

Ruling Out Novel H1N1 Influenza Virus Infection with Direct Fluorescent Antigen Testing

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We evaluated the ability of direct fluorescent antigen (DFA) influenza tests to identify novel H1N1 influenza virus. DFA results were compared with polymerase chain reaction results. The negative predictive value of DFA testing was at least 96%. Therefore, when performed on specimens of adequate quality, DFA tests can effectively rule out infection due to novel H1N1 virus.

In the setting of the novel H1N1 influenza virus infection outbreak in the United States, a major barrier to effective control of the outbreak has been a lack of knowledge about the performance of commercially available influenza tests for detection of novel H1N1 virus. Even for seasonal influenza, rapid antigen test formats, such as enzyme-linked immunosorbent assay, optical immunoassay, or lateral flow have a wide range of sensitivity and specificity (24%–95% and 69%–100%, respectively) [1], and results can be greatly influenced by specimen type, study population, and duration of symptoms before testing. Detection of influenza by direct fluorescent antigen (DFA) testing involves sedimentation of respiratory epithelial cells onto a slide well and subsequent staining with influenza-specific antibodies conjugated to fluorescent dye; the test takes 1–4 h. The sensitivity of DFA tests for seasonal influenza is higher than that of point-of-care tests [2–5], but DFA tests require technical expertise and a fluorescence microscope. Culture of influenza virus from a respiratory specimen has historically

been the gold standard for diagnosis but can take days to complete. Polymerase chain reaction (PCR) testing is highly sensitive (lower limit of detection, 1–10 infectious units) [1], but it also requires technical expertise and expensive equipment. Also, as in the case of novel H1N1 virus, PCR testing may require rapid production and validation of new primer and probe sets.

At our hospital, where we perform DFA testing as our rapid test of choice for influenza diagnosis, we knew that our test had sufficient sensitivity ($\geq 95\%$ sensitivity compared with viral culture) to allow us to effectively rule out seasonal influenza, but we were uncertain how the test would perform for detection of novel H1N1 virus. Without knowledge of test sensitivity or negative predictive value for diagnosis of novel H1N1 virus infection, it was at first impossible to interpret a negative test result in a patient with suspected “swine flu.” To assess the performance characteristics of DFA testing for novel H1N1 virus, we therefore compared the results of our hospital laboratory’s DFA tests with the results of PCR testing for novel H1N1 influenza virus performed by the Massachusetts (Hinton) State Laboratory Institute (HSLI).

Methods. Analyses include all specimens that were (1) collected between 18 May and 29 May 2009 and (2) subjected to both DFA testing for influenza and PCR testing for novel H1N1 virus. This date range was selected because before this time period no specimens from our institution had tested positive for H1N1 virus by PCR, and after this time period, we no longer submitted DFA-negative specimens for PCR testing. All specimens which were DFA-positive for influenza A virus were forwarded to the HSLI for PCR testing. In accordance with testing guidelines set up by the HSLI, specimens that had DFA test results that were negative for influenza virus were only forwarded for PCR testing if they had been collected from inpatients or health care workers.

Specimens were collected only from symptomatic health care workers or patients who met Centers for Disease Control and Prevention criteria for influenza-like illness, as part of their routine clinical evaluation. Flocked swabs (Copan Diagnostics) (109 specimens) or, rarely, nasopharyngeal aspiration (3 specimens) were used to collect respiratory epithelial cells from the posterior nasopharynx. Swabs (2 per patient) were immediately placed after collection in either phosphate buffered saline for DFA or M4-RT viral transport medium (Microtest) for PCR. Specimens were put immediately on ice after collection and were stored at 4°C until processed for testing. Specimens were

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almost exclusively collected by trained hospital respiratory therapists.

Two commercial DFA kits were used for influenza testing in the Beth Israel Deaconess Medical Center clinical laboratory: (1) Simulfluo^r influenza A/B (Chemicon/Millipore) and (2) D3 DuetTM DFA RSV/Respiratory Virus Screening Kit (RVP; Diagnostic Hybrids), which tests for influenza A and B viruses, adenovirus, respiratory syncytial virus, and parainfluenza virus. The Simulfluo^r kit was used for influenza testing unless the RVP test was specifically ordered by the clinician. The presence of ≥ 30 columnar epithelial cells per test well was required for the specimen to be considered adequate for DFA testing; results from inadequate specimens were not reported, unless the results were positive (which occurred extremely rarely). Inadequate nasopharyngeal swab specimens were infrequent (3% of total specimens obtained during the time frame of the study); higher rates were observed with other specimen types that were infrequently submitted (eg, nasopharyngeal aspirates and bronchoalveolar lavage specimens).

Confirmatory PCR testing for novel H1N1 virus was performed at HSLI with PCR reagents and protocols provided by the Centers for Disease Control and Prevention Influenza Branch [6, 7]. A specimen was confirmed as positive for novel H1N1 virus if all 3 targets (panA, SwH1, and SwA) had positive results and the controls within the kit met the kit specifications. A specimen was confirmed as negative for novel H1N1 virus if all 3 targets had negative results. A specimen that had positive test results for only 1 or 2 of the 3 targets was reported as having inconclusive results.

Results. Results for specimens tested by both DFA and PCR were compared. From 18 May through 29 May 2009, 112 nasopharyngeal specimens (109 swab specimens and 3 aspirate specimens) were collected from patients with a mean age of 44.1 years; only 2 patients were <18 years old (1 patient was 1 year of age, and 1 patient was 13 years of age). A total of 87 specimens (78%) were tested with the Simulfluo^r kit, 21 (19%) were tested with the RVP kit, and 4 (3%) were tested with both tests. In subset analyses, the 2 assay kits performed comparably, and therefore data for the 2 assays were combined. Data are summarized in table 1. On the basis of these data, the DFA was calculated to have a sensitivity of 93% (95% confidence interval [CI], 8%), specificity of 97% (95% CI, 4%), negative predictive value 96% (95% CI, 5%), and positive predictive value of 95% (95% CI, 7%) relative to PCR.

The 3 DFA-negative, PCR-positive specimens were reviewed in detail. All were nasopharyngeal swab specimens. In the first specimen, there were <30 columnar epithelial cells in the slide well. However, neutrophils had been incorrectly interpreted as columnar epithelial cells. Therefore, the specimen should have been rejected on the basis of the criteria for specimen adequacy that were in place at the time. The second specimen also had

Table 1. Direct Fluorescent Antigen (DFA) versus Polymerase Chain Reaction (PCR) Test Results for Detection of Novel H1N1 Influenza Virus in Nasopharyngeal Specimens

DFA result	No. of specimens, by PCR result	
	Positive	Negative
Positive	39	2
Negative	3	67

NOTE. Of the 112 specimens collected, 42 (37%) had PCR results that were positive for novel H1N1, 69 (62%) had negative PCR results (for both novel H1N1 and pan-influenza A targets), and 1 (1%) had inconclusive PCR results and was excluded from the analysis (this DFA-negative specimen was panA positive, SwA positive, and SwH1 negative and potentially had levels of novel H1N1 virus that were below the limit of detection for both tests).

high numbers of neutrophils and was similarly inadequate. The third specimen also had high numbers of neutrophils that were misinterpreted as columnar epithelial cells, but there were 30–60 columnar epithelial cells. This review demonstrated that a cutoff value for specimen adequacy of ≥ 60 columnar epithelial cells per well (rather than ≥ 30 columnar epithelial cells per well) would have classified all 3 DFA-negative, PCR-positive specimens as inadequate for DFA testing, resulting in a sensitivity and negative predictive value that approached 100%.

Conclusions. Effective control of any outbreak of infection due to a novel influenza strain will rely on the ability of available testing modalities to detect and, critically, to rule out infection due to the novel strain. During the recent outbreak of novel H1N1 disease in Boston, Massachusetts, we found that we were able to effectively rule out novel H1N1 infection in symptomatic adults using DFA testing, which is a rapid, relatively low-cost, and commercially available method. Even before optimization of criteria for specimen adequacy, the negative predictive value of our DFA tests was 96%.

Our specimens were predominantly nasopharyngeal swab specimens collected using flocced swabs (which contain brush-like nylon fibers to improve cell collection), and this collection method produced excellent yield of columnar epithelial cells. Any discrepant results (negative DFA results but positive PCR results) that we did see were explained by 1 or more of the following factors: (1) borderline insufficient numbers of respiratory columnar epithelial cells in the specimen or (2) misinterpretation of neutrophils as columnar epithelial cells and, as a result, incorrect assessment of specimens as having adequate numbers of cells.

In response to these findings, we have now increased our laboratory's cell threshold for considering a specimen to be adequate for DFA testing from ≥ 30 to ≥ 60 columnar epithelial cells per test well. Past laboratory data (internal data from the 2007–2008 influenza season; data not shown) indicated that use of this more stringent criterion for specimen adequacy would not significantly increase the frequency of specimen rejections. Furthermore, we have emphasized with our technol-

ogists the need to reliably distinguish respiratory columnar epithelial cells (the cell type infected by influenza virus) from neutrophils and squamous epithelial cells in assessing specimen adequacy.

We expect that with these changes we will have very few false-negative DFA results going forward. Importantly, immunofluorescent staining (eg, DFA testing) is a standard method for identifying viruses in clinical virology laboratories. Therefore, clinical virology laboratories should be readily able to apply this prior experience to the implementation of highly sensitive DFA testing for novel H1N1 virus. It should be noted that our study examined test performance primarily for adult inpatients and health care workers (out patients) who met Centers for Disease Control and Prevention criteria for influenza-like illness. Therefore, our results may not similarly apply to other patient populations (eg, pediatric patients or other specific outpatient groups) in whom the performance characteristics of the DFA test may differ. Furthermore, our negative predictive value is specific to the disease prevalence associated with this period of the H1N1 epidemic; if the prevalence of H1N1 virus infection in adults increases in the future, the negative predictive value of the DFA tests may decrease to some degree. Nevertheless, our results demonstrate that DFA testing can be used to effectively exclude infection due to the novel H1N1 influenza strain in a hospital setting, allowing resources and infection-control efforts to be focused on those individuals who have positive test results.

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Potential conflicts of interest. All authors: no conflicts.

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