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Title: Development of Loop-mediated isothermal amplification (LAMP) assay for rapid detection of Neisseria meningitidis

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Abstract: Background and Objectives: Detection of Neisseria meningitides using conventional method is time consuming and laborious. Development of a rapid method is required for prompt control and prevention of meningococcal disease. A novel method known as Loop-mediated isothermal amplification (LAMP) is considered suitable for molecular diagnosis in laboratories, hospitals and even field due to its simplicity, rapidity, specificity, sensitivity and cost-effectiveness. We aimed to develop and evaluate a LAMP assay for rapid and simple detection of N. meningitidis.

Materials and Methods: A set of four primers; two inner and two outer; were designed according to the conserved region of the crgA gene of N. meningitidis. The LAMP reaction was set up using the bacterial genomic DNA as the template. The same LAMP reactions were done but in the presence of genomes of various negative control bacteria for evaluation of test specificity. The product reactions were analyzed by agarose gel electrophoresis. The reaction was monitored by real-time measurement and end point observation of turbidity, which is correlated with the production of magnesium pyrophosphate.

Results: Reaction time and temperature were optimized for 60 min and 65 °C, respectively. In the agarose gel electrophoresis of the crgA gene LAMP products, the characteristic ladder of multiple bands was observed. Result of amplification using negative control genomes as template was negative. The specificity of the amplification was confirmed by restriction endonuclease digestion with XbaI.

Conclusion: Our currently developed crgA LAMP assay is a rapid, simple and specific method for detection of N. meningitidis. After further validation studies, this assay will have the diagnosis potential of N. meningitides in laboratories, hospitals and also many other fields.

Keywords: LAMP, rapid detection, diagnosis potential

Presentation: Poster