

Development of an isothermal assay for the detection of *Schistosoma haematobium* and human papillomavirus DNA in cervical samples

Introduction

Female genital schistosomiasis (FGS) is a gynaecological condition caused by infection with the trematode *Schistosoma haematobium*, which is endemic across sub-Saharan Africa (SSA). FGS is known to lead to cervical cancer and other chronic reproductive health problems. FGS has also shown an association with high-risk human papillomavirus (HR-HPV) type infections, the causative agent of over 99% of cervical cancer cases. Difficulty accessing diagnostics for both FGS and HR-HPV prevent timely case management.

This project aims to develop a multiplex recombinase polymerase (RPA) assay for the low-resource diagnosis of FGS and HR-HPV to support timely interventions.

Methodology and Results

RPA Primers and probes were designed targeting the L1 region of HR-HPV types 16, 35, 52, and 18/45 in combination. Limited genomic conservation between HR-HPV types prevents the development of a multi-type target. These were incorporated into multiplex RPA assays with a previously developed RPA assay for *S. haematobium* (*Sh-Dra1-RPA*), and a human Beta globin gene (HBG) internal control assay. Analytical and clinical sensitivity and specificity for each target were tested as both singleplex and multiplex assays.

The assays will be optimised for use on self-swabs cervico-vaginal samples, increasing accessibility in endemic settings. To accommodate the three target limitations of RPA, a two-step diagnostic pathway was designed. The index test includes *S. haematobium*, HPV-16, and HBG control: HBG to determine optimal sample taking and preparation, HPV-16 as the most prevalent and highest risk HPV type, and *S. haematobium* to identify if the patient has FGS. If the patient tests positive for HBG, but negative for HPV-16, a second test including HPV types 35, 52, and 18/45 combined will be performed, as these are high-prevalence HR-HPV types in sub-Saharan Africa.

Discussion

Consideration of the challenges to healthcare access in target populations is essential for diagnostic development. Due to their shared sample type, range, and impact on women's health, FGS and HPV are ideal candidates for combined testing. Low-resource tests allow testing at home or in community settings, increasing healthcare accessibility for women in rural areas. The two-step test algorithm allows for quick decision-making and cost-effective screening for these two highest causes of cervical cancer in Africa.