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Short Communication

Loop-mediated isothermal amplification for human influenza A viruses

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Influenza diagnostic testing is a pivotal public health tool because this viral infection causes enormous morbidity, mortality and financial burden. Laboratory diagnosis of influenza infection also plays an important role in the individual patient management and outbreak control that all resulting in significant financial benefits (1). There are a number of molecular tests developed for rapid detection and typing of influenza virus. Reverse transcriptase PCR using specific primers recommended by WHO has been set up and used in diagnostic laboratories worldwide for rapid detection and typing of influenza viruses (2).

In the last decades several modern molecular techniques have been innovated that provides the new alternatives for molecular diagnosis of infectious disease (3). In 2000 Notomi et al. reported a novel nucleic acid amplification method, loop-mediated isothermal amplification (LAMP) working based on self-recurring strand-displacement DNA synthesis primed by a specially designed set of target-specific primers (4). Soon after LAMP assay as a more rapid, accurate and cost-effective method has been taken into consideration in Influenza molecular detection. Several studies have been reported the successfully application of LAMP/RT-LAMP method for detection of different subtypes of influenza viruses such as H1N1, H3N2, H5N1, H9N2 (5, 6, 7).

Here, we carried out LAMP approach for detection of the human influenza A viruses (H1 and H3) using primers introduced by Poon et al in 2005, as mentioned in table 1 (8).

This set of primers is specific for M fragment but by designing degenerated nucleotides in their sequences, it just detects human H1, H2 and H3 isolates. Two standard seasonal influenza virus strains, H1N1 (New Caledonia 20/1999) and H3N2 (A/H3N2/Panama/2007/99) were obtained from Pasteur Institute, Influenza Research Lab. 150 microliter of infected culture supernatant of each virus sample, equivalent to 4×10^3 PFU/ml, was subjected to RNA extraction with a commercial RNX-PLUS^{TM} solution (SinaClone-Iran). The viral RNA was eluted in 50 microliter DEPC-treated water. The cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit (Fermantas-Lithuania) according to manufacturer's instructions. The Uni12 primer, 5'-AGCRAAAGCAGG-3', which is complementary to the conserved 3'end of all influenza A virus RNA segments was used for cDNA synthesis (9). Five microliter of cDNA was serially diluted to 10^{-4}. Two microliter of each dilution was amplified in a 25 microliter reaction mixture containing 0.4 mM deoxynucleoside triphosphates, 1.6 μM from each forward and backward inner primers, 0.2 μM from each F3 and B3c primers, 8U of Bst DNA polymerase (New England Biolabs), and 1X Bst polymerase reaction buffer. The reaction mixtures were incubated at 60˚C for 1 hour. Figure 1 shows the ladder-like pattern of amplicones in gel electrophoresis that considers as a positive result for LAMP test. However, any visual turbidity differences

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Table 1. Names and sequence of primers for Human Influenza Viruses LAMP assay.

Primer	Sequence (5' to 3')
F3	TGGTGC(A/G)CTTGCCAGTTG
B3c	CCAGCCATTTGCTCCATAGC
FIP(F1c+F2)	TGCTGGGAGTCAGCAATCTGTTACAG(G/A)ATGGGGGCTGT(A/G)ACC
BIP(B1+B2c)	AGGCAAATGGTG(G/A)CAACAACCTGTAGTGCTGGCCA(A/G)AACC

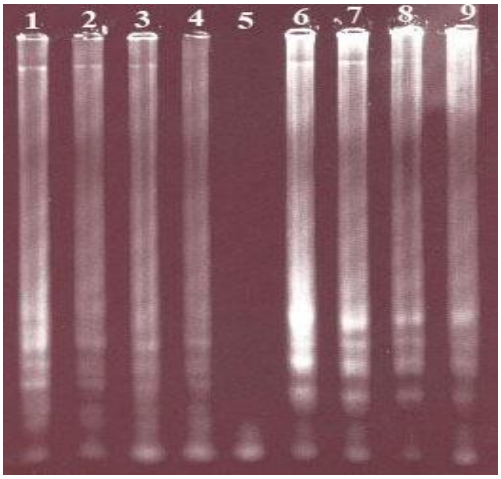


Fig. 1. Gel agarose electrophoresis of LAMP products which were done on cDNA derived from H1N1 (New Caledonia 20/1999) and H3N2 (A/H3N2/ Panama/2007/99) run in left (lanes 1-4) and right (lanes 6-9) panels respectively. The concentration of cDNA used in the first lanes of each panels (lanes 1 and 6) was equivalent to 4×10^3 PFU/ml that logarithmic serially diluted and used in the reactions respectively (lanes 2-4 and 7-9). Lane 5, blank negative control.

could be observed between cases and the negative control, neither the H1 nor H3 cases.

Adequate Mg^{++}concentration is essential to form Magnesium pyrophosphate salt in LAMP reaction, the insoluble salt that cause in growing turbidity as the reaction proceeds (10). Therefore we tried different concentration of $MgSo_4$ in LAMP mixture (4mM-8mM) to experiment its effect on appearing visual turbidity but we did not observe turbidity in any concentration of $MgSo_4$. Although we could not set visual judgment for detection of positive samples and did not find the exact threshold detection limit of the test, it seems that the LAMP test experimented here is an effective, simple and quick method to laboratory diagnosis of human influenza cases. The procedure should be improved in ongoing experiments.

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