ASSESSMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAYS FOR *ESCHERICHIA COLI* DETECTION

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INTRODUCTION:

As of lately, loop-mediated isothermal amplification (LAMP) has become a powerful alternative to the polymerase chain reaction (PCR) in the molecular diagnostic world, especially for pathogen detection in clinical and food samples. LAMP is a powerful and new method of nucleic acid amplification, with the ability to detect DNA at a very low level. In comparison to other molecular methods such as PCR and Real-Time PCR, LAMP eliminates the need for sophisticated thermal cyclers. High efficiency of DNA amplification by LAMP significantly shortens the whole amplification process.

OBJECTIVES:

This study aims to assess the efficacy of two different LAMP kits, WarmStart (Real-Time LAMP) and Colorimetric LAMP in detection of *Escherichia coli*. We tested and optimized the kits for the detection of *E. coli* ATCC® 25922 directly from bacterial suspension and indirectly using bacterial genomic DNA (gDNA). LAMP results were then compared with the results of *E. coli* detection using standard PCR methodology.

METHOD / DESIGN:

For use in LAMP detection of *E. coli* directly from the bacterial suspension, we prepared eight bacterial dilutions in concentrations ranging from 1.5×10^{8} - 1.5×10^{1} CFU/mL. All bacterial dilutions were then used for LAMP detection using WarmStart Colorimetric LAMP 2X Master Mix (New England BioLabs) for colorimetric LAMP and WarmStart LAMP Kit (New England BioLabs) for Real-Time LAMP. Real-Time LAMP reactions were done on the Genie® II, an instrument for isothermal nucleic acid amplification (Figure 1). LAMP primers (F3, B3, FIP, BIP, LF, BF) (Figure 2) for the *malB* gene are taken from the literature. For indirect detection we extracted genomic DNA from *E. coli* using GeneJET Genomic DNA Purification Kit (Thermo Scientific). Extracted gDNA with the concentration 50 ng/µl was used for preparation of ten-fold dilutions (10^{-1} - 10^{-4}), and these dilutions were used in both Colorimetric and WarmStart LAMP assays in order to compare limits of detection with and without DNA extraction. Finally, we used *E. coli* genomic DNA serial dilutions for standard PCR, using F3 and B3 primers for *malB* gene.

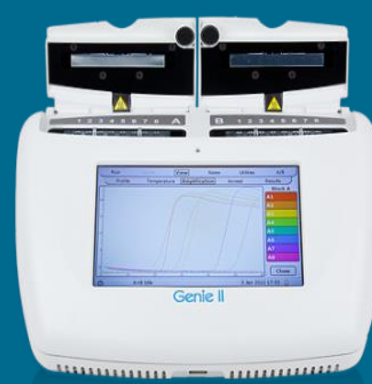

Figure 1. Genie® II instrument for Real-Time LAMP. (http://www.optigene.co.uk/instruments/instrument-genie-ii/)

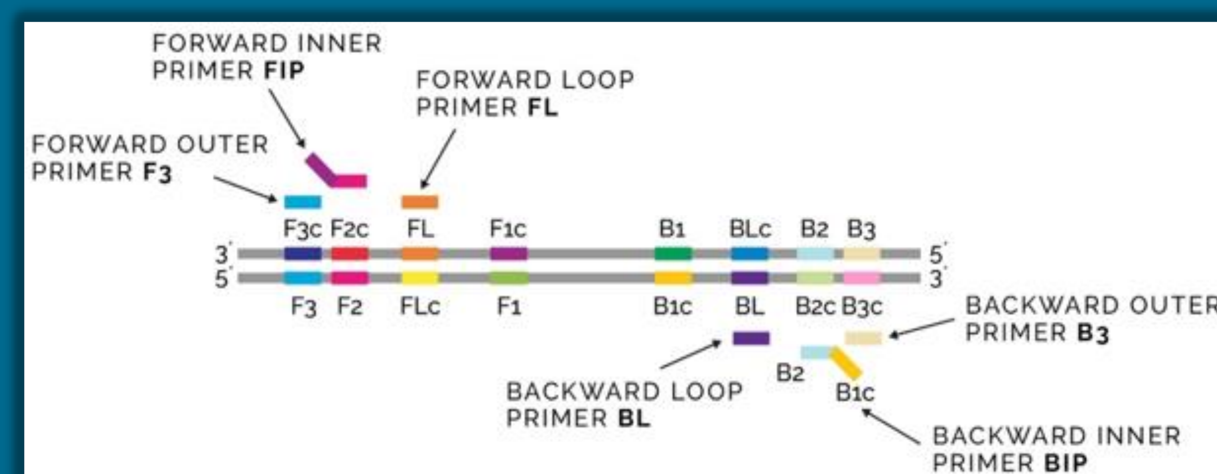

Figure 2. Schematic representation of LAMP primers.

RESULTS:

The results showed that both LAMP methods (WarmStart and Colorimetric LAMP) enabled the detection of *E. coli* directly, in suspension (without prior DNA isolation) for bacterial concentration of 1.5×10^{8} CFU/mL (Figure 3, Chart 1). Additionally, the results of Colorimetric LAMP using serial dilutions of *E. coli* gDNA showed detection in all tested concentrations, except the lowest (10^{-4}) (Figures 4-5). On the other hand, the WarmStart LAMP kit enabled *E. coli* gDNA detection in all tested gDNA dilutions (Chart 2, Figure 6). The conventional PCR methods failed to detect *E. coli* malB gene product for the lowest DNA concentration (Figure 7.). Additionally, DNA bends on agarose gels are more intensive in the LAMP products compared to those obtained with PCR products.

LAMP detection of *E. coli* without prior gDNA isolation

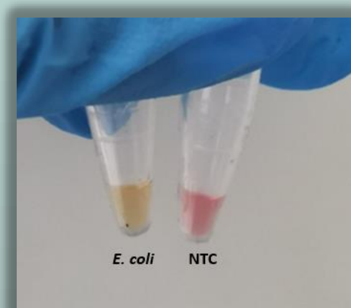

Figure 3. Results of Colorimetric LAMP detection directly from the bacterial suspension: Left tube (yellow) – suspension with *E. coli* concentration 1.5×10^{8} CFU/mL; Right tube (pink) – non-template control.

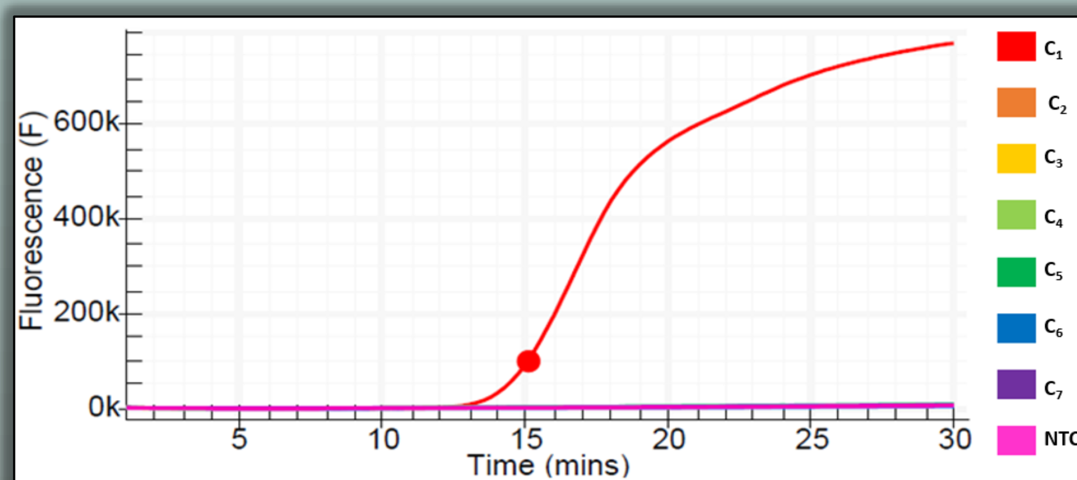

Chart 1. Results of Real-Time LAMP detection directly from the bacterial suspension. C_1 (red line) represents *E. coli* concentration 1.5×10^{8} CFU/mL; C_2 = 1.5×10^{7} CFU/mL; C_3= 1.5×10^{6} CFU/mL; C_4 = 1.5×10^{5} CFU/mL; C_5 = 1.5×10^{4} CFU/mL; C_6 = 1.5×10^{3} CFU/mL; C_7 = 1.5×10^{2} CFU/mL; NTC-non-template control. Only red line is visible since all the others could not be detected i.e are overlapping at 0.

Colorimetric LAMP detection of tested concentrations of *E. coli* gDNA

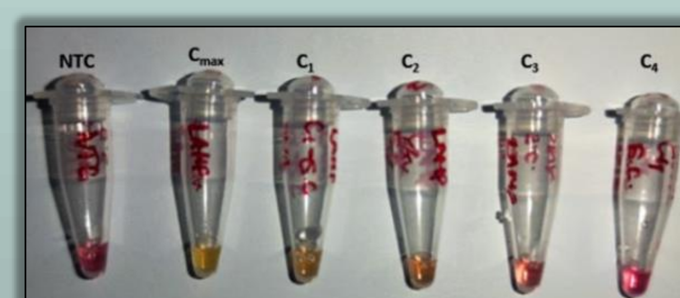

Figure 4. Results of Colorimetric LAMP detection of *E. coli* gDNA concentrations: C_{max} = 50 ng/µl; C_1 = 50 x 10^{-1} ng/µl; C_2 = 50 x 10^{-2} ng/µl; C_3 = 50 x 10^{-3} ng/µl; C_4 = 50 x 10^{-4} ng/µl; NTC – non-template control.

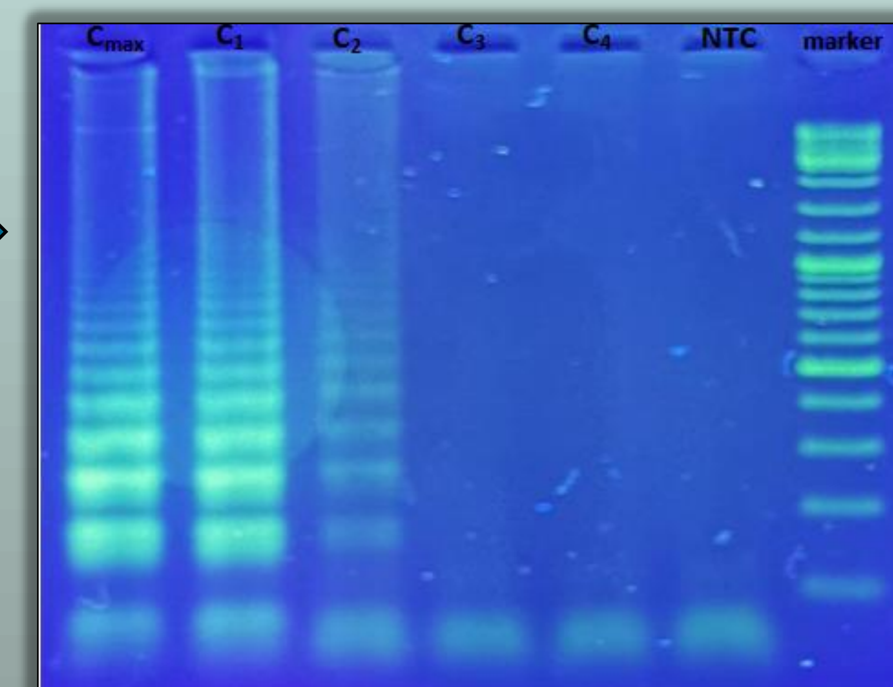

Figure 5. Agarose gel electrophoresis of Colorimetric LAMP products of ampilification of tested *E. coli* gDNA concentrations

Real-Time LAMP detection of tested concentrations of *E. coli* gDNA

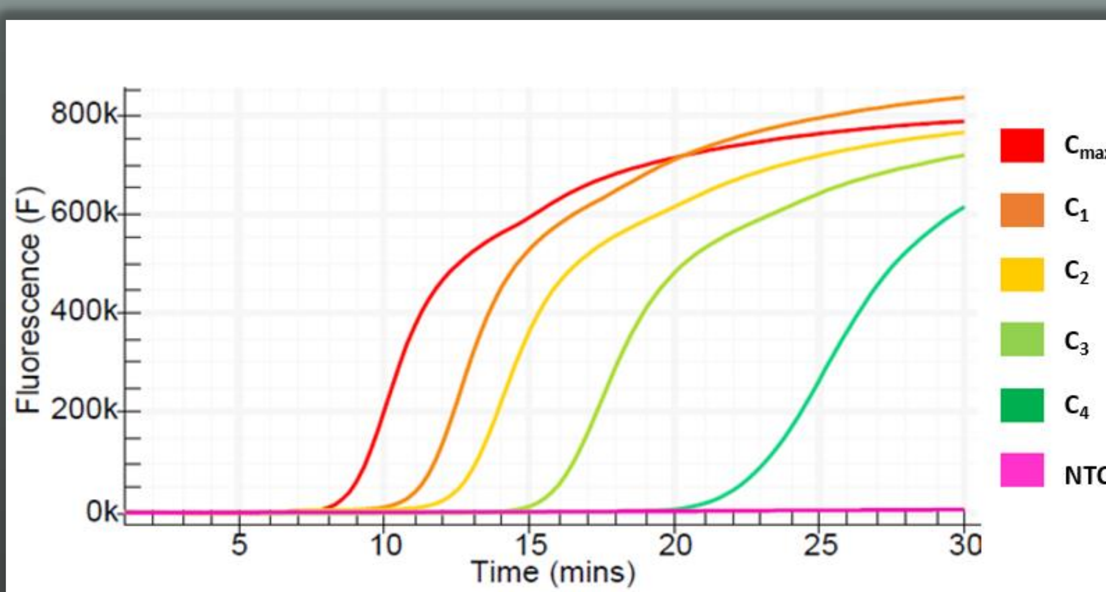

Chart 2. Results of Real-Time LAMP detection of *E. coli* gDNA concentrations: C_{max} = 50 ng/µl; C_1 = 50 x 10^{-1} ng/µl; C_2 = 50 x 10^{-2} ng/µl; C_3 = 50 x 10^{-3} ng/µl; C_4 = 50 x 10^{-4} ng/µl; NTC – non-template control.

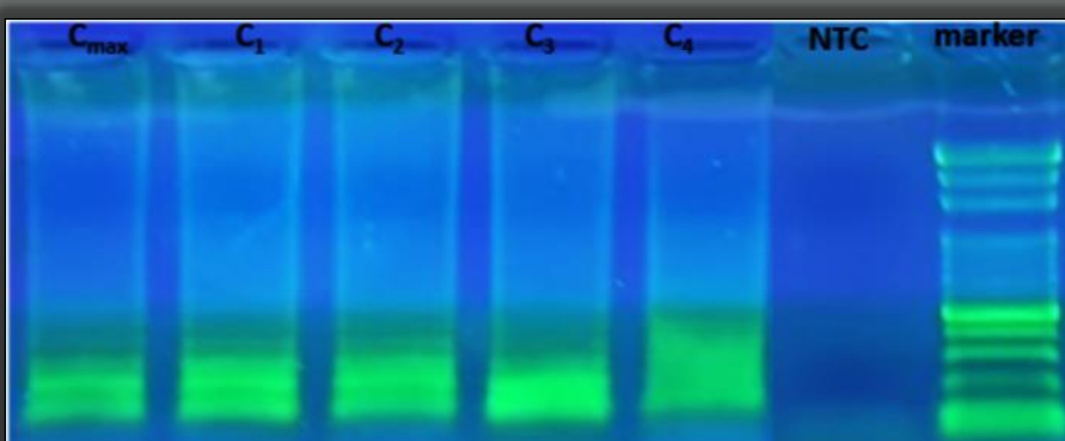

Figure 6. Agarose gel electrophoresis of Real-Time LAMP products of ampilification of tested *E. coli* gDNA concentrations.

PCR detection of tested concentrations of *E. coli* gDNA

Figure 7. Agarose gel electrophoresis of PCR products of ampilification of tested *E. coli* gDNA concentrations in the range 50 to 50 x 10^{-4} ng/µl.

CONCLUSIONS:

Presented research showed the incredible power of the LAMP methodology (both, Colorimetric and Real-Time) to detect *E. coli* even without prior isolation of bacterial gDNA. Also, LAMP enabled *E. coli* detection even at an extremely low concentration of tested gDNA (50 x 10^{-4} ng/µl), while with conventional PCR that was not the case. PCR managed to detect bacterial gDNA up to a concentration of 50 x 10^{-3} ng/µl. Based on these results, LAMP was proved to be more sensitive than PCR, with a large capacity to be used in molecular diagnostics. Also, LAMP is easier to handle since it is more time-efficient and can be performed using portable small devices suitable for on Point-of-Care (POC) research.

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