Novel Fast Reverse Transcriptase PCR Assay for Molecular Detection of Human Influenza A (H1N1) Virus[▽]

A novel subtype of influenza A pandemic virus, A(H1N1)v, was recently reported by the Centers for Disease Control and Prevention (CDC) (Atlanta, GA) and WHO in April 2009 (1). Infection most commonly results in a mild respiratory tract infection, but there are reports of severe cases with mortalities even in patients without chronic diseases (2). Early detection of infection is important in programs aiming to minimize the spread of the disease (3). There are only a few validated laboratory diagnostic methods for the diagnosis of the virus A(H1N1)v; reverse transcriptase PCR (RT-PCR)-based detection methods are the fastest of these (4).

This communication describes the development of a fast protocol for a real-time RT-PCR assay for viral detection ("fast RT-PCR"). Assay performance was then compared with the results obtained with the standard CDC real-time protocol. A retrospective study of 228 previously diagnosed samples was performed. Samples were randomly selected: 155 samples provided negative results, and 73 provided positive results for the new pandemic virus. RNA extraction was performed from nasopharyngeal or oropharyngeal swabs collected with a synthetic tip and plastic shaft (Virocult) using QIAmp virus RNA minikit (Qiagen) and EZ1 virus minikit (Qiagen), following the manufacturer's instructions.

In the CDC protocol for real-time PCR amplification and detection, the InfA primer and probe set is designed for universal detection of type A influenza viruses, the swInfA primer/probe set is directed to all swine influenza A viruses, and the swH1 primer/probe set specifically detects swine H1 influenza. The fourth primer/probe set targets the human RNase P gene and serves as an internal control for human nucleic acid (6).

The RT-PCR assays were performed using the AgPath-ID one-step RT-PCR kit (Applied Biosystems). Briefly, 5 μ l purified RNA was reverse transcribed and amplified in a 25- μ l reaction mixture containing 12.5 μ l of 2 $\times$ RT-PCR buffer (Applied Biosystems), 1 μ l of 25 $\times$ RT-PCR enzyme mix (Applied Biosystems), 300 nM forward primer, 300 nM reverse primer, and 75 nM probe (see the CDC protocol for the primer/probe description [5]). Fast RT-PCR was performed in a 7500 fast real-time PCR system (Applied Biosystems) and analyzed by 7500 software v2.0.1. The thermal cycling conditions comprised a 10-min RT step at 50°C, a 10-min initial PCR activation step at 95°C, and 45 cycles of 95°C for 3 s and 55°C for 30 s each.

The results for the CDC and fast RT-PCR were in complete agreement, with all 73 (H1N1)v-positive samples and 155 negative samples having the same findings with both assays (Table 1). Threshold cycle (C_T) means were similar in all 4 sets, with standard deviation means ranging from 1.1113 to 2.7951. The fast RT-PCR assay took an average of 67 min to run, nearly an hour less than the CDC assay, which took 122 min to run. Overall, the studies herein reveal a significant benefit to the substitution for the standard RT-PCR method by the fast RT-PCR method, with a significant reduction in response time, without any loss of consistency. These are major advantages during an influenza pandemic, where accurate and rapid diagnosis is critical for minimizing laboratory time consumption (5). The described protocol modification is easily introduced if using a fast real time thermal cycler and proper enzyme such as the 7500 fast real-time PCR system and the AgPath-ID one-step RT-PCR kit, respectively.

TABLE 1. Results of RT-PCR analyses of 228 samples according to the standard or fast RT-PCR protocol^a

Method	C_T mean or SD				No. of samples			Run time (min)
	InfA	SwInfA	SwH1	RNaseP	A(H1N1)v positive	A(H1N1)v negative	Total	
Standard RT-PCR	27.21	29.49	27.35	28.05	73	155	228	122
Fast RT-PCR	27.66	28.18	27.67	29.04	73	155	228	67
SD	1.7684	2.7951	1.7011	1.1113				

^a Comparison of the standard PCR (CDC protocol) and fast RT-PCR showed the fast RT-PCR to have 100% accuracy.

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