Detection of Mumps Virus RNA by Real-Time One-Step Reverse Transcription PCR Using the LightCycler Platform

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Received 28 July 2008/Returned for modification 24 September 2008/Accepted 9 October 2008

A real-time reverse transcriptase PCR (RT-PCR) assay that targeted both the mumps virus F gene and human RNase P in clinical samples was adapted for use with the LightCycler platform. LightCycler RT-PCR is as sensitive as conventional nested RT-PCR and significantly less expensive and labor-intensive, making it ideal for mumps diagnosis during outbreaks.

Mumps is a vaccine-preventable disease caused by an RNA virus of the family Paramyxoviridae. Despite vaccination strategies, recent outbreaks in the United Kingdom, United States, and Canada highlight the need for rapid diagnosis (1, 3, 9, 12). Serological methods have limited (if any) utility for the diagnosis of mumps in partially immunized individuals (7, 10). Detection of mumps virus RNA by reverse transcriptase PCR (RT-PCR) and a subsequent second round of nested PCR (RT-n-PCR) is the most sensitive method for laboratory confirmation (4; G. Tipples et al., http://www.nml-lnm.gc.ca/guide/docs/Mumps_Lab_guide.pdf), but it is not recommended for use on the LightCycler platform. LightCycler RT-PCR detection of mumps virus RNA targeting the F gene and incorporated the internal control RNase P described by Boddicker et al. (2). This assay was evaluated and compared to conventional RT-n-PCR for the detection of mumps virus RNA in clinical specimens submitted during an outbreak in Nova Scotia.

Unlike methods targeting the highly variable small hydrophobic (SH) gene (2), a real-time PCR with a target amplifying the most conserved region of the mumps virus genome, the fusion (F) gene, was described by Uchida et al. (11). In order to decrease sample turnaround times and minimize the potential for amplicon contamination, real-time RT-PCR assays have recently evolved from a two-step process (a first round of RT-PCR followed by a round of real-time PCR) (2, 5, 11) to one-step processes (RT-PCR only) (2, 6, 8). Despite their increased sensitivity and speed, few real-time RT-PCR assays have been validated for clinical use (2, 5, 6, 8, 11). Boddicker et al. (2) developed a one-step real-time RT-PCR assay targeting both the mumps virus SH gene and the endogenous internal control Homo sapiens RNase (RNase P) that could be used on clinical specimens. However, among others, this assay was not validated for use on the LightCycler platform. Jin et al. (5) described a real-time method compatible with the LightCycler platform which targeted hemagglutinin-neuraminidase using micro-groove binder technology. However, this assay involved a two-step real-time PCR approach. This study adapted methods described by Uchida et al. (11) for one-step real-time RT-PCR detection of mumps virus RNA targeting the F gene and incorporated the internal control RNase P described by Boddicker et al. (2). This assay was evaluated and compared to conventional RT-n-PCR for the detection of mumps virus RNA in clinical specimens submitted during an outbreak in Nova Scotia.

Nucleic acid extraction was performed on a MagNA Pure LC (Roche Diagnostics, Branchburg, NJ) from 140 μl of specimens with a total nucleic acid isolation kit and a 60-μl elution volume. Real-time RT-PCR was performed on a LightCycler 2.0 instrument using a QuantiTect Multiplex NR RT-PCR kit in 20-μl reaction mixtures consisting of 1× master mix, 2 μl enzyme, 300 nM of primers, and 100 nM of probes. Sequences for the primers and probes have been described elsewhere (2, 11). To better complement the LightCycler platform, the RNase P probe (2) was modified (the 3’ and 5’ ends were labeled with Pulsar 650 and BHQ2, respectively). Thermocycling conditions were as follows: reverse transcription at 48°C for 45 min, initial denaturation at 95°C for 15 min, and 45 cycles of denaturation at 95°C for 15 s and combined annealing/extension steps at 59°C for 60 s. Fluorescence analyses for the F gene and RNase P were performed at 530 and 705 nm, respectively.

Ten arbitrarily chosen buccal and urine specimens were evaluated to compare primer/probe combinations targeting either the SH (2) or F (11) gene. Crossing point (Cp; cycle in which the fluorescence crosses the threshold) values for the SH target were consistently lower than those for the F gene, resulting in failure to detect mumps virus in three cases. Where highly conserved targets like the F gene would be able to detect mumps virus RNA (6, 11), sequence variation in primer or...
buccal swabs, 51 (of 214) urine specimens, and 1 (of 3) cerebrospinal fluid (CSF) samples were positive, representing 73 (of 261) specimens. Of 478 specimens, RT-n-PCR was performed using a Qiagen One-Step RT-PCR kit by members of Public Health Agency of Canada, the NML, and providing various genotypes of mumps virus used in the specificity panel. We thank Li Jin (Health Protection Agency, United Kingdom) for donating members of the Roche LightCycler instrument could easily be implemented in other laboratories using this platform.

Real-time RT-PCR technology offers a number of advantages compared to conventional RT-n-PCR. Following extraction, an average experiment by real-time one-step RT-PCR can be performed in approximately 2.5 h, compared to 8 h for conventional RT-n-PCR, and the average cost per specimen (without labor) for a typical run of 32 samples with controls was $15.60 for conventional RT-n-PCR compared to $9.31 for real-time RT-PCR. When labor cost, in addition to faster turnaround times, is accounted for, real-time RT-PCR undoubtedly offers additional cost savings.

In summary, the real-time RT-PCR assay described herein is sensitive, specific, and far more rapid than conventional RT-n-PCR. In addition, it is significantly less expensive and labor-intensive, making it ideal for mumps diagnosis. Since mumps outbreaks have been documented worldwide, the one-step real-time RT-PCR validated for the Roche LightCycler instrument could easily be implemented in other laboratories using this platform.
REFERENCES


