Sample-to-Answer Molecular Diagnostic Platform for Rapid Point-of-Care detection of Chlamydia

Chelsea Qinjie Zhou, Rohan Pais and Andrea Pais
Novel Microdevices, LLC, Annapolis, MD

Introduction

Chlamydia trachomatis (CT) is an obligate intracellular human pathogen and the most common bacterial sexually transmitted disease (STD). CDC recommends Nucleic acid amplification tests (NAATs) as the tests of choice due to their high sensitivity and specificity, however they require expensive laboratory equipment and skilled technicians to run the test. Current point-of-care tests (POCTs) that produce rapid results are immunoassay based tests which lack sufficient sensitivity. Here we present a simple, fully automated, sample-to-result, hand-held POC NAAT platform for point-of-care detection of CT in under 40 minutes. The test can be performed by a layperson in three easy steps.

Materials and Methods

Microfluidic cartridges were prototyped by aligning and sealing laser-patterned sheets of acrylic with pressure-sensitive silicone adhesive. A low-power (2.5 W) heating element was fabricated by embedding a resistor and thermocouple into a custom built aluminum block and potting it in place using thermally conductive epoxy. The heater element is activated during the isothermal amplification to provide uniform heating.

Microfluidic cartridges were designed with a Sample-extraction component that is used to process the sample into a single-use microfluidic cartridge that contains all of the assay components and is then used in the heater to generate the test result.

Technology Features:

- Self-contained disposable microfluidic cartridge
- Low-cost, USB or battery powered instrumentation
- Single assay automation actuator that is designed to perform multiple assay steps in a precise sequence along its path of motion

Assay Features:

- Sample-to-Answer in 40 minutes or less
- Analytical sensitivity of amplification assay is 5 EB/reaction
- High specificity against microbial organisms commonly present in vaginal samples
- Sample-to-Answer assay sensitivity is 50 EB/swab for CT

Results

A. Concept illustration of the proposed hand-held microfluidic device. B. Schematic diagram describing the working principle of our low power microfluidic assay automation technology using a single rotational motion. As the microfluidic chip rotates relative to the actuator element, multiple assay steps are performed in a precise timing sequence, including dispensing stored reagents, opening/closing micro-valves and moving magnetic beads or liquids between chambers. C. Photograph of a prototype self-contained microfluidic cartridge that rotates to co-extract and template the sample-to-result assay sequence. Colored liquid is filled in the reagent pouches for better visualization.

Heat-inactivated CT cells and purified CT DNA were provided by Johns Hopkins Center for POCTs for STDs. Negative swabs spiked with CT cells were used in all experiments. The swab was first inserted into the sample extraction container. The sample extraction container is designed to separate and elute the CT DNA from the swab to maximize the elution of its contents into the solution containing lysis buffer present inside it. The lysate from the sample extraction container was transferred to the microfluidic cartridge where the DNA was extracted and purified using magnetic beads conjugated with an ionizable functional group that binds or elutes nucleic acids depending on the pH of the buffer present. Isothermal LAMP amplification was carried out in a 25 µL reaction volume and incubated at 64°C for 30 minutes with Biotin and FAM labeled FIP and LB primers respectively. Reaction products were subjected to 2% agarose gel electrophoresis and Nucleic Acid Lateral Flow (NALF) strips (Forisite Diagnostics).

Conclusions

- We have developed an inexpensive, low power, sample-to-answer molecular diagnostic platform for point-of-care applications; and demonstrated the accurate detection of CT on the platform in under 40 minutes.
- Future work will include pre-clinical studies with patient swab samples to benchmark the assay and platform performance with the gold standard clinical NAATs for CT.

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For questions and additional information please contact Andrea Pais at andrea@novelmicrodevices.com