Optimising sample storage and preparation for the low-resource DNA-based diagnosis of active female genital schistosomiasis

Background

Female genital schistosomiasis (FGS) is a neglected gynaecological disease affecting ~56 million women across sub-Saharan Africa. It is caused by eggs of the parasitic trematode *Schistosoma haematobium* becoming trapped in the female genital organs and surrounding tissues. Diagnostic advancements for FGS, such as low-resource DNA-based diagnostics, can decentralise screening. However, they are often limited in low-resource settings due to high-resource sample storage and extraction methods used alongside. This study investigated optimal sample storage and DNA extraction methods that can be coupled with low-resource tests for closer-to-the user diagnosis of active FGS.

Methodology

Nylon flocked swabs were spiked with single *S. haematobium* eggs. and stored in four different storage media at four different temperatures for six different longevities of time (up to 28 days), before being processed with a standard high resource DNA extraction method.
To further investigate the impact of low-resource preservation and DNA extraction methods swabs were stored at 22°C for 24 hours, in a total of five storage conditions- three commercially available storage media, 200µl of distilled water, or completely dry. Swabs were processed using 11 DNA extraction methods that could theoretically be implemented in low-resource laboratories (i.e. needing minimal equipment). All DNA extracts were analysed using the *Schistosoma* ITS-2-qPCR to measure CT value. Samples with a CT value below a 35 CT cut-off were considered positive. All data was statistically analysed using StataNow.

Results

All conditions including time point ($F(5, 284) = 6.80$, $p < .001$), storage media ($F(3, 284) = 9.66$, $p < .001$), and temperature ($F(3, 284) = 7.4$, $p = .016$) had a statistically significant impact on CT value when analysed using one-way ANOVA tests. Notably, detectable DNA was obtained from swabs exposed to all storage variables. When two-way ANOVA analyses were performed to see if combinations of conditions had compound effects on CT value, only the combination of temperature and timepoint had a significant impact ($F(15, 264) = 3.65$ $p = .032$), with a decline In CT value with an increase in temperature and time.
When comparing storage media and extraction method combinations, both storage and extraction methods had a statistically significant impact on CT value through one-way ANOVA analysis (storage media: ($F(4, 115) = 7.4$, $p < 001$, extraction method: ($F(10, 154) = 19.99$, $p<0000$). When combined, storage media and extraction method had a significant effect on CT value ($F(40, 164) = 3.13$, $p < 001$).

Significance

Our experiment shows that DNA can be obtained from *S. haematobium* eggs/DNA on swabs preserved and stored under very low-resource conditions, including no cold-chain or media, supporting the accessibility of molecular diagnostics for FGS.
High-resource extraction methods produced CT values below cutoff regardless of storage method. However, combinations of dry swab sample storage with low-resource extraction

methods produced similar CT values. This suggests a need for further consideration of new low-resource extraction methods.