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Rapid Subtyping of Influenza A Virus by Loop-Mediated Isothermal Amplification: Two Cases of Influenza Patients Who Returned from Thailand

Naoko Nakagawa* and Masahiro Ito

Kobe Institute of Health, Kobe 650-0046, Japan

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Influenza is an important infectious disease that is responsible for considerable morbidity and mortality in elderly and compromised individuals (1,2). Once a patient has been diagnosed, an anti-influenza virus drug such as oseltamivir can be administered. Therefore, immunochromatography rapid diagnosis tests are popularly used as diagnostic tools at bedside. However, hemagglutinin (HA) subtypes of influenza A virus are not detected by the immunochromatography tests, and new subtypes of influenza A virus may emerge at any time. When clinic patients are diagnosed as influenza A-positive by the immunochromatography tests and are found to have just returned from countries where human avian influenza cases have been reported, rapid virus subtyping is essential. We experienced 2 such cases and determined virus subtypes rapidly by applying a reverse transcriptase (RT)-loop-mediated isothermal amplification (LAMP) assay.

LAMP is a novel nucleic acid amplification method (3). The specificity is extremely high, because 4 primers are used recognizing 6 distinct regions on the target DNA. This method is faster than polymerase chain reaction, because amplification is carried out under isothermal conditions. LAMP has been used for the rapid diagnosis of infectious agents including bacteria such as *Mycobacterium tuberculosis* (4) and DNA viruses such as herpes viruses (5,6). RT-LAMP has been used for the detection of RNA viruses such as SARS coronavirus (7,8). We established RT-LAMP for the detection of influenza A virus subtype 1 (AH1), subtype 3 (AH3) and influenza B virus. The total procedure was completed within 3 h, and the sensitivity was higher than that of the immunochromatography test (9).

Case 1: A 30-year-old Japanese man, a resident of Bangkok, Thailand, came back to Japan for summer vacation on Friday, August 5, 2005. He was engaged in trading and had no history of contact with living or dead birds. On Saturday, August 6, fever (temperature 38.5°C), sore throat and myalgia developed, and on Sunday, August 7 he visited the hospital. He was diagnosed with influenza A by the immunochromatography test. Oseltamivir was administered, and the patient returned home. The case was reported to Kobe Health Center on Monday, August 8. The nasopharyngeal sample obtained for the immunochromatography test was kept in the refrigerator, and transported to Kobe Institute of Health for further investigation (2:00 p.m.). RNA was extracted from the sample by QIAmp Viral RNA Mini Kit (Qiagen, Tokyo,

Japan) in the BSL-3 laboratory. The specimen was examined by RT-LAMP as previously described (9). The sample was positive with subtype H3 specific primers. AH1 positive and AH3 positive controls were positive with subtype H1 and H3 specific primers, respectively. The negative control did not respond to any primers (Figure 1). We immediately reported to the hospital that the HA subtype of influenza A virus in the patient's sample was H3 (4:30 p.m.). Thereafter, the sample was inoculated to Madin-Darby canine kidney (MDCK) cells in 24-well plates and incubated at 35°C in the BSL-2 laboratory. On the 3rd day, a cytopathic effect was detected and the

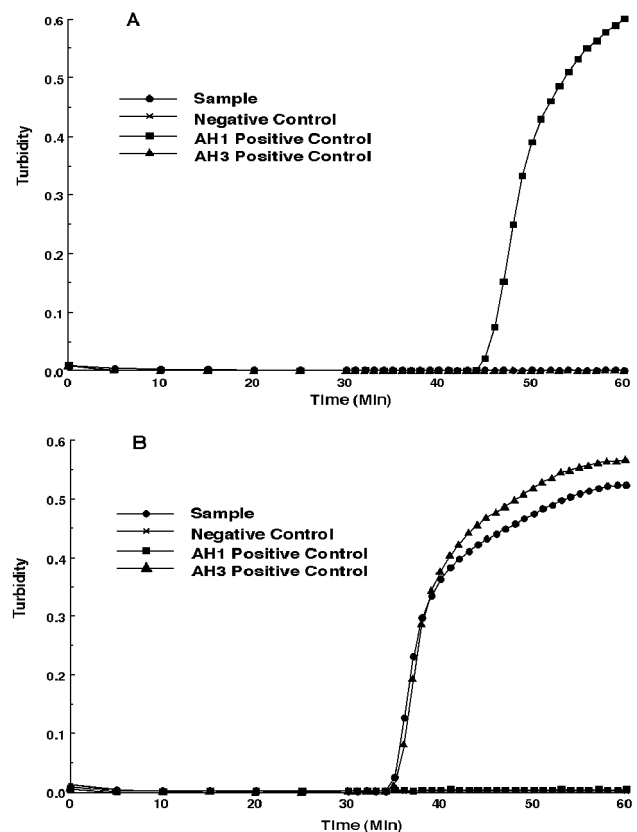


Fig. 1. Influenza virus detection by RT-LAMP. Turbidity caused by the existence of magnesium pyrophosphate, the amplification by-products was detected by the real-time turbidimeter LA320-C (Y-axis). X-axis shows time (min). (A) Absence of AH1 in the sample from Case 1. A sample from Case 1 as well as positive controls (AH1, AH3) and a negative control were subjected to RT-LAMP using primers specific for AH1. (B) Detection of AH3 in the sample from Case 1. A sample from Case 1 as well as positive controls (AH1, AH3) and a negative control were subjected to RT-LAMP using primers specific for AH3.

*Corresponding author: Mailing address: Kobe Institute of Health, 4-6, Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan. Tel: +81-78-302-6260, Fax: +81-78-302-0894, E-mail: naoko_nakagawa@office.city.kobe.jp

virus was identified as influenza A virus H3 subtype by hemagglutination inhibition (HI) test. The viral antigenicity was serologically similar to that of A/Wyoming/3/2003.

Case 2: A 31-year-old Japanese man who had traveled in Thailand for 5 days came back to Japan on Monday, September 26, 2005. The next morning, he developed fever. In the late afternoon on September 29, he visited the hospital with high fever, chilliness, shivering and sore throat. Leukocyte count ($6100/\text{mm}^3$) and C-reactive protein level (7.3 mg/dl) were at normal levels. He was diagnosed with influenza A by the immunochromatography test. The nasopharyngeal specimen was transported to Kobe Institute of Health, and RT-LAMP was undertaken as described above. The patient's sample was positive with H3 specific primers. The virus was isolated from sample-inoculated MDCK cells on the 4th day and was identified as influenza A virus H3 type. The antigenicity was serologically similar to that of A/Wyoming/3/2003.

Thus, RT-LAMP was demonstrated to be a useful rapid diagnostic and subtyping method for influenza viruses. By designing primer pairs, RT-LAMP is applicable to the detection of other subtypes. Detection kits for H5 subtype are now commercially available. Human influenza by other subtypes such as AH9 may also emerge at any time. RT-LAMP will serve as a useful diagnostic tool.

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REFERENCES

1. Choi, K. and Thacker, S. B. (1981): An evaluation of influenza mortality surveillance, 1962 - 1979. I. Time series forecasts of expected pneumonia and influenza death. *Am. J. Epidemiol.*, 113, 215-226.
2. Choi, K. and Thacker, S. B. (1981): An evaluation of influenza mortality surveillance, 1962 - 1979. II. Percentage of pneumonia and influenza deaths as an indicator of influenza activity. *Am. J. Epidemiol.*, 113, 227-235.
3. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000): Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.*, 28, e63.
4. Iwamoto, T., Sonobe, T. and Hayashi, K. (2003): Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* Complex, *M. avium*, and *M. intracellulare* in sputum samples. *J. Clin. Microbiol.*, 41, 2616-2622.
5. Ihira, M., Yoshikawa, T., Enomoto, Y., Akimoto, S., Ohashi, M., Suga, S., Nishimura, N., Ozaki, T., Nishiyama, Y., Notomi, T., Ohta, Y. and Asano, Y. (2004): Rapid diagnosis of human herpesvirus 6 infection by a novel DNA amplification method, loop-mediated isothermal amplification. *J. Clin. Microbiol.*, 42, 140-145.
6. Okamoto, S., Yoshikawa, T., Ihira, M., Suzuki, K., Shimokata, K., Nishiyama, Y. and Asano, Y. (2004): Rapid detection of varicella-zoster virus infection by a loop-mediated isothermal amplification method. *J. Med. Virol.*, 74, 677-682.
7. Fujino, M., Yoshida, N., Yamaguchi, S., Hosaka, N., Ota, Y., Notomi, T. and Nakayama, T. (2005): A simple method for the detection of measles virus genome by loop-mediated isothermal amplification (LAMP). *J. Med. Virol.*, 76, 406-413.
8. Thai, H. T. C., Le, M. Q., Vuong, C. D., Parida, M., Minekawa, H., Notomi, T., Hasebe, F. and Morita, K. (2004): Development and evaluation of a novel loop-mediated isothermal amplification of method for rapid detection of severe acute respiratory syndrome coronavirus. *J. Clin. Microbiol.*, 42, 1956-1961.
9. Ito, M., Watanabe, M., Nakagawa, N., Ihara, T. and Okuno, Y.: Rapid detection and typing of influenza A and B by loop-mediated isothermal amplification: comparison with immunochromatography and virus isolation. *J. Virol. Methods* (in press).