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Development of a Cas13a-based lateral flow assay for detecting Neisseria gonorrhoeae

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Background

The prevalence of Neisseria gonorrhoeae infection is highest among resource-limited settings. Importantly, such areas lack laboratory infrastructure necessary to perform pathogen-specific testing, resulting in syndromic management, which misses the high proportion of cases that are asymptomatic and contributes to antimicrobial resistance via the overuse of antibiotics. Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) uses isothermal amplification via recombinase polymerase amplification (RPA) paired with a CRISPR-Cas13a enzyme linked with a programmable RNA guide to produce low-cost lateral

flow tests. Such tests can obviate the costs of standard polymerase chain reaction testing. We aimed to develop a Cas13a-based lateral flow Neisseria gonorrhoeae detection assay.

Aim/Methods
We used an online software to predict guide sequences to the porA gene used in Neisseria gonorrhoeae detection. We evaluated guides targeting three regions of the porA gene as well as two different primer sets per guide. We evaluated the performance of those guides and primer sets on 23 purified Neisseria gonorrhoeae isolates using fluorescence detection to quantify assay performance. We then substituted the standard quenched FAM reporter used in fluorescent readout detection for a biotinylated FAM reporter compatible with lateral flow readouts, and tested 14 of those specimens in triplicate on lateral flow strips. Finally, we evaluated the analytic sensitivity of that assay via serial dilutions in water.

Results
Of the three guides and two primer sets tested, we selected the guide-primer set combination with the greatest discrimination of Neisseria gonorrhoeae compared to negative controls and the highest fluorescence signal. All 23 isolates were detected on fluorescence readout and all 14 of the isolates tested were detected using lateral flow (Figure). Our Neisseria gonorrhoeae lateral flow assay was able to detect down to 10,000 DNA copies per milliliter. The lateral flow assay ran over 90 minutes.

Conclusions
We report on the development of a novel Cas13a-based lateral flow assay for detecting Neisseria gonorrhoeae. Our assay was able to detect all Neisseria gonorrhoeae isolates analyzed. Further work will aim to characterize the performance of that assay among other sexually transmitted pathogens and other non-gonococcal Neisseria specimens, as well as incorporate detection of molecular markers of antimicrobial resistance.

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Cas13a-Based Detection of Neisseria gonorrhoeae Isolates

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