Direct Fluorescent-Antibody Testing Followed by Culture for Diagnosis of 2009 H1N1 Influenza A

Paul Bakerman,* Lilanthi Balasuriya, Ora Fried,† David Tellez, Pamela Garcia-Filion, and Heidi Dalton

Phoenix Children’s Hospital, Pediatric Intensive Care Unit, Phoenix, Arizona 85016

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During the 2009 H1N1 influenza A outbreak, 773 children were tested for influenza by direct fluorescent-antibody testing with PCR confirmation. Direct fluorescent-antibody testing has a specificity of 99.6% but a sensitivity of only 65.0%. Physicians should recognize diagnostic limitations of direct fluorescent-antibody testing, which missed one-third of infected individuals. We retrospectively reviewed influenza testing data at a large tertiary urban pediatric hospital during the 2009 H1N1 influenza A outbreak. Patients were hospitalized, severely ill, or immunocompromised. Hospital protocol was to obtain nasopharyngeal swabs for direct fluorescent-antibody (DFA) testing. If the test was negative, viral culture was performed. All samples underwent PCR testing for the 2009 H1N1 strain of influenza A virus. Diagnostic accuracies of the testing methods were compared for identification of this novel strain.

Samples were obtained between 30 September and 1 December 2009. Duplicate nasopharyngeal swabs were obtained simultaneously from a nares using Copan nylon-tipped flocked swabs (Microreologics Srl, Brescia, Italy). DFA testing was performed for multiple viruses. Sample swabs were directly applied to a microscope slide and then placed in MicroTest M4-RT viral transport medium (Thermo Fisher Scientific, Lenexa, KS). Slides were stained with a D3 Ultra DFA respiratory virus screening reagent (Diagnostic Hybrids, Athens, OH). Positive samples were stained with influenza A virus-specific fluorescein-labeled monoclonal antibody (Light Diagnostics, Millipore Corp., Billerica, MA).

All DFA test-negative samples underwent viral respiratory culture. Influenza virus culture was performed by inoculation of samples from the viral transport medium into RMix shell and RhMK tubes (Diagnostic Hybrids, Athens, OH). RMix shell samples were stained with fluorescein-labeled monoclonal antibody at 2 days. RhMK tubes were observed for hemagglutination and stained with fluorescein-labeled monoclonal antibody.

Duplicate nasopharyngeal samples were sent to the Arizona state laboratory for 2009 H1N1 influenza A virus-specific testing by real-time reverse transcriptase PCR. PCR for 2009 H1N1 influenza A virus was performed using the World Health Organization-Centers for Disease Control and Prevention protocol. The assay utilized a panel of oligonucleotide primers and dually labeled hydrolysis (TaqMan) probes for qualitative detection and characterization. The swInfA primer and probe set was used to detect swine influenza A virus (7).

Nasopharyngeal swabs were obtained from 773 children ranging from 5 days to 26 years of age. Median age was 3.04 years (5th and 95th percentiles, 1.7 months and 15 years). Eighty-one percent (n = 626) of the tested patients were hospitalized.

PCR identified 2009 H1N1 influenza A virus in 31.8% (n = 246) of patients. DFA testing was positive in 162 patients, 160 of whom were also PCR positive. DFA testing was negative in 611 patients, 86 of whom were positive by PCR. This resulted in a sensitivity of 65.0%, a specificity of 99.6%, a positive predictive value (PPV) of 98.8%, and a negative predictive value (NPV) of 85.9%. Among those with a negative influenza DFA test (n = 611), 92.0% (n = 562) underwent viral culture.

Twenty-six cultures were not done despite negative DFA testing and 23 cultures were cancelled since the DFA test was positive for other viruses. Forty-two (7.5%) were culture positive for influenza A virus; the sensitivity, specificity, PPV, and NPV were 51.8%, 99.6%, 95.6%, and 92.3%, respectively. Sequential testing (DFA positive or DFA negative/culture positive) increased sensitivity to 81.3% with a specificity of 99.2%, a PPV of 98.0%, and an NPV of 91.9% (Table 1).

In 2 patients, viral culture was positive and PCR was negative; culture demonstrated typical cytopathic effect and was identified utilizing influenza A virus-specific monoclonal antibody. In 2 patients, DFA testing was positive and PCR was negative; viral culture was not performed.

Reports of the diagnostic accuracy of DFA testing for diagnosis of 2009 H1N1 influenza A vary widely. There are case reports of false-negative DFA tests even in severely ill adult patients with 2009 H1N1 influenza A and respiratory failure (5). In a study involving 112 primarily adult patients, DFA testing had a sensitivity of 93%, a specificity of 97%, an NPV of 96%, and a PPV of 95% relative to 2009 H1N1 influenza A virus-specific PCR (6). In a larger study involving 6,090 patients, outpatients, and emergency department visits, DFA testing had a sensitivity of 47.2%, a specificity of 99.6%, an NPV of 90.6%, and a PPV of 96.2% for the diagnosis of 2009 H1N1 influenza A. Ages ranged from 4 days to 98 years, but the authors did not differentiate between adult and pediatric populations (3). Another study involving 172 specimens re-
ported a DFA test sensitivity of 38.7%, a specificity of 100%, an NPV of 82.2%, and a PPV of 100% (2).

PCR is the most sensitive and specific test for the diagnosis of influenza and can differentiate between influenza virus serotypes (1, 4). However, this test may be too strain specific when multiple strains of influenza virus are circulating in the community. In 2 patients, viral culture was positive and PCR was negative. PCR was specific for 2009 H1N1 influenza A virus; positive culture may represent infection with non-H1N1 serotypes. False-positive culture or false-negative PCR is a possible explanation but is less likely. In 2 patients, DFA testing was positive and PCR was negative. These results may represent false-positive DFA, false-negative PCR, or infection with a different strain of influenza A virus. DFA test results are generally more difficult to interpret, making a false-positive DFA test a possibility. Hospital protocol for viral testing did not require confirmation of a positive DFA test with culture, which could have clarified whether a positive DFA test represented infection with non-2009 H1N1 influenza A virus. The hospital protocol dictated that swabs were obtained and manually applied to slides prior to being placed in viral transport medium. While the purpose of this procedure is to accelerate rapid DFA testing, this procedure potentially introduces bias since test samples were not equally distributed for DFA culture and PCR.

This study reports a DFA test sensitivity of 65.0%; with addition of viral culture, sensitivity improved to 81.3% compared to PCR as the gold standard. Both DFA testing and culture had excellent (>95%) specificity and positive predictive value. DFA testing alone missed 1/3 of infected patients later identified by PCR. Addition of viral culture increased diagnostic sensitivity, but results were not available rapidly. Overreliance on test results can lead to misdiagnosis and lost opportunity for early initiation of therapy.

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