A Duplexed Lateral Flow Assay for Schistosome cfDNA Detection in Urine

Towards low-cost, point-of-care, nucleic acid diagnostics.

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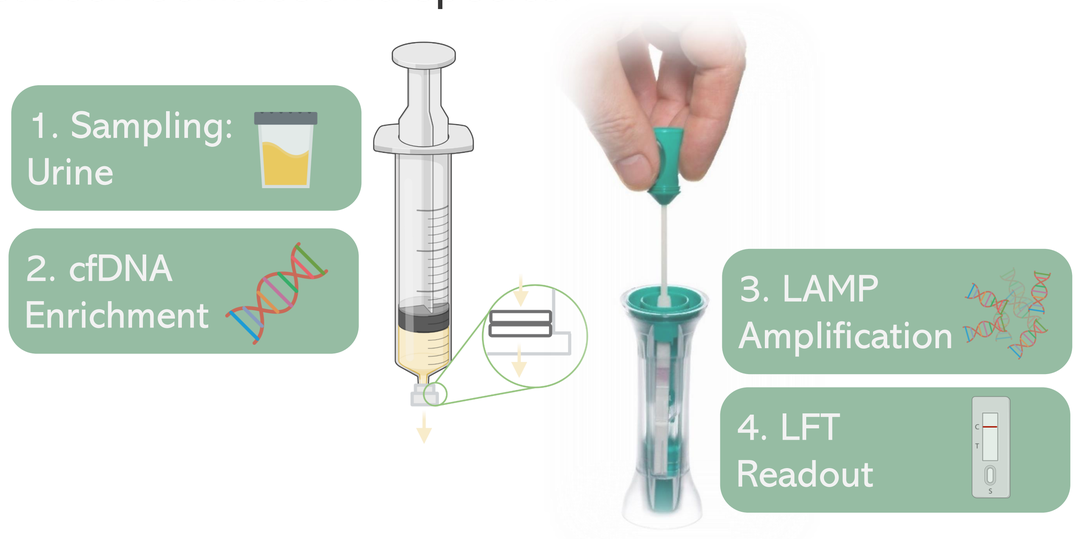
Currently available diagnostics for schistosome infections do not offer the required sensitivity in low prevalence settings to support elimination campaigns [1]. Nucleic acid amplification tests (NAATs) could fill this requirement but must be simple and cost-effective.

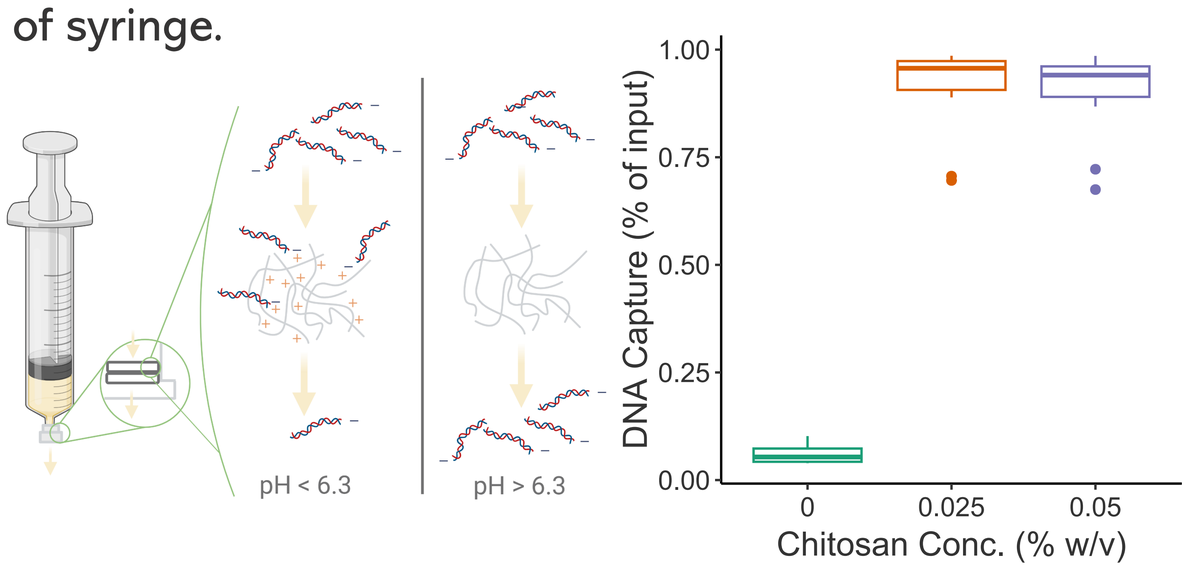
Parasite cell-free DNA (cfDNA) in urine could present an opportunity to simultaneously detect multiple *Schistosoma* species in a single sample [2].

Here we present a simple filter-based sample preparation method, and duplexed isothermal NAAT assays for *S. haematobium* and *S. mansoni.* Assay readout is on a dual-labelled lateral flow strip (LFT) to differentiate between *Schistosoma* species.



2. cfDNA Enrichment

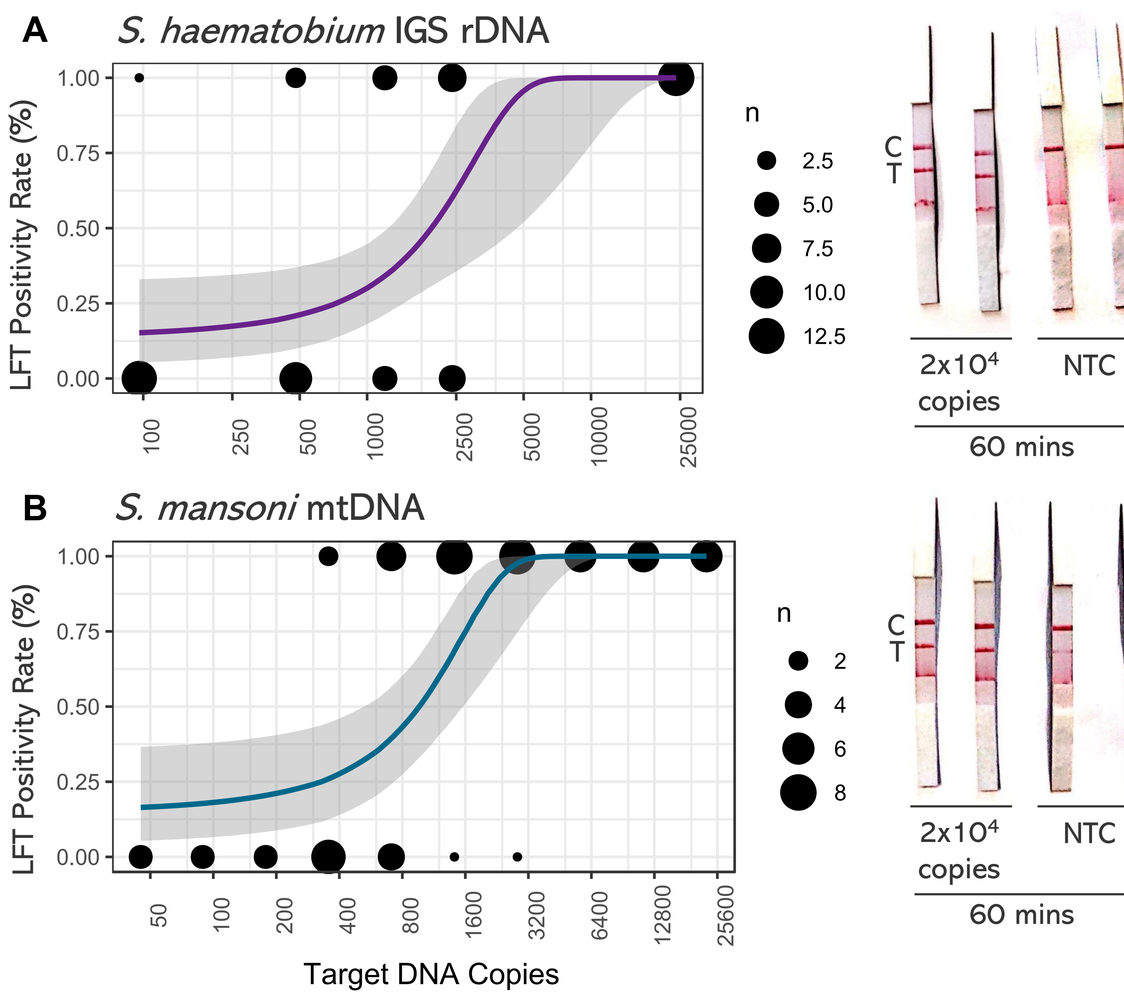
The pH-dependent charge switching properties of chitosan allow for electrostatic capture of DNA onto a membrane when pH < 6.3. DNA can subsequently be eluted in a buffer of pH > 6.3 [3]. Human gDNA was diluted, buffered to pH 5.0, and filtered through a chitosan-functionalised glass fibre membrane at the end of syringe.



Membranes functionalised in solutions of 0.05 and 0.025 % (w/v) chitosan capture a greater percentage of DNA compared to untreated membranes (0% chitosan).

3. LAMP Amplification

4. LFT Readout



LAMP assays specific for (A) *S. haematobium* and (B) *S. mansoni* were modified for use with a lateral flow readout. Probit regression was used to model the relationship between LFT positivity rate and target DNA copy number. The 95% LoDs were $5.0x10^4$ and $2.5x10^4$ target copies per reaction, respectively.

Using dual-labelled LFTs, the assays can be duplexed into a single tube and differentiate between *S. haematobium, S. mansoni,* and samples containing gDNA from both species.

Next Steps

The cfDNA enrichment device and duplexed LAMP assays will be validated using clinical urine samples to determine if *S. haematobium* and *S. mansoni* cfDNA can be detected in a urine sample using a simple prototype point-of-care diagnostic.

[1] Diagnostic target product profiles for monitoring, evaluation and surveillance of schistosomiasis control programmes. Geneva: World Health Organization; 2021
[2] Archer J, LaCourse JE, Webster BL, Stothard J Russell (2020). Parasitology 147, 873–888.
[3] Rosenbohm JM, Robson JM, Singh R, Lee R, Zhang JY, Klapperich CM, Pollock NR, Cabodi M. Anal. Methods, 2020,12, 1085-1093

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