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Comparison of Reverse Transcription-PCR with Tissue Culture and Other Rapid Diagnostic Assays for Detection of Type A Influenza Virus

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We applied a reverse transcription (RT)-PCR assay for influenza A virus to combined nasal wash-throat swab specimens previously obtained from an outpatient pediatric population with acute respiratory illness during concurrent epidemics of influenza A virus and respiratory syncytial virus. The results of the RT-PCR assay were compared with those previously reported with virus cultivation and commercially available rapid diagnostic kits (E. A. Dominguez, L. H. Taber, and R. B. Couch, J. Clin. Microbiol. 31:2286–2290, 1993). With virus cultivation as the "gold standard," the RT-PCR assay had a sensitivity, specificity, and efficiency of 95, 98, and 97%, respectively, compared with 75, 100, and 93%, respectively, for the best diagnostic kit (Becton Dickinson Directigen). RT-PCR is an effective alternative to virus isolation for the detection of influenza A virus in clinical specimens.

Influenza A virus (Flu A) is a major cause of acute respiratory disease in children and can cause a clinical syndrome similar to that of respiratory syncytial virus infection (8). The prompt diagnosis of infection due to Flu A would allow the use of specific antiviral therapy. Rapid diagnostic kits for the detection of Flu A infections include those utilizing direct and indirect immunofluorescence (IF) and enzyme immunoassays (EIAs). We previously reported a comparison of three kits (Baxter Bartels Microscan [indirect IF], Becton Dickinson Directigen EIA [BD-EIA], and Imagen [direct IF]) with tissue culture isolation for the diagnosis of Flu A infections (6). This report compares the results of a Flu A reverse transcription (RT)-PCR assay applied to those specimens with the results obtained previously with tissue culture and the commercially available diagnostic kits.

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The Flu A RT-PCR assay was a modification of a previously described assay by Donofrio et al. (7) with primers specific for the matrix gene (segment 7) of Flu A. Primers were slightly shorter than those used by Donofrio et al. (7) (sense nucleotides 101 to 123 and antisense nucleotides 291 to 312). Viral nucleic acids were extracted from stock cultures and clinical specimens (combined nasal wash-throat swab) by previously described methods (1, 10). Precipitated nucleic acids were suspended in 15 μl of water and used for cDNA synthesis in a mixture containing 10 mM Tris hydrochloride (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 3.3 μM primer FAM1 (5′-CAGAGACTTGAAGATGTCTTTGC-3′), 667 μM deoxynucleoside triphosphates, 20 U of RNasin (Promega Corp, Madison, Wis.), and 5 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.). The RT mixture was incubated for 1 h at 43°C and then placed on ice. Seventy microliters of PCR mixture was added to the RT mixture to yield a solution containing 10 mM Tris hydrochloride (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 1 μM (each) primers FAM1 and FAM2 [5′-GCTCTGTCCATGTTATTTG(GA)AT-3′], 200 μM deoxynucleoside triphosphates, and 5 U of *Taq* polymerase (Perkin Elmer Corp., Norwalk, Conn.). The final mixture was overlaid with mineral oil, and the cDNA was amplified with a PTC-100 thermal cycler (MJ Research, Inc., Cambridge, Mass.). After an initial 4-min heat denaturation at 94°C, 40 cycles of heat denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min 30 s, and primer extension at 72°C for 1 min were followed by a final primer extension step at 72°C for 15 min. Amplified products were 212 bp in length. Ten microliters of the PCR products was analyzed by agarose gel electrophoresis with 1.5% SeaKem (FMC BioProducts, Rockland, Maine) agarose in 1× TBE buffer (89 mM Tris hydrochloride, 2.5 mM

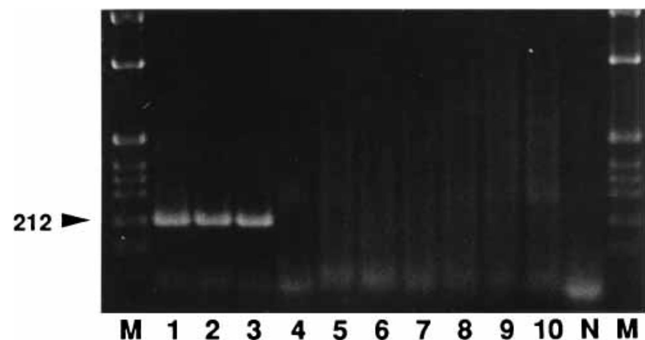

FIG. 1. Detection of laboratory strains of respiratory viruses. Lanes: M, molecular weight marker (1-kb DNA ladder [Gibco BRL, Gaithersburg, Md.]), with marker sizes from the arrowhead up 201 and 220, 298, 344, 396, 506 and 517, and 1,018 bp; 1 to 5 influenza A/Chile/1/83 (H1N1), A/Shanghai/16/89 (H3N2), A/Beijing/353/89 (H3N2), B/Panama/45/90, and B/Yamagata/88 viruses, respectively; 6 human rhinovirus type 15; 7, = respiratory syncytial virus 18537; 8 to 10, parainfluenza virus types 1, 2, and 3, respectively; N, negative reagent control. The Flu A-specific amplicons are 212 bp in length.

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TABLE 1. Comparison of diagnostic tests with virus isolation as the gold standard for evaluation[a]

Assay[b]	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value	% Efficiency
BB-IF	40 (19–63)	88 (74–96)	57 (27–84)	79 (63–90)	75 (62–85)
I-DF	65 (41–85)	92 (79–98)	76 (49–93)	87 (72–95)	85 (73–93)
BD-EIA	75 (51–92)	100 (91–100)	100 (83–100)	91 (78–98)	93 (83–98)
RT-PCR	95 (75–100)	98 (88–100)	95 (75–100)	98 (88–100)	97 (89–100)

[a] Values for commercially available kits vary slightly from those reported by Dominguez et al. (6) because of the availability of only 72 of 81 specimens for RT-PCR evaluation. Values in parentheses represent 95% confidence intervals.
[b] BB-IF, Baxter Bartels Microscan indirect IF; I-DF, Imagen direct fluorescence.

disodium EDTA, 89 mM boric acid). Amplified products were visualized by UV transillumination after staining with ethidium bromide. Results were confirmed by slot blot hybridization with an oligonucleotide, AH2 (5′-TCCTGTCACCTCTGACT AAGGGGATTTTG-3′; sense nucleotides 177 to 205), which was end labeled with digoxigenin (2, 7). Precautions were taken for the prevention of carryover contamination (9). Negative reagent controls were run for each test and were always free of detectable amplification products.

Initial studies with the primers examined their sensitivity and specificity for the detection of Flu A. Both H1N1 (A/Taiwan/1/86 and A/Chile/1/83) and H3N2 viruses (A/Los Angeles/2/87, A/Shanghai/16/89, and A/Beijing/353/89) were amplified successfully. As few as 3 50% tissue culture infective doses of influenza A/Taiwan/1/86 and 6 50% tissue culture infective doses of A/Beijing/353/89 viruses were detected. Influenza B virus (B/Ann Arbor/86, B/Victoria/87, B/Yamagata/88, and B/Panama/45/90), respiratory syncytial virus (18537), parainfluenza virus (types 1, 2, and 3), coronavirus (229E and OC43), adenovirus (types 1, 4, and 5), and rhinovirus (types 1b, 13, and 15) RNA did not yield specific amplified products. Figure 1 shows the results of amplification of representative samples containing either Flu A (positive) or other respiratory viruses (negative).

Seventy-two of the 81 specimens evaluated by Dominguez et al. (6) were available for RT-PCR analysis. Four specimens from which RSV was isolated and five from which no virus was identified were unavailable. Nineteen of the 20 specimens culture positive for Flu A were also positive by RT-PCR. The one culture-positive sample that was RT-PCR negative also was negative by each of the other rapid diagnostic tests. Fifty-one of 52 specimens which were culture negative for Flu A were also negative by RT-PCR, including samples that were culture positive for respiratory syncytial virus, rhinovirus, adenovirus, enterovirus, cytomegalovirus, and herpes simplex virus. One specimen from a febrile asthmatic child was culture positive for rhinovirus and was also positive by RT-PCR for Flu A. This specimen was negative by the other rapid diagnostic kits. The sensitivity, specificity, positive and negative predictive values, and efficiency, with 95% confidence intervals, of the RT-PCR assay and other rapid tests are shown in Table 1, with the results of virus cultivation as the "gold standard" (11).

Several investigators have previously reported on RT-PCR assays for the detection of influenza virus in clinical specimens (3–5, 7, 12–15). In several different assay formats and with primers that amplify different portions of the Flu A genome, RT-PCR results have been comparable to those of tissue culture. This study found similar results with clinical samples obtained from an acutely ill, pediatric clinic population. Previous reports have not compared RT-PCR assays for Flu A with other diagnostic kits. Although the effect of freezing the clinical specimens prior to performance of the RT-PCR assay or of removal of cellular material for direct fluorescent antibody assay cannot be assessed, the RT-PCR assay performed somewhat better than the best commercial kit, Becton Dickinson Directigen (BD-EIA). The sensitivity of BD-EIA may have been lowered because of removal of much of the cellular material for IF studies (6). The increased sensitivity of the RT-PCR assay was offset by the longer time required to get a result (24 to 36 h versus 20 to 30 min for BD-EIA. However, both assays yielded results much sooner than those obtained by tissue culture (3 to 14 days).

In conclusion, an RT-PCR assay was applied to clinical specimens obtained from an outpatient pediatric population with acute respiratory illness and detected the presence or absence of Flu A with a sensitivity of 95%, specificity of 98%, and efficiency of 97%. These results were better than those obtained with commercially available diagnostic kits. Application of this RT-PCR assay should aid in identifying illnesses associated with Flu A infection.

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