An Ultra-rapid Molecular Assay for Detection of Pathogenic Bacteria

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ABSTRACT

- Infectious diseases driven by bacteria are among the first causes of mortality in remote, isolated, and low-resource settings.
- The current gold standard diagnostics technique, polymerase chain reaction (PCR), is complex, expensive and is mainly suited to centralized laboratories.
- We have developed an ultra-rapid molecular assay which employs loop-mediated isothermal amplification (LAMP), that can be integrated into a microfluidic device to diagnose nucleic acids within 30 min.
- This method provides a more feasible, time-effective, and cost-effective diagnostic technique compared to conventional PCR, circumventing delays in screening and test results.

BACKGROUND

The Problem:

- Reducing time delay between bacteria sample collection and treatment could be lifesaving (1)
- Direct detection of bacterial nucleic acids takes at least 24 hours for definitive results by standard methods (2, 3)
- Goal is to reduce cost and time of standard diagnostic techniques, while maintaining their sensitivity and accuracy

Proposed Approach:

- We have developed an ultra-rapid molecular assay which employs loop-mediated isothermal amplification (LAMP)
- This assay has detected *Escherichia coli* (*E. Coli*) and *Pseudomonas aeruginosa* (*P. A.*), with noticeable color changes within 30 minutes.
- Reaction changes colour within 30 minutes due to transition from basic to acidic pH in presence of phenol red, when DNA amplifies
  - DNA releases H+ ions during amplification
- Reaction changes colour in 3-10 minutes in microfluidic device (on-chip)

METHODS

1. Primer Design (5' to 3')

ECOLI-F3	GCCATCTCCTGATGACGC
ECOLI-B3	ATTTACCGCAGCCAGACG
ECOLI-FIP	CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT
ECOLI-BIP	CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT
ECOLI-LF	CTTTGTAACAACCTGTCATCGACA
ECOLI-LB	ATCAATCTCGATATCCATGAAGGTG

- Primers target *E. Coli* malB gene; sequence represents position 3204 to 3407 in GenBank sequence (GDB J01648) (4)

PA-F3	GCGTTGCCGCCAACAATG
PA-B3	CATGCGGGCAACCTCTC
PA-FIP	GTTGTCACCCCACCTCCGGGCGGCAACGTTCCTCC
PA-BIP	CTCCGTGCAGGGCGAACTGCAGGCGAGCCAACTC
PA-LF	ACCTGCCGTGCCATACC
PA-LB	GTTCATGCAGCTCCAGCAG

- Primers target *P.A.* oprL gene; sequence represents position 801 to 1000 (5)

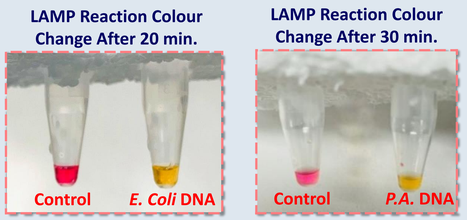
2. LAMP Reaction Contents

- 12.5 µL Warmstart 2X Colourimetric MasterMix, 2.5 µL 10X Primer Mix, 9 µL DNAse free H2O, 1 µL DNA sample*

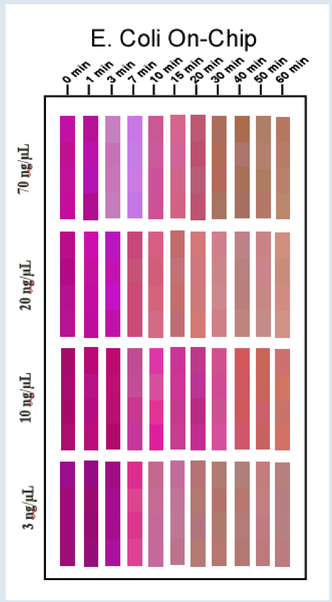
*DNA sample extracted by boiling *E. Coli* cultures grown overnight at 95 ºC for 10 minutes
*chemical lysis method used for *P.A.* cultures

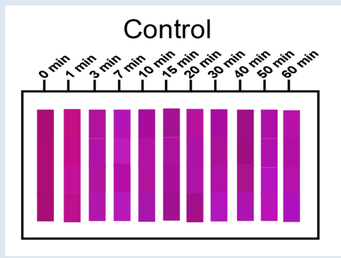
3. Validation of Primers

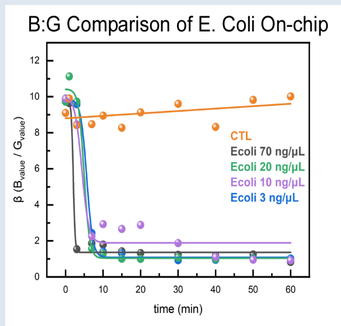
- Placed LAMP reaction in sealed tube in 60-65 ºC water bath for 1 hour
- Colour in *E. Coli* DNA sample (70 ng/µL) changed from pink to yellow in 20 minutes
- Colour of *P.A.* DNA sample (50 ng/µL) changed from pink to yellow in 30 min



E. COLI RESULTS ON-CHIP







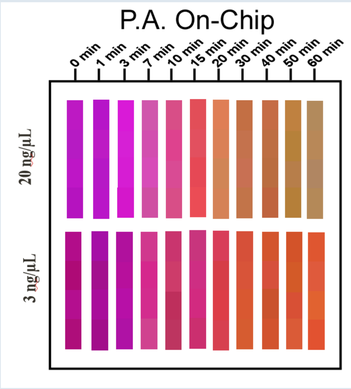
All *E. Coli* DNA samples changed colour from pink to orange, compared to control which showed no colour change.

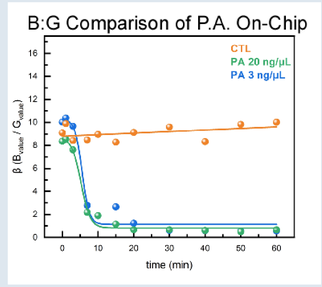
Measured using β (B:G) values.

70 ng/µL showed β reduction in **3 min**, compared to 20, 10, and 3 ng/ µL which showed β reduction in **7 min**.

β reduced from ~10:1 to ~2:1.

P.A. RESULTS ON-CHIP





P.A. DNA samples changed colour from pink to orange, compared to control which showed no colour change.

Both 20 ng/µL and 3 ng/µL samples showed β reduction in **10 min**.

β reduced from ~10:1 to ~2:1.

CONCLUSION & FUTURE DIRECTIONS

- This molecular assay for bacteria detection is significantly more rapid compared to conventional methods
- Combined with microfluidics, bacteria diagnosis can be achieved in 3-10 minutes
- With this approach healthcare systems in low-resource settings will be well-prepared to combat future infectious disease outbreaks
- Next steps include testing assay across all relevant *P.A.* concentrations & testing *MRSA* (gram-positive bacteria)

This approach shows a proof-of-concept for an optimized LAMP assay for bacteria diagnosis in healthcare settings

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