WATER RESEARCH INSTITUTE
Biomedical & Public Health Research Unit

The design and evaluation of a new SYBR-green quantitative PCR assay for the diagnosis of Schistosomiasis.

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Introduction

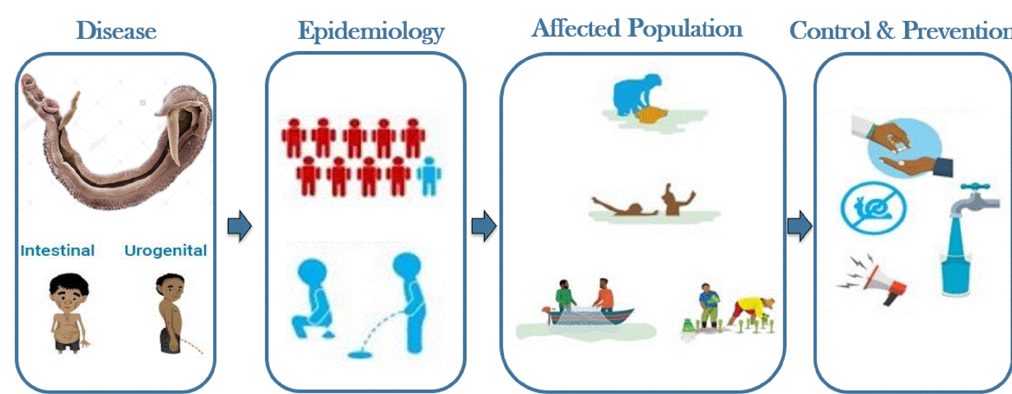
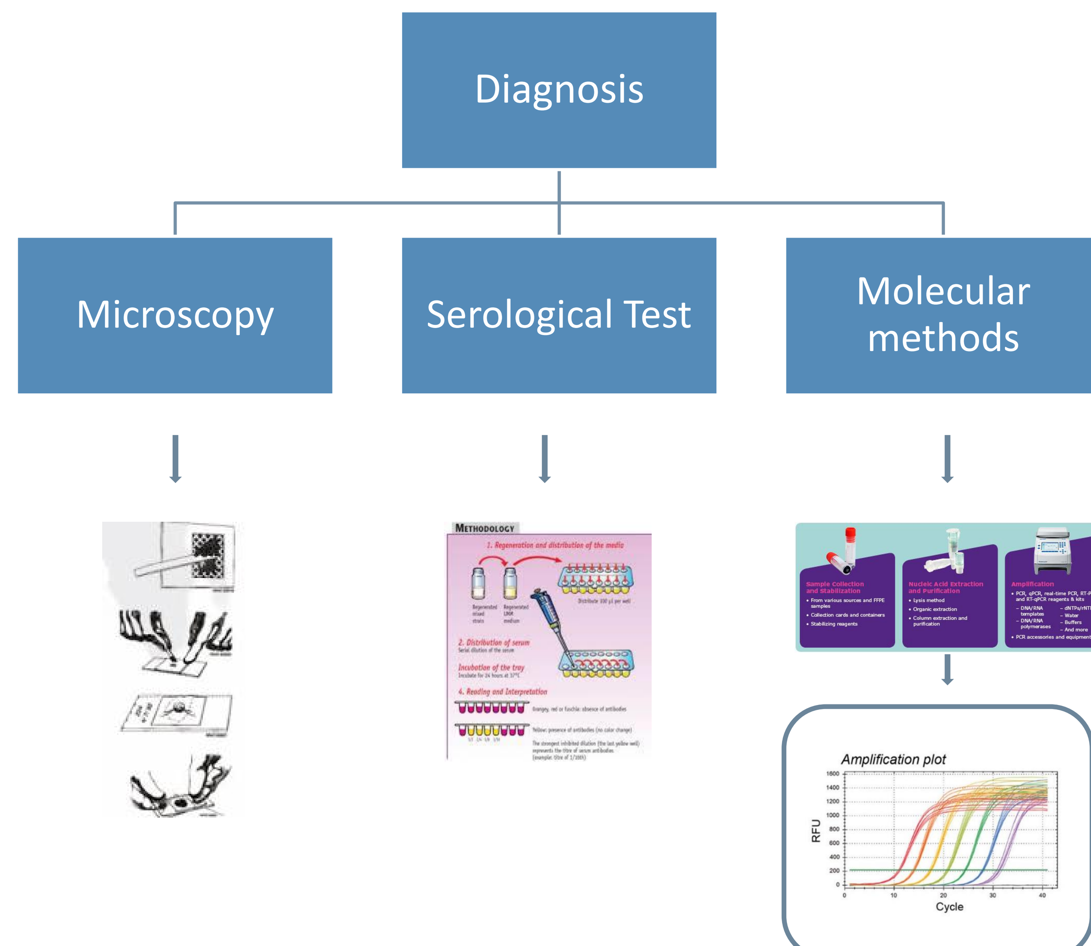


Figure 1. Chart on schistosomiasis (adopted from WHO)



Methods and Materials

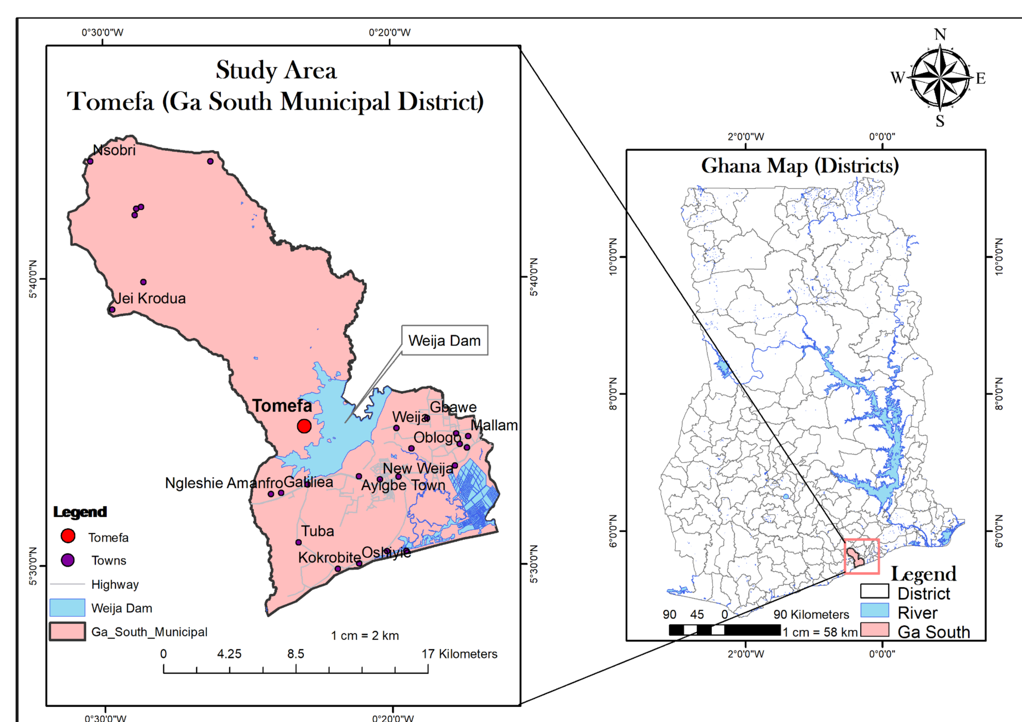


Figure 2. Tomefa in the Ga South Municipal District of Ghana

Study design
Study used archived 150 urine and 200 stool DNA samples from previous study.
Primers were designed from the COX1gene of each Schistosoma sp. with OligoExplorer v 1.5.

Table 1. qPCR Primer sets used for Schistosome amplification

Organism	Primer Name	Sequence, 5' ⟶ 3'
S. haematobium	Sh-COX1-RTF	TCGTTTGGGTATTATGGATT
	Sh-COX1-RTR	GATGGGCTCAAACTACACTT
S. mansoni	Sm-COX1-RTF	CCTCGTCGTGTAAGTTGCTATG
	Sm-COX1-RTR	ACAACCAAAGACTCCCACAAA

Figure 3. *S. haematobium* [A] and *S. mansoni* [B] COX1 primers

Results

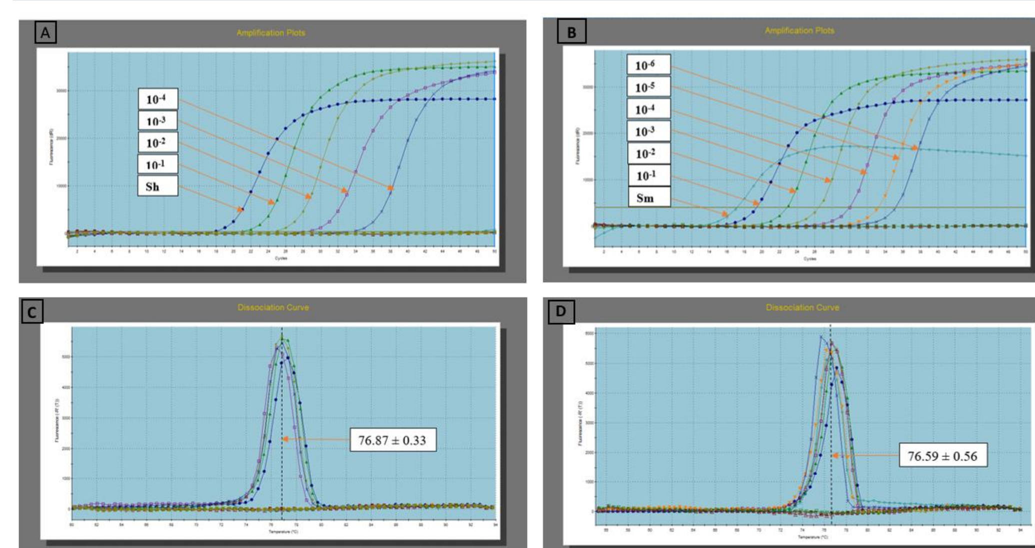


Figure 4. Lowest DNA detection limit for *S. haematobium* [A] (1.216pg) and *S. mansoni* [B] (0.122pg) with their respective dissociation curve [C&D].

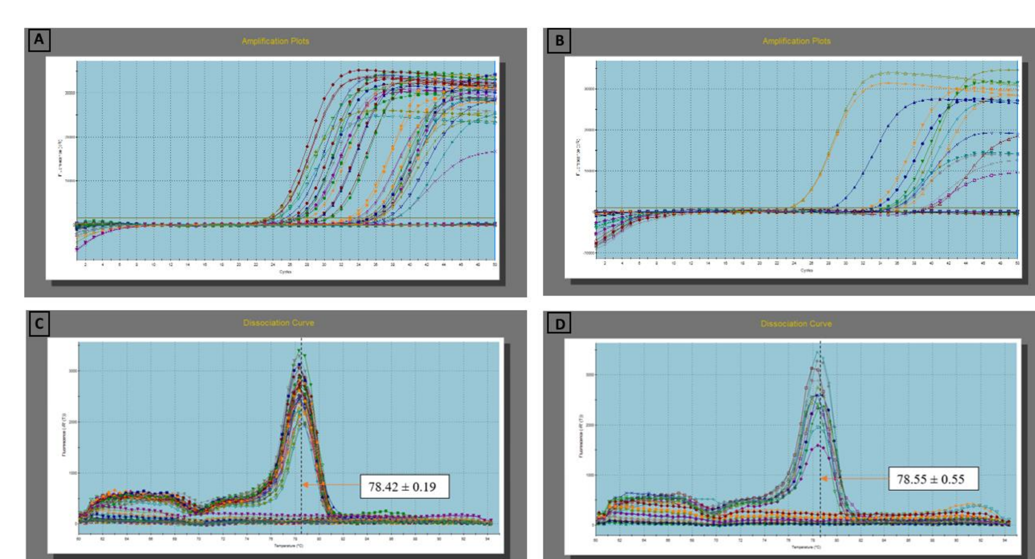


Figure 5. qPCR amplification plot of *S. haematobium* microscopy positives [A] and negatives [B] with their respective dissociation curves [C&D].

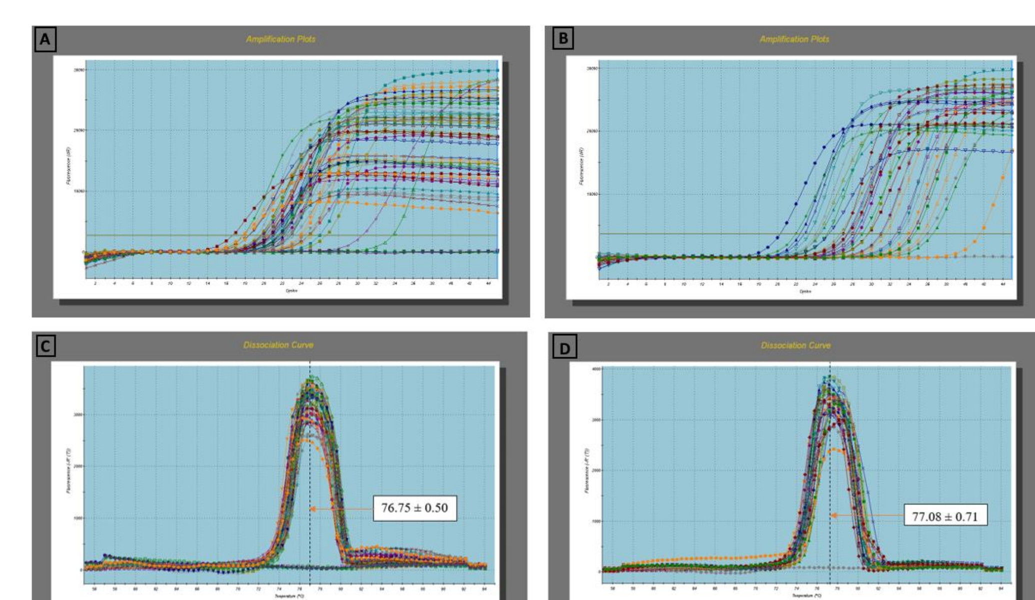


Figure 6. qPCR amplification plot of *S. mansoni* microscopy positives [A] and negatives [B] with their respective dissociation curves [C&D].

Table 2. Diagnostic accuracy of qPCR for Schistosomiasis.

	Microscopy (ref. method)		qPCR			
	S. h.	*S. m.*	*S. h.*		*S. m.*	
			+ve	-ve	+ve	-ve
Positive	73	150	70	3	150	0
Negative	77	50	14	63	47	3
Prevalence			36.0%		91.7%	
Sensitivity	-	-	93.7%		82.6%	
Specificity	-	-	92.3%		90.3%	

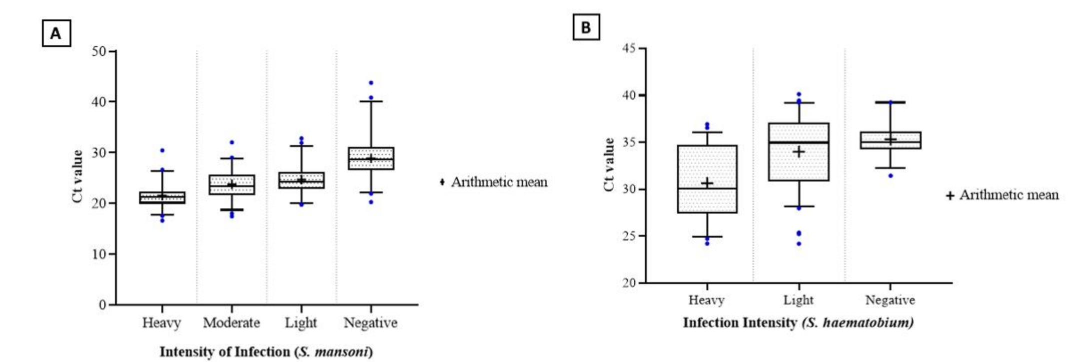


Figure 7. Boxplot of all *S. mansoni* qPCR positives (A) and *S. haematobium* qPCR positives (B), comparing the distribution of the Ct-values in microscopy-negative and microscopy-positive samples.

Discussion

The present study was focused on developing an alternative *COX1-based* SYBR green PCR assay for the detection of schistosomiasis which is not dependent on HRM. In this designed assay, schistosome species-specific primers were used for the discrimination between *S. haematobium* and *S. mansoni* detection in urine and stool samples, respectively.

The lowest DNA detection limit for the detection of *S. haematobium* and *S. mansoni* via the application of the qPCR assay was 1.216pg and 0.122pg, respectively. This clearly shows that the newly designed qPCR assay is very sensitive, especially since a similar outcome was observed in the study conducted by Sady and colleagues (2015). In addition, a comparison with the TaqMan probe assay conducted by Hove and colleagues (2008) revealed a similar trend.

The extra detected positives from the microscopy negative samples, especially in the case of intestinal schistosomiasis, as well as the high prevalence of schistosomiasis from the BLCA show clearly that the community, Tomefa is highly exposed and as a results, requires a wholistic intervention to control the spread of the disease.

Finally, the outcome of sensitivity and specificity from the BLCA confirms that this qPCR assay designed from the *COX1 gene* is highly sensitive and can match existing qPCR assays used for the detection of schistosomiasis from different target sequences.

Conclusions

The present study extensively evaluated the performance of qPCR for the detection of schistosomiasis by using designed primers which targets the *COX 1* gene of the each schistosome species.

The developed *COX1* SYBR Green qPCR assay *for S. mansoni* and *S. haematobium* detections presented in this paper has proven effective and could be applicable to epidemiological surveys for treatment monitoring. It is a quick, efficient, and accurate procedure, which can be a good substitute for schistosomiasis qPCR assays that rely on probes.

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References

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