Lateral flow biosensor combined with loop-mediated isothermal amplification for detection of legionella pneumophila.

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Abstract

Legionella pneumophila (*L. pneumophila*) is the most pathogenic species of *Legionella* , which can cause Legionella disease. It can cause pneumonia, or Pontiac fever. In severe cases, it can lead to respiratory failure and kidney failure, with a high fatality rate. Here, a novel molecular diagnosis method, a loop-mediated isothermal amplification coupled with lateral flow biosensor (LFB) method (LAMP-LFB) was successfully established and evaluated for the identification of *L. pneumophila* . A set of 6 primers was designed specifically based on the *L. pneumophila* -specific gene *mip*. The optimized time and temperature conditions for the LAMP was 50 min and 64°C respectively. The minimum concentration that can be detected by this method was 100fg. Using the protocol, we could observe the LAMP amplification within 2min by LFB. The whole process, including the preparation of DNA (20 min), LAMP reaction (50 min) and results reporting (2 min), could be finished within 75 min. Among 50 alveolar lavage fluid samples, 5(10%) were *L. pneumophila* -positive by the LAMP-LFB, and the diagnostic accuracy was 100% when compared to the culture method. While only 4 samples were positive using PCR method. In a word, the LAMP-LFB assay is a rapid, sensitive and specific detection method that can detect *Legionella pneumophila* , and it can be used as a new molecular method for the detection of target pathogens in water, environmental and clinical samples.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and [accessed as a PDF](#).

Figures

Figure 1

The sequences, positions and modifications of the *L. pneumophila*-specific gene mip, Right arrows and left arrows indicate sense and complementary sequences that were used in the study.

Figure 2

Confirmation and detection of *L. pneumophila*-LAMP products. A, Color change of *L. pneumophila*-LAMP tubes; B, LFB applied for visual detection of *L. pneumophila* -LAMP products. Tube A1, positive amplification; tube A2, negative amplification (*Enterobacter aerogenes* (ATCC49701)), tube A3 negative amplification (*klebsiella pneumoniae* (ATCC27736)), tube A4, negative control (DW); Biosensor B1, positive amplification; biosensor B2, negative amplification (*Enterobacter aerogenes* (ATCC49701)), biosensor B3, negative amplification (*klebsiella pneumoniae* (ATCC27736)), biosensor B4, negative control (DW).

Figure 3

Optimizing the reaction temperature of LAMP-LFB assay. We analyzed the kinetic curves at different temperatures by real-time turbidity detection. The threshold value was 0.1 and the turbidity of >0.1 was considered to be positive. Eight kinetic graphs (A–H) were generated at various temperatures (61–68°C, 1°C intervals) with target pathogens DNA at the level of 10pg per reaction.

Figure 4

Sensitivity of *L. pneumophila*-LAMP-LFB assay Serially diluted genomic templates of *L. pneumophila* (ATCC43661) were used to detect the sensitivity of the LAMP-LFB assay. The LoD (limit of detection) was analysed by colorimetric indicator detection(A) and LFB (B). The serial dilutions (10pg,1pg,100fg,10fg,1fg and 100ag,10ag) of target templates were used for standard LAMP reactions. Tubes (A) /Biosensors (A) 1–8 represented the DNA levels of 10ng *L. pneumophila* (ATCC43661) templates, 1ng *L. pneumophila* (ATCC43661) templates, 100pg *L. pneumophila* (ATCC43661) templates, 10pg *L. pneumophila* (ATCC43661) templates, 1pg *L. pneumophila* (ATCC43661) templates and 100fg *L. pneumophila* (ATCC43661) templates, 10fg *L. pneumophila* (ATCC43661) templates, 1fg *L. pneumophila* (ATCC43661) templates and blank control (DW). The genomic DNA levels of 10pg to 10fg per reaction produced the positive results.

Figure 5

Optimal Duration Time for *L. pneumophila*-LAMP-LFB assay. Four different reaction times (A, 30min; B, 40min; C, 50min; D, 60min) were tested and compared at 64°C. Biosensors 1, 2, 3, 4 represent DAN levels of 100fg/μl (LoD level). The best time was 50min.

Figure 6

Specificity of *L. pneumophila*-LAMP-LFB assay The LAMP assay was conducted using different genomic DNA templates and these results were analysed visually. Tube A1, *L. pneumophila* (ATCC43661); Tube A2-21 represented the species of *Stenotrophomonas maltophilia*; *Staphylococcus aureus*; *Vibrio parahaemolyticus*; *Enterobacter aerogenes* (ATCC49701); *Enterobacter aerogenes*; *Klebsiella pneumoniae* (ATCC27736); *Klebsiella pneumoniae*; *Enterococcus faecium*; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; *Penicillium italicum*; *Dung enterococcus*; *Proteus singularis*; *S. aureus* (MRSA) (ATCC43300); *Streptococcus pharyngitis*; *Serratia marcescens* (ATCC14756); *Serratia marcescens*; *Pseudomonas fluorescens*; group B streptococcus; *Haemophilus influenzae*. Tube A21, blank control (DW). Biosensor B1, *L. pneumophila* (ATCC43661); Biosensor B2-21, negative amplification; Biosensor B21, negative control (DW).