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Novel Detection Methods for Sexually Transmitted Infection (STIs) from M. Genitalium

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Abstract

BACKGROUND: Mycoplasma genitalium is a sexually transmitted pathogen, causing 25-35% of nongonococcal urethritis in males and linked to premature rupture of placental membranes in females. This pathogen is difficult to culture, requiring an alternative identification method for diagnosis. Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification technique that uses a set of 4 to 8 DNA primers, allowing for rapid target amplification and a viable detection method.

METHODS: Primer sets were designed using available pdhD gene G37 strain of M. genitalium (L43967.2). Alignments of the pdhD genes of four M. genitalium types were performed using Clustal Omega. LAMP primers (F3, B3, FIP, and BIP) were designed using NEB's LAMP Primer design tool and synthesized through Integrated DNA Technologies. Loop primers (LF, LB) were designed separately to increase the specificity and sensitivity of the LAMP assay using the NEB LAMP Primer design tool. The final primer set was chosen based on ΔG values. The specificity of all primers was confirmed using BLAST.

Colorimetric LAMP reactions were performed using the WarmStart® Colorimetric (or Fluorescent) LAMP 2X Master Mix (DNA & RNA) (M1800S) from New England Biolabs (NEB, Ipswich, MA, USA) or the WarmStart® Fluorescent LAMP/RT-LAMP Kit (E1708S) with LAMP Fluorescent Dye (NEB #B1700). Reaction mixes were prepared as described by the manufacturer (NEB) at 65ºC for 30 minutes. A lateral flow assay (LFA) using the Milenia HybriDetect LFA kit was also used Genomic copies were calculated based on the M. genitalium genome size (580,076 base pairs).

RESULTS: The pdhD Color LAMP reaction allowed target detection of 15.97 genomic copies at a concentration of 1x10-2 pg/µL in 30 minutes. Controls that contained no target DNA or off-target DNA and did not result in any noticeable amplification. Fluorescent LAMP allowed detection of a much lower target DNA copy amount, 1.6 genomic copies, which equates to a concentration of 1x10- 7ng/µL, in a shorter amount of time (18 min).

A preliminary lateral flow assay (LFA) experiment for the detection of Mycoplasma genitalium successfully detected Mycoplasma genitalium DNA but not the negative control Mycoplasma hominis (ATCC 23114D).

DISCUSSION/CONCLUSION: LAMP assays maintain the high sensitivity of normal PCR assays while allowing for a more rapid diagnosis with similar sensitivity, eliminating the need for thermal cycling. To visualize our detection results, another method called LFA was used to compare our detection results to a close genomic relative, Mycoplasma hominis, which showed no detection.

We found that this assay is specific to Mycoplasma genitalium when compared to Mycoplasma hominis. The pdhD fluore-

scent LAMP was found to be more sensitive (1.6 genomic copies) than the Colorimetric LAMP assay (15.97 genomic copies). Fluorescent LAMP reactions were monitored for 32 minutes at 65°C with the fastest time to detection of 14 minutes containing the highest concentration of the target. Although the colorimetric method was rapid, it had the innate flaw of being indirect, whereas LFA is specific and a direct method of visualizing the product of amplification. Similarly, fluorescent lamp is a specific and direct method of measurement but requires cumbersome equipment.

Current work is underway to establish a point of care LAMP assay. We project that screening pregnant women for Mycoplasma and other organisms could potentially be a preventative measure precluding chorioamnionitis and possibly fetal loss.