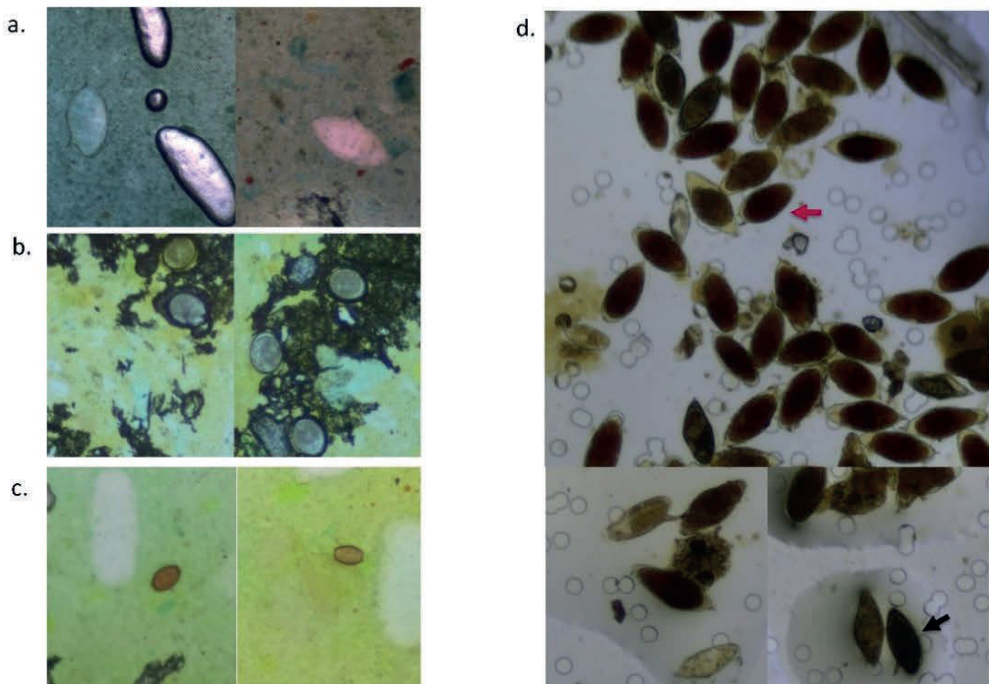


Figure 3. Digital image of Kato-Katz smear (a, b & c) and urine filtration slide (d) captured with the AiDx Assist showing *S. mansoni* eggs (a), *A. lumbricoides* eggs b) *T. trichiura* eggs (c) and several *S. haematobium* eggs (indicated with a red arrow) and *S. mansoni* eggs (indicated with a black arrow) (d).

Qualitatively, the agreement between the AiDx Assist (semi- and fully-automated) and conventional microscopy was moderate to substantial for the detection of *S. mansoni* eggs (K=0.538 and K=661, P<0.05 respectively). Figure 4a illustrates the diagnostic agreement between conventional microscopy and the semi- and fully-automated mode of the AiDx Assist. Based on conventional microscopy, the sensitivity of the semi-automated AiDx Assist (86.8%) for the detection *S. mansoni* egg was significantly higher than the fully-automated AiDx Assist (56.9%) although their specificities were comparable (81.4% and 86.8%, respectively).

Table 2. Diagnostic performance of the semi-automated and fully-automated AiDx Assist for the detection of *Schistosoma* eggs performed on urine and stool.

Index test	<i>S. mansoni</i>		<i>S. haematobium</i>	
	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Semi-automated AiDx Assist	86.8 (80.2-91.9)	81.4 (75.8-86.2)	94.6 (91.1-97.0)	90.6 (84.4-94.9)
Fully automated AiDx Assist	56.9 (48.4-65.2)	86.8 (80.2-91.9)	91.9 (87.9-94.9)	91.3 (85.3- 95.4)

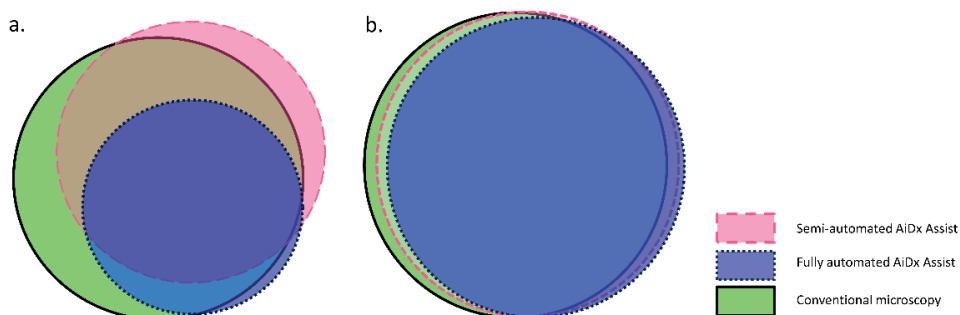


Figure 4. Proportional Venn diagram demonstrating agreement of percentage positive by semi-automated AiDx Assist, fully-automated AiDx Assist and conventional microscopy for the detection *S. mansoni* and *S. haematobium* egg on a) stool and b) urine respectively.

A moderate correlation was observed between the semi- and fully-automated mode of the AiDx Assist and conventional microscopy for quantification of *S. mansoni* eggs ($r = 0.64$ P<0.05, $r = 0.78$, P<0.05 respectively) (Figure 5a & b).

AiDx Assist performance for detection of *S. haematobium* eggs on urine slides

A total of 398 urine samples had results for all three diagnostic methods (Figure 1). The proportion of *S. haematobium* infection was comparable among the semi- and fully-automated AiDx Assist and conventional microscopy (65.1%, 63.1% and 65.3% respectively) (Table 1). The median egg count for *S. haematobium* by all three methods; semi-automated, fully-automated

AiDx Assist and conventional microscopy were comparable (18 eggs/10ml, 19 eggs/10ml, 16 eggs/10ml respectively). Figure 3d shows digital images of urine slides where *S. haematobium* and *S. mansoni* eggs are visualized.

An almost perfect agreement was observed between the semi-automated AiDx Assist and conventional microscopy ($K=0.820$, $P<0.05$) as well as between the fully-automated AiDx Assist and conventional microscopy ($K=0.851$, $P<0.05$) for the detection of *S. haematobium* eggs (Figure 3b). Using conventional microscopy as the reference, the sensitivity and specificity of the semi-automated (94.6% and 90.6% respectively) and fully-automated AiDx Assist (91.9% and 91.3% respectively) for the detection of *S. haematobium* eggs were comparable (Table 2).

Also, a very strong correlation was observed between egg counts of the semi- and fully-automated AiDx Assist and conventional microscopy ($r = 0.93$ $P<0.05$, $r = 0.95$ $P<0.05$, respectively) (Figure 5c & d).

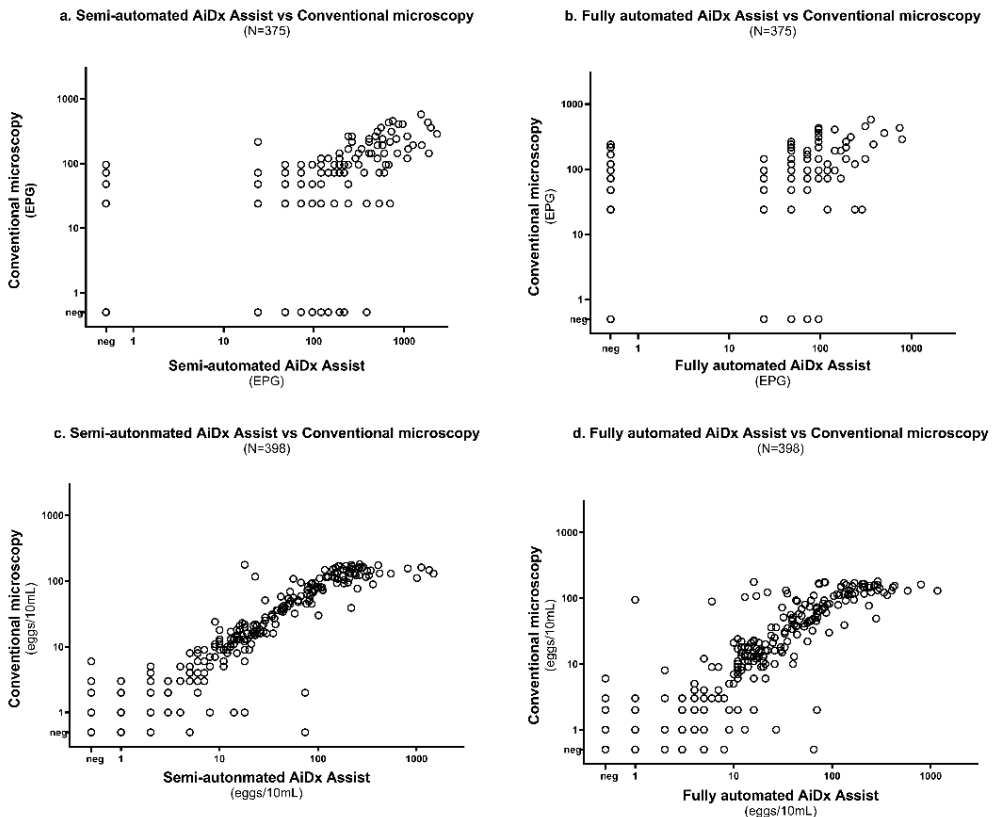


Figure 5. Correlation plot on a log scale between semi-automated AiDx Assist, fully-automated AiDx Assist and conventional microscopy for a & b) *S. mansoni* egg count c & d) *S. haematobium* egg count.

Discussion

For the first time we report the performance of the semi- and fully-automated AiDx Assist, as a single device in a field setting for detecting and quantifying *S. mansoni* and *S. haematobium* eggs in stool and urine samples, respectively, using conventional microscopy as the reference. Overall, the performance of semi-automated and the fully-automated AiDx Assist was found to be modest for the detection of *S. mansoni* eggs and requires optimization to improve the performance. Furthermore, the performance of the semi-automated AiDx Assist and the fully-automated AiDx Assist for the detection of *S. haematobium* eggs in urine samples was consistent with Makau-Barasa et al [5]. We also demonstrated that the AiDx Assist has a multi-diagnostic potential as other helminth eggs were visualized in digital images of some of the stool samples.

The significantly higher sensitivity of the semi-automated AiDx Assist (86.8 %) over the fully-automated AiDx Assist (56.9 %) for the detection of *S. mansoni* eggs on KK slides could be due to the fact that stool samples often contain dirt (artifacts) which interferes with the AI detection algorithm, while a trained expert would be able to ignore this and only detect *S. mansoni* eggs a phenomenon also observed in Dacal et al [14]. Another reason could be the difference in stool properties such as colour, texture and consistency as well as variation in stain preparation leading to variable KK smears (as observed in Figure 3a-c). The AiDx Assist AI algorithm for the detection of *S. mansoni* eggs in stool samples requires optimization, and such factors need to be taken into consideration. That is, a high quality and diverse data set covering as many different KK smear variations as possible, is required to further train the AI algorithm. Also, validation of the AiDx Assist using alternative stool preparation methods, e.g. the floatation preparation methods which results in relatively clean slides, could be a solution to the challenges with dirt or artifacts. However, such methods are not very field compatible [15, 16].

Additional analysis revealed that majority of the stool samples (68.8 %) based on conventional microscopy had low infection intensity (1-99 EPG, Table 1) of which more than half were missed by the AI algorithm. Therefore, further optimizing the AI algorithm for accurately detecting light infections would significantly improve the overall performance of the fully-automated AiDx Assist. The comparable specificity of the semi-automated AiDx Assist (81.4 %) to the fully-automated AiDx Assist (86.8 %) is due to the additional validation step by the operator during the fully-automated AiDx Assist as the positive cases detected by the AI are further checked and ruled out if false positive.

The sensitivity and specificity between the semi-automated AiDx Assist and fully-automated AiDx Assist for the detection *S. haematobium* eggs based on conventional microscopy were comparable (Table 2) and was consistent with previously reported findings [5]. Despite differences in the positive rates and similarities in the infection intensity observed between studies the consistent outcome provides more evidence on the reliability and robustness of the AiDx Assist for the detection of *S. haematobium* infection in urine. It also provides more evidence that, the AiDx Assist meets the required WHO diagnostic Target Product Profile (TPP) (WHO76) for sensitivity and specificity of *S. haematobium*.

Although a strong to moderate correlation was observed between the semi-automated AiDx Assist, fully-automated AiDx Assist and conventional microscopy for *S. mansoni* and *S. haematobium* egg quantification, at high infection intensities microscopy tended to underestimate egg count. This observation is in contrary to that of Meulah et al. [6] where for ≥ 100 eggs/10ml of urine the AI-algorithm integrated in the Schistoscope underestimated egg counts due to egg overlap. This contradicting observation could be partly due the differences in AI architecture used as well as the level of experience of the microscopists between studies. Images of samples with high egg counts (≥ 100 eggs/10ml for *S. haematobium* and ≥ 1000 EPG for *S. mansoni*) based on the semi-automated AiDx Assist were re-analyzed manually by an independent experienced microscopist which confirmed that conventional microscopy underestimated egg counts for samples with high infection intensity. This could be due to less experience by the microscopist in estimating egg count for sample with high infection intensity. Moreover, a better correlation scatter between the semi- and fully-automated AiDx Assist was observed across all infection intensities for both *S. mansoni* and *S. haematobium* egg quantification (Supplementary Figure 1).

Through retrospectively analysing digital images of KK slides prepared from various samples, it was possible to visualize other stool helminth parasites. The design of the AiDx Assist optical system theoretically enables the visualization of parasite features within a size range of approximately 15-400 μ m. This implies that, parasites and/or their eggs within this size range can be manually detected as also demonstrated by other studies [4, 10, 14, 18, 19]. However, the current AI-powered prototype of the AiDx Assist has been specifically developed for detecting eggs of *S. mansoni* and *S. haematobium*. While visualizing other helminth eggs on digital images captured with the AiDx Assist demonstrates its potential for detecting additional parasites, further development and optimization are necessary for these parasites. This process would involve generating datasets for different stool helminth parasites to train an AI algorithm to recognize these eggs, followed by validation. The digital images generated in this study could serve this purpose. Also, with the different variations in the digital image data set collected within this study, it could be used to optimise the diagnostic performance of the fully-automated AiDx Assist for detecting *S. mansoni* eggs and to develop the device for other helminth parasites in stool.

The limitation of this study is the fact that during conventional microscopy, the technicians were only asked to mark the number of *Schistosoma* eggs. Consequently, when exploring the AiDx Assist's capability to identify other stool helminths through digital images, no comparison could be made to conventional microscopy. This missed opportunity could have provided further evidence regarding the device's potential to detect other stool parasites.

In conclusion, the overall diagnostic performance of the semi- and fully-automated AiDx Assist for the detection of *S. mansoni* infection was found to be modest and requires improvement to meet the WHO TPP in terms of diagnostic accuracy. The consistent finding on *S. haematobium* detection and the additional observation of *A. lumbricoides* and *T. trichiura* revealed its potential for screening multiple diseases in endemic settings.

Author contributions

BM: Writing – original draft, Writing – review & editing, Data curation, Formal Analysis, Conceptualization, Methodology, Software, Validation, Visualization. PTH: Writing – review & editing, Methodology, Validation, Visualization. SP: Conceptualization, Validation, Data curation, Writing – review & editing. SJ: Data curation, Software, Validation. MA: Conceptualization, Writing – review & editing. JOF: Methodology, Writing – review & editing. JAO: Methodology, Writing – review & editing. DB: Conceptualization, Writing – review & editing. CH: review & editing. LvL: Conceptualization, Methodology, Writing – review & editing. GV: Conceptualization, Writing – review & editing. JC: Conceptualization, Writing – review & editing. TA: Conceptualization, Data curation, Writing – review & editing. JCD: Writing – review & editing, Conceptualization. LMB: Conceptualization, Writing – review & editing. JS: Conceptualization, Methodology, Writing – review & editing.

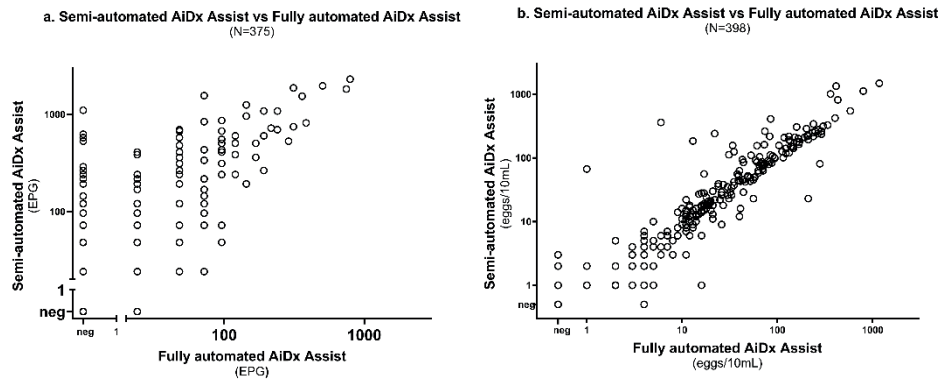
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Chapter 6

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Supplementary information



S1 Figure. Correlation plot on a log scale between semi-automated AiDx Assist and fully-automated AiDx Assist for the for a) *S. mansoni* egg count b) *S. haematobium* egg count.