JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2006, p. 3366–3367 Vol. 44, No. 9
0095-1137/06/$08.00+0 doi:10.1128/JCM.00314-06

Relevance of Influenza A Virus Detection by PCR, Shell Vial Assay, and Tube Cell Culture to Rapid Reporting Procedures

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Received 13 February 2006/Returned for modification 10 April 2006/Accepted 26 June 2006

Influenza A virus was detected at higher rates and for more extended time periods with real-time PCR than with cell cultures. We show here that, using the theranostic approach, rapid viral detection and reporting can provide for early implementation and assessment of available antiviral therapy.

Influenza virus infects 5 to 20% of the population and results in 30,000 to 50,000 deaths each year in the United States. The control of influenza relies on rapid, sensitive diagnostic assays necessary for targeted treatment with Tamiflu and infection control.

We compared real-time PCR with shell vial and conventional tube cell cultures for the laboratory diagnosis of influenza virus infections. The goals of the present study were (i) to determine the diagnostic laboratory assay (real-time PCR versus shell vial and conventional cell culture) that provided the shortest turn-around time for processing, testing, and reporting and (ii) to determine which assay was most sensitive for detecting the persistence of influenza in hospitalized patients. These results would provide data-based information correlating laboratory testing with rapid result reporting that could be used for antiviral intervention for patients with influenza virus infections. In addition, an understanding of how long influenza virus can persist in infected hospitalized patients may enable hospital infection control teams to limit nosocomial transmission.

Respiratory specimens (throat swabs) from patients ≥18 years of age hospitalized with clinical symptoms of influenza at our institute from December 2004 through February 2005 were collected and tested by PCR for influenza A virus. If the initial PCR assay was positive, subsequent throat swabs were obtained after the original laboratory diagnosis at four time periods (dependent on the length of hospital stay)—48 h, 72 h, 5 days, and 7 days—and tested by PCR, shell vial, and tube cell culture.

Respiratory specimens from patients were added to 3 ml of M5 medium (MicroTest, Inc., Lilburn, GA). A total of 200 μl of patient specimen was extracted by using a MagNA Pure automated instrument (Roche Applied Sciences, Indianapolis, IN) and eluted in a final volume of 100 μl. Then, 5 μl of specimen extract was reverse transcribed and amplified by one-step real-time reverse transcription-PCR (RT-PCR) using the RNA Master HybProbe kit (catalog no. 03018954001; Roche). The transcribed product was amplified by asymmetric PCR using primers and probes specific for the matrix gene of influenza A virus and monitored for the development of target nucleic acid sequences after the annealing step during real-time RT-PCR (LightCycler; Roche) cycling using fluorescence resonance energy transfer technology. Analysis of the real-time RT-PCR amplification and probe melting curves was done by using LightCycler software.

Primer and probe sets. The primer and probe sets were as follows: primer 1, 5′-TAA CCG AGG TCG AAA CGT ATG TTC T-3′; primer 2, 5′-GGC ATT TTG GAC AAA GCG TCT A-3′; Probe-FL, 5′-CGA AAT CGC GCA GAG ACT TGA AGA TGT-3′; and Probe-Red, 5′-TTG CTG GGA AAA ACA CAG ATC TTG AGG C-3′.

Aliquots of respiratory specimens added to 3 ml of M5 medium (0.2 ml) were inoculated into each of two shell vial cell cultures containing a cell monolayer of R-Mix (mixed monolayer of human adenocarcinoma cells [A549] and mink lung cells [M_v1Lu]) on a 12-mm circular coverslip (Diagnostic Hybrids, Athens, OH) (7).

Each specimen in M5 medium from study patients also was inoculated in a 0.2-ml volume into a primary rhesus monkey kidney tube cell culture and incubated at 35 to 37°C for up to 14 days (Diagnostic Hybrids, Athens, OH; Viromed Laboratories, Minneapolis, MN).

Turnaround times were calculated from the time the initial specimens were received into the laboratory to the time the result was reported for real-time RT-PCR and shell vial assays or completed for tube cell cultures. The average day zero turnaround times for real-time RT-PCR assay were significantly shorter (14.8 h) than the average day zero times for shell vial (49.3 h; $P < 0.0001$) and tube cell culture (199.2 h; $P < 0.0001$).

Of the 50 patients initially positive by PCR, 43 of 50 (86%) were determined to be positive by both shell vial and cell culture (Table 1). Influenza virus type A nucleic acid was detected by PCR in 34 of 50 (68%) patients at 48 h and in 13 of 41 (31.7%) patients at 72 h after the original positive test. Shell vial and tube cell culture detection were significantly lower at the 48-h (22 of 50 [44%] and 18 of 50 [36%], respectively; $P < 0.001$) and 72-h (6 of 41 [14.6%] and 4 of 41 [9.8%], respectively; $P < 0.05$) time points. Detection of influenza A virus at day 5 was limited to PCR; there were no culture-

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TABLE 1. Comparison of real-time RT-PCR versus shell vial and tube cell culture for detection of influenza virus infection over 7 days[a]

Day	No. of positive specimens/total no. of specimens sampled (%)		
	Real-time RT-PCR	Shell vial assay	Tube cell culture
0	50/50 (100)	43/50 (86)	43/50 (86)
2	34/50 (68)	22/50 (44)	18/50 (36)
3	13/41 (32)	6/41 (14.6)	4/41 (9.8)
5	2/8 (25)	0/8 (0)	0/8 (0)
7	2/10 (20)	1/10 (10)	0/10 (0)

[a] $P < 0.001$.

positive patients detected at this time period. By day 7, 2 of 10 (20%) patients tested were positive by PCR; 1 of 10 (10%) of these patients were also found to be positive by shell vial assay. No samples were determined to be positive by tube cell culture after day 5.

During the 2005-2006 influenza season, we implemented the scheduling of PCR assay runs for influenza viruses A and B (four times daily); this reduced turnaround times to an average of 5.5 h after the specimen was received in the laboratory. Ambulatory patients selected a pharmacy to which their prescription could be faxed (by the lab) in the event of a positive PCR. Patients could then access their test results and prescription information via an automated telephone system. This laboratory service allowed the clinician to target therapy and the patient to easily access their results and rapidly start treatment.

R-Mix shell vial cell cultures have been reported to reduce the detection of influenza virus from several days to 1 or 2 days after receipt of the specimen into the laboratory (2, 5, 9); however, this assay was substantially less sensitive than PCR. In a previous evaluation of 557 respiratory tract specimens in our laboratory, we detected influenza A virus in 92 specimens (16.5%) by PCR, 49 specimens (8.8%) by R-Mix, and 24 specimens (4.3%) by the Binax NOW Flu A rapid enzyme immunoassay test (3). Thus, PCR yielded 88% and 283% increases in sensitivity compared to R-Mix shell vial cell culture and Binax NOW antigen detection, respectively, in agreement with other publications (1, 4, 6, 8, 10, 11). The negative predictive value (available within hours) of the influenza A PCR assay (99.9%) also made this assay superior to culture techniques.

In our study, we detected influenza A virus infections by sensitive real-time PCR technology in patients after 7 days of hospitalization. It is important to mention that symptom onset was at least 24 h prior to the laboratory diagnosis of influenza in all of the patients included in the study. These data suggest that patients can shed influenza A virus beyond the 5-day droplet isolation period recommended by the Centers for Disease Control and Prevention. Importantly, PCR results may not equate with active virus; however, a positive PCR result may indicate infectious or active virus since cell culture techniques have reduced sensitivity compared to PCR.

Using the theranostic approach, prompt reporting of influenza A virus results to the clinician can provide the advantages of early detection, documented laboratory diagnosis, and accurate implementation and assessment of available antiviral therapy.

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