A New Real-Time Reverse Transcription-PCR Assay for Detection of Human Enterovirus 68 in Respiratory Samples

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A global reemergence of human enterovirus 68 (EV-D68) associated with severe respiratory illness occurred in 2014. We developed and validated an EV-D68-specific real-time reverse transcription-PCR (RT-PCR) for the detection of EV-D68 in respiratory samples. The rapid diagnosis of EV-D68 may contribute to better management of EV-D68 infections.

Human enterovirus 68 (EV-D68) infections are associated with severe respiratory illness in pediatric patients (1). In the last decade, increased circulation of this virus has been reported worldwide (2–5). From mid-August 2014 to 13 October 2014, an outbreak with 691 laboratory-confirmed EV-D68 infections was observed in the United States (6, 7).

At present, positive EV-D68 samples are identified using a generic human enterovirus (HEV)/human rhinovirus (HRV) real-time reverse transcription-PCR (RT-PCR), followed by sequencing of amplicons. The aim of the present study was to develop and validate an EV-D68-specific real-time RT-PCR for the detection and quantification of EV-D68 in respiratory samples.

The primers and the probe (Table 1) were designed with Primer Express software, version 3.0 (Applied Biosystems, Foster City, CA) using all available EV-D68 sequences (n = 133) (see Table S1 in the supplemental material) of the 5′ untranslated region retrieved from the GenBank database. The primer and probe sequences were evaluated in silico by querying the NCBI nucleotide database for related sequences (n = 45) (see Table S1). No matches with enteroviruses (HEVs) or rhinoviruses (HRVs) were found for the forward primer and probe, while the reverse primer showed 62% to 87% identity with some HEVs, such as EV-A90 and coxsackievirus A24.

A DNA plasmid used as the standard calibrator was produced by cloning a 610-bp fragment of EV-D68 into a plasmid vector pCR2.1 (Invitrogen, Carlsbad, CA) and coxsackievirus A24.

A linear correlation between the log starting copy number and cycle threshold (Cp) was achieved with 8 to 0.1 log10 DNA plasmid copies/µl (Fig. 1A). At each concentration, four replications were tested in a single run. Liner regression analysis of the Cp values against the log10 EV-D68 plasmid concentration resulted in an R2 value of 0.9886 (Fig. 1A). The precision of the assay, defined as the coefficient of variation (CV), was measured using the linearity test results and the average intra-assay CV value, 2.7% ± 1.4% (Fig. 1A), while the average interassay variability was 2.9% ± 0.9% (data not shown).
The analytical sensitivity of the assay was assessed by determining the lower limit of detection (LOD) using a dilution series of standard DNA plasmids at the following concentrations: 1, 10, 50, 100, and 500 copies/reaction. From each dilution, 10 replicates were tested in 3 different assays on different days. The proportion of the positive results obtained from each input concentration was analyzed using probit regression analysis. The probit analysis showed a 95% LOD of 13 copies/reaction corresponding to 715 copies/ml in bronchoalveolar lavage fluid and 286 copies/ml in nasal swab (Fig. 1B) samples for our current extraction protocol.

This new real-time RT-PCR is a useful tool for detecting EV-D68 in respiratory samples. The rapid diagnosis of EV-D68 may contribute to better management of EV-D68 infections.

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