Ultra-Rapid Isolation and Detection of *Neisseria gonorrhoeae* by Microwave-Accelerated Methods

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**INTRODUCTION**

*Neisseria gonorrhoeae* (GC) is the second most commonly reported sexually-transmitted infections (STIs). In 2013, there were 333,304 cases of gonorrhea reported to the CDC. This infection has serious sequelae among women: PID, tubal factor infertility, chronic pelvic pain, and ectopic pregnancy. Gonorrhea infections can increase HIV transmission 3-5 fold. The most vulnerable groups for STIs and their sequelae are women, adolescents, and racial and ethnic minorities.

Often persons who present to a clinic for STI testing never return to the clinic to receive their STI test results. Thus the availability of a point-of-care test which can be resulted to them immediately is highly desirable. We have previously reported on Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) assays for the detection of Chlamydia. The objective of this study was to develop a microwave-based approach for the isolation of GC DNA, which when combined with our MAMEF assay can lead to the sensitive and rapid detection of gonorrhea infection.

**MATERIALS AND METHODS**

Rapid isolation and fragmentation of genomic DNA from either bacterial cells or vaginal swabs elution (2 mL aliquot) were carried out in lysing chambers (Figure 1) using microwave irradiation (Figure 2). The lysing chambers are composed of gold triangles deposited on glass slides, and a self-adhesive silicon isolator placed over the gold triangles to create a lysing chamber (Figure 3).

Immediately following the lysis step, detection of GC genomic DNA is carried in silver-covered wells (Figure 3), which have been shown to enhance the fluorescence signal. Detection of genomic DNA is mediated by the complementary binding of probes to the target sequence (Figure 4). The application of low-power microwaves drives the hybridization of probes and target to completion within seconds. This process is known as Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF).

1. **Heating-based DNA fragmentation**

2. **Microwave-based DNA fragmentation**

3. **Microwave-focusing triangles and DNA fragmentation**

4. **MAMEF-based detection of gonorrhea DNA**

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5. **Microfluidic-based microwave-assisted lysing**

**RESULTS**

- Rapid lysis of cells, fragmentation, and detection of target DNA can be carried out in less than 10 minutes.
- The assay is highly sensitive and no signal was detected in the absence of target DNA.
- Detection of GC target DNA is mediated by a fluorescent probe-based approach, which is enhanced by the intrinsic properties of the MAMEF approach.

**CONCLUSIONS**

- Microwal lysing and DNA fragmentation can be achieved in as little as 30 seconds by microwave irradiation.
- Microwave-based DNA isolation is cheaper and faster than chemical lysing approaches.
- MAMEF-based detection of GC DNA is fast and the assay shows good sensitivity and specificity.
- Microwave-accelerated methods have the potential to be useful in the development of point-of-care (POC) diagnostics.

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