JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2010, p. 665–666 Vol. 48, No. 2
0095-1137/10/$12.00 doi:10.1128/JCM.02318-09

Fast-Track Communication

Unique Finding of a 2009 H1N1 Influenza Virus-Positive Clinical Sample Suggests Matrix Gene Sequence Variation[▽]

Received 25 November 2009/Accepted 30 November 2009

The 2009 H1N1 influenza virus has rapidly spread all over the world. At this time, regions in the northern hemisphere are at the beginning of the typical annual respiratory virus season, although the 2009 H1N1 virus has been highly prevalent for the last several months. This year, it is likely that the 2009 H1N1 and seasonal influenza viruses will coexist for a period of time. Rapid and accurate laboratory detection of influenza virus and its subtype is very important for selection of appropriate antiviral therapy and initiation of infection control measures for hospitalized patients. However, with limited useful assays available, this is a challenging task. While quick and easy to perform, rapid influenza virus antigen detection assays are known to suffer from low sensitivity for the 2009 H1N1 virus (1, 2). Reverse transcription-PCR (RT-PCR) is not only sensitive compared to virus culture but also much more rapid than culture and, therefore, is widely used (3). Here, we report our finding of a highly unusual case of 2009 H1N1 influenza.

The patient was a 4-year-old girl who presented with a weeklong history of persistent wheezing. On 25 October, she was admitted through the emergency room because of 1 day of fever, cough, rhinorrhea, and labored breathing. The patient was treated with oseltamivir and supportive therapy. Her condition substantially improved, and she was discharged 1 day after admission. While the history was unremarkable, the laboratory results from viral assays were very interesting. The nasopharyngeal (NP) aspirate/wash fluid as well as the NP swab collected at admission tested positive for influenza A virus by the Binax rapid antigen detection assay (Inverness Medical, Princeton, NJ). However, RT-PCR analysis of the same NP specimen with the ProFlu+ assay (Gen-Probe Prodesse, Inc., Waukesha, WI) was neg-

TABLE 1. Laboratory test results

Sample type tested	Assay	Kit/materials/reagents (manufacturer) or protocol(s)	FDA status[a] or description of assay	Target(s) for influenza A virus[b]	Result
NP aspirate	Rapid antigen assay	BinaxNOW Influenza A&B (Inverness)	IVD	Influenza virus nucleoprotein antigens	Positive for influenza A virus
NP aspirate	Virus culture	R-Mix cells (Diagnostic Hybrids) and Bartels staining reagents (Trinity Biotech)	Standard shell vial culture	Unspecified influenza A virus antigen	Positive for influenza A virus
NP aspirate	Real-time RT-PCR	ProFlu+ for influenza A and B viruses and RSV (Gen-Probe Prodesse)	IVD	Matrix gene of influenza A virus	Negative for influenza A and B viruses and RSV
NP aspirate	Real-time RT-PCR	ProFlu-ST for identifying 2009 H1N1 and seasonal H1 and H3 viruses (Gen-Probe Prodesse)	EUA	Nucleoprotein gene of 2009 H1N1 virus	Positive for 2009 H1N1 virus
Cell culture supernatant	Real-time RT-PCR	ProFlu+ for influenza A and B viruses and RSV (Gen-Probe Prodesse)	IVD	Matrix gene of influenza A virus	Negative for influenza A and B viruses and RSV
Cell culture supernatant	Real-time RT-PCR	ProFlu-ST for identifying 2009 H1N1 and seasonal H1 and H3 viruses (Gen-Probe Prodesse)	EUA	Nucleoprotein gene of 2009 H1N1 virus	Positive for 2009 H1N1 virus
Cell culture supernatant	Real-time RT-PCR	CDC real-time RT-PCR protocol for influenza A virus (H1N1)	EUA	Matrix, HA, and nucleoprotein genes	Positive for 2009 H1N1 virus
Cell culture supernatant	Real-time RT-PCR	MultiCode-RT*x* influenza A/B virus reagents (EraGen Biosciences)	RUO	Matrix gene	Positive for influenza A virus
Cell culture supernatant	Real-time RT-PCR	Influenza virus detection and subtyping by melting-curve analysis (performed at Evanston Hospital)	Laboratory developed	Matrix gene	Positive for 2009 H1N1 virus

[a] EUA, emergency use authorization; RUO, research use only.
[b] HA, hemagglutinin.

ative for influenza A and B viruses and respiratory syncytial virus (RSV). To investigate these findings further, influenza A virus subtyping by PCR and virus culture were conducted. Surprisingly, the same nucleic acid extract that had been negative for influenza virus by the ProFlu+ assay was strongly positive for 2009 H1N1 influenza virus by the ProFlu-ST assay (Gen-Probe Prodesse, Inc., Waukesha, WI), with a cycle threshold (C_T) of 17. Both a shell vial culture with R-Mix cells (Diagnostic Hybrids, Athens, OH) and a tube culture with rhesus monkey kidney cells stained positive for influenza A virus with a Bartels viral respiratory screening and identification kit (Trinity Biotech, Carlsbad, CA). Upon repeated RT-PCR assays of the NP cell culture supernatant, results were the same as those obtained by testing the initial NP specimen, i.e., the ProFlu+ assay was negative for influenza A and B viruses but the ProFlu-ST assay was positive for 2009 H1N1 influenza virus. Split samples from the same cell culture supernatant were also run independently in two other institutions, and the results remained the same. The same cell culture supernatant was referred to Illinois Department of Public Health laboratories for testing using the CDC RT-PCR assay. This sample was positive for novel influenza A virus (H1N1) RNA (all three reactions with InfA, swInfA, and swH1 primer-probe sets were positive). The same sample tested positive for influenza A virus by using MultiCode-RT*x* influenza A/B reagents (EraGen Biosciences, Madison, WI) and was also identified as positive for 2009 H1N1 influenza virus by using a laboratory-developed assay for subtyping performed at Evanston Hospital of the NorthShore University Health Systems (4). A summary of laboratory viral test results is given in Table 1.

Although further study of this 2009 H1N1 influenza virus isolate which was not detected by the ProFlu+ assay is ongoing, one possible cause of the detection failure may be sequence variation in the primer and/or probe binding regions in the virus matrix gene. It is interesting that the performance of some other PCR assays targeting the same gene were not affected, indicating that the hypothesized sequence variation may be limited. Previous experience with the ProFlu+ and ProFlu-ST assays has demonstrated excellent correlation between the two assays, with only a few discordant results (ProFlu+ positive and ProFlu-ST negative) and no previous cases of ProFlu+-negative and ProFlu-ST-positive results found. The ProFlu+ assay does not differentiate among influenza A virus subtypes. Because of its good performance, ability to detect influenza A and B viruses and RSV simultaneously, relative ease of use, and status of being the only in vitro diagnostic (IVD) product in the class, this assay is widely used by laboratories for influenza diagnosis. Many laboratories perform a subtyping assay to determine the virus type only when the ProFlu+ test is positive for influenza A virus. Therefore, a negative result from this assay would likely miss the diagnosis of 2009 H1N1 influenza unless other assays, such as virus culture, were performed.

We believe that this is the first report of a sample that may indicate a mutation in the influenza A virus matrix gene. At this time, although it seems to be very rare, the true prevalence of this variant among all 2009 H1N1 viruses is unknown until more data are available. Because of the implication of misidentification with a single assay, this case underscores the need for cautious interpretation and additional testing when a negative RT-PCR result does not seem to fit the clinical presentation.

ADDENDUM

Culture supernatant from this sample was analyzed by genetic sequencing of the matrix gene. Preliminary results demonstrated no mismatch in primer binding regions. However, a unique single-base mismatch in the middle of the ProFlu+ influenza A virus probe and a single-base mismatch at the 3′ end were found. The mismatch at the 3′ end is present in nearly all 2009 H1N1 influenza A virus sequences deposited in the NCBI database, but the mismatch in the middle of the probe is present in only a few of the many hundreds of sequences deposited. The ProFlu+ manufacturer is conducting more studies of the virus isolate to assess the effect of the mismatch on the detectability of this unique isolate (E. Tyler, K. Harrington, and S. R. Visuri, Gen-Probe Prodesse, personal communication).

REFERENCES

1. **CDC.** 2009. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) virus—United States, 2009. MMWR Morb. Mortal. Wkly. Rep. **58:**826–829.
2. **Ginocchio, C. C., F. Zhang, R. Manji, S. Arora, M. Bornfreund, L. Falk, M. Lotlikar, M. Kowerska, G. Becker, D. Korologos, M. de Geronimo, and J. M. Crawford.** 2009. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J. Clin. Virol. **45:**191–195.
3. **Nowak, J. A., and K. L. Kaul.** 2009. The role of community molecular diagnostics laboratories in the H1N1 pandemic. J. Mol. Diagn. **11:**369–370.
4. **Stone, B., J. Burrows, S. Schepetiuk, Geoff Higgins, A. Hampson, R. Shaw, and T. Kok.** 2004. Rapid detection and simultaneous subtype differentiation of influenza A viruses by real time PCR. J. Virol. Methods **117:**103–112.

Xiaotian Zheng*
Children's Memorial Hospital
Northwestern University Feinberg School of Medicine
Chicago, IL

Kathleen M. Todd
Children's Memorial Hospital
Chicago, IL

Belinda Yen-Lieberman
Cleveland Clinic
Cleveland, OH

Karen Kaul
Kathy Mangold
Evanston Hospital
NorthShore University Health Systems
University of Chicago
Evanston, IL

Stanford T. Shulman
Children's Memorial Hospital
Northwestern University Feinberg School of Medicine
Chicago, IL

*Phone: (773) 880-6910
Fax: (773) 880-4687
E-mail: x-zheng@northwestern.edu

▽ Published ahead of print on 9 December 2009.