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# A One-Step, Real-Time PCR Assay for Rapid Detection of Rhinovirus

Duc H. Do,* Stella Laus,† Amy Leber,‡ Mario J. Marcon,‡ Jeanne A. Jordan,§¶ Judith M. Martin,†‖ and Robert M. Wadowsky*†¶

*From the Department of Infectious Diseases and Microbiology,* Graduate School of Public Health, Pittsburgh, Pennsylvania; the Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center,† Pittsburgh, Pennsylvania; the Department of Laboratory Medicine,‡ Nationwide Children's Hospital, Columbus, Ohio; the Magee Women's Research Institute,§ Pittsburgh, Pennsylvania; and the Departments of Pathology¶ and Pediatrics,‖ School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania*

**One-step, real-time PCR assays for rhinovirus have been developed for a limited number of PCR amplification platforms and chemistries, and some exhibit cross-reactivity with genetically similar enteroviruses. We developed a one-step, real-time PCR assay for rhinovirus by using a sequence detection system (Applied Biosystems; Foster City, CA). The primers were designed to amplify a 120-base target in the noncoding region of picornavirus RNA, and a TaqMan (Applied Biosystems) degenerate probe was designed for the specific detection of rhinovirus amplicons. The PCR assay had no cross-reactivity with a panel of 76 nontarget nucleic acids, which included RNAs from 43 enterovirus strains. Excellent lower limits of detection relative to viral culture were observed for the PCR assay by using 38 of 40 rhinovirus reference strains representing different serotypes, which could reproducibly detect rhinovirus serotype 2 in viral transport medium containing 10 to 10,000 $TCID_{50}$ (50% tissue culture infectious dose endpoint) units/ml of the virus. However, for rhinovirus serotypes 59 and 69, the PCR assay was less sensitive than culture. Testing of 48 clinical specimens from children with cold-like illnesses for rhinovirus by the PCR and culture assays yielded detection rates of 16.7% and 6.3%, respectively. For a batch of 10 specimens, the entire assay was completed in 4.5 hours. This real-time PCR assay enables detection of many rhinovirus serotypes with the Applied Biosystems reagent-instrument platform.** ***(J Mol Diagn 2010, 12:102–108; DOI: 10.2353/jmoldx.2010.090071)***

Rhinoviruses are the most common cause of viral upper respiratory tract infections and have been associated with more severe lower tract infections in compromised patients.[1–3] Several real-time, RT-PCR assays have been developed; these have improved the diagnosis of rhinovirus infection over traditional culture methods, which are slow and insensitive.[4–7] However, only a few published PCR assays have combined reverse transcription and PCR in the same real-time reaction (ie, one-step assay).[8,9] The advantages of the one-step assay over the two-step assay include improved workflow, reduction in assay preparation time, and elimination of cross contamination from the transfer of cDNA from the reverse transcription reaction into the PCR reaction. One-step assays have been developed for a limited number of PCR amplification platforms and chemistries and may not necessarily perform optimally with other platforms.[8] In addition, cross-reactivity with genetically similar enteroviruses has been reported with some assays.[8,9]

A one-step, real-time PCR assay has not been described for the ABI Prism Sequence Detection System (Applied Biosystems; Foster City, CA) though this platform is widely used in clinical and research laboratories. High PCR efficiency with this platform generally requires the use of primers and a TaqMan probe (Applied Biosystems) with melting temperatures of 58°C to 60°C and 68°C to 70°C, respectively, and an amplicon size of 50 to 150 bp. The 5′ noncoding region of the rhinovirus genome is most commonly targeted in PCR assays. It consists of six subregions (designated A through F) of approximately 20 bases that are highly conserved in picornaviruses and separated by longer, intervening variable sequences.[10] These characteristics complicate the design of efficient one-step, real-time assays. We have observed that a 16-base sequence immediately downstream from subregion E is reasonably well-conserved in rhinoviruses but not in enteroviruses, suggesting that this sequence could serve as a target for a TaqMan probe in a one-step, real-time PCR assay for rhinovirus.

The aim of this study was to develop a rapid, sensitive, and specific one-step, real-time PCR assay for rhinovirus for use with the ABI Prism Sequence Detection System.

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Address reprint requests to Robert M. Wadowsky, Sc.D., Department of Pathology, Children's Hospital of Pittsburgh of UPMC, 45th St and Penn Ave, Pittsburgh, PA 15201. E-mail: Robert.Wadowsky@chp.edu.

**Table 1.** Detection Limits of the One-step PCR Assay for Various Rhinovirus Serotypes

| Rhinovirus serotype | Rhinovirus species* | Source | $TCID_{50}$ equivalents per reaction,† mean (SD) | Negative $log_{10}$ titer for assay,‡ mean (SD) | |
|---|---|---|---|---|---|
| | | | | PCR | Culture |
| 1A | A | ATCC | 0.00001 (0.00000) | — | — |
| 1B | A | NCH | — | 5.5 (0.7) | 5.5 (0.7) |
| 2 | A | ATCC | 0.001 (0.000) | — | — |
| 3 | B | ATCC | 0.0055 (0.0064) | — | — |
| 4 | B | NCH | — | 5.0 (0.0) | 4.0 (0.0) |
| 5 | B | NCH | — | 4.0 (0.0) | 2.5 (0.7) |
| 6 | B | NCH | — | 4.5 (0.7) | 4.0 (0.0) |
| 7 | A | ATCC | 0.001 (0.000) | — | — |
| 8 | A | NCH | — | 4.0 (1.4) | 4.5 (0.7) |
| 9 | A | NCH | — | 6.0 (0.0) | 4.0 (0.0) |
| 13 | A | NCH | — | 4.0 (0.0) | 5.0 (1.4) |
| 15 | A | NCH | — | 5.0 (0.0) | 4.0 (0.0) |
| 17 | B | ATCC | 0.055 (0.064) | — | — |
| 21 | A | ATCC | 0.01 (0.00) | — | — |
| 23 | A | NCH | — | 5.0 (0.0) | 4.0 (0.0) |
| 29 | A | ATCC | 0.0001 (0.0000) | — | — |
| 31 | A | NCH | — | 6.0 (0.0) | 4.5 (0.7) |
| 35 | B | NCH | — | 4.0 (0.0) | 4.5 (0.7) |
| 37 | B | ATCC | 0.055 (0.064) | — | — |
| 38 | A | NCH | — | 5.0 (0.0) | 4.5 (0.7) |
| 39 | A | ATCC | 0.01 (0.00) | — | — |
| 40 | A | ATCC | 0.001 (0.000) | — | — |
| 41 | A | NCH | — | 3.0 (0.0) | −1.0 (0.0)§ |
| 44 | A | NCH | — | 4.5 (0.7) | 3.0 (0.0) |
| 49 | A | NCH | — | 4.0 (0.0) | 2.0 (0.0) |
| 50 | A | NCH | — | 5.0 (0.0) | 4.0 (0.0) |
| 53 | A | NCH | — | 4.0 (0.0) | 5.0 (0.0) |
| 55 | A | NCH | — | 4.5 (0.7) | 1.5 (0.7) |
| 58 | A | ATCC | 0.0055 (0.0064) | — | — |
| 59 | A | NHC | — | 1.5 (0.7) | 5.0 (0.0) |
| 62 | A | ATCC | 0.0001 (0.0000) | — | — |
| 64 | A | NCH | — | 3.0 (0.0) | 3.5 (0.7) |
| 66 | A | ATCC | 0.001 (0.000) | — | — |
| 69 | B | NCH | — | 2.5 (0.7) | 4.5 (0.7) |
| 72 | B | ATCC | 0.01 (0.00) | — | — |
| 74 | A | NCH | — | 4.0 (0.0) | 5.0 (0.0) |
| 86 | B | NCH | — | 5.0 (0.0) | 5.0 (0.0) |
| 92 | B | NCH | — | 4.0 (0.0) | 4.0 (0.0) |
| 97 | B | NCH | — | 3.0 (0.0) | 0.0 (0.0)¶ |
| 99 | B | NCH | — | 4.0 (0.0) | 4.0 (0.0) |

*Ledford et al.[11]
†Determined from testing dilution series of rhinovirus stock extract in duplicate PCR reactions in each of two separate experiments.
‡Determined from testing dilutions series of rhinovirus stock suspension in duplicate PCR reactions (following extraction) and in duplicate WI-38 cell culture tubes in each of two separate experiments.
§Value signifies that no cytopathic effect was observed from the undiluted and diluted suspensions.
¶Value signifies that cyptopathic effect was observed only from undiluted suspension.

## *Materials and Methods*

### *Source and Cultivation of Rhinovirus Reference Strains*

Reference strains of rhinoviruses were obtained as frozen suspensions from the American Type Culture Collection (ATCC; Manassas, VA) and the Clinical Virology Laboratory at Nationwide Children's Hospital (NCH; Columbus, OH) (Table 1).[11] WI-38 human embryonic lung fibroblast cell monolayers in 16 × 125-mm cell culture tubes (Viromed Laboratories; Minnetonka, MN) were used in preparing replicate frozen aliquots for limit of detection studies and for measuring the concentration of virus in stock suspensions. Frozen virus suspensions were thawed in a 37°C water bath and, if necessary for the experiment, diluted serially in 10-fold steps by using Eagle's minimal essential medium containing 2% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 10 μg/ml gentamicin, 2.5 μg/ml Fungizone, and 20 mmol/L HEPES buffer (Viromed Laboratories). After removing the medium from the cell culture tubes, 0.2-ml portions from the virus suspensions were inoculated onto duplicate monolayers. The cell culture tubes were then incubated stationary at 33°C for 1 hour to promote adsorption of virus to the cells. After adsorption, 1 ml of the medium was added to the tubes. To enhance infection of the cells by the virus, the tubes were incubated on a roller drum (0.5 to 0.75 rpm) for 10 days at 33°C. The tubes were examined microscopically for the appearance and progression of a cytopathic effect. Virus concentration in