Evaluation of Indirect Fluorescent Antibody Assays Compared to Rapid Influenza Diagnostic Tests for the Detection of Pandemic Influenza A (H1N1) pdm09

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Abstract
Performance of indirect fluorescent antibody (IFA) assays and rapid influenza diagnostic tests (RIDT) during the 2009 H1N1 pandemic was evaluated, along with the relative effects of age and illness severity on test accuracy. Clinicians and laboratories submitted specimens on patients with respiratory illness to public health from April to mid October 2009 for polymerase chain reaction (PCR) testing as part of pandemic H1N1 surveillance efforts in Orange County, CA; IFA and RIDT were performed in clinical settings. Sensitivity and specificity for detection of the 2009 pandemic H1N1 strain, now officially named influenza A(H1N1)pdm09, were calculated for 638 specimens. Overall, approximately 30% of IFA tests and RIDTs tested by PCR were falsely negative (sensitivity 71% and 69%, respectively). Sensitivity of RIDT ranged from 45% to 84% depending on severity and age of patients. In hospitalized children, sensitivity of IFA (75%) was similar to RIDT (84%). Specificity of tests performed on hospitalized children was 94% for IFA and 80% for RIDT. Overall sensitivity of RIDT in this study was comparable to previously published studies on pandemic H1N1 influenza and sensitivity of IFA was similar to what has been reported in children for seasonal influenza. Both diagnostic tests produced a high number of false negatives and should not be used to rule out influenza infection.

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Ethics Statement
During the period of this study, influenza A/H1N1/pdm09 infection was reportable as part of enhanced surveillance in California. The information collected for this study is consistent with activities performed during a public health response and did not require institutional review board approval. Therefore, no consent was obtained, as the specimens included in this study were tested as part of public health surveillance. Technical and physical safeguards to ensure the privacy of protected health information were followed as required by the Health Insurance Portability and Accountability Act of 1996, including maintaining electronic files on secure servers, storing records in locked cabinets and limiting access to authorized personnel.

Materials and Methods
During the period of this study, influenza A/H1N1/pdm09 infection was reportable as part of enhanced surveillance in California. The information collected for this study is consistent with activities performed during a public health response and did not require institutional review board approval. Therefore, no consent was obtained, as the specimens included in this study were tested as part of public health surveillance. Technical and physical safeguards to ensure the privacy of protected health information were followed as required by the Health Insurance Portability and Accountability Act of 1996, including maintaining electronic files on secure servers, storing records in locked cabinets and limiting access to authorized personnel.
The Orange County Health Care Agency initiated enhanced surveillance for human cases of pandemic H1N1 on April 24, 2009. Clinicians and community partners were asked to report patients with influenza-like illness meeting certain epidemiologic criteria, which evolved over time based on Centers for Disease Control and Prevention (CDC) and California Department of Public Health guidance, and to submit specimens to the Orange County Public Health Laboratory (OCPHL) for testing. During the initial stages of the 2009 influenza pandemic, surveillance focused on case finding activities. Specimens were accepted at OCPHL if patient had influenza-like illness (ILI), defined as fever $$\geq$$100°F, cough and/or sore throat, and met one of the following conditions: (1) had contact with a confirmed case, (2) traveled to areas with pandemic H1N1 activity in the seven days preceding illness onset, (3) had contact with someone with ILI who traveled to areas with pandemic H1N1 activity, (4) had contact with pigs, (5) was part of a defined cluster or outbreak of people with ILI, or (6) was hospitalized with ILI or pneumonia. Submission criterion was revised on June 25, 2010. Patients met the new criteria for testing if they had ILI, pneumonia or severe, unexplained febrile respiratory illness, or sepsis-like syndrome (in infants, adults over 64 years of age, or persons with compromised immune systems) and one of the following: (1) was a health care worker, (2) was pregnant, (3) was part of a defined cluster or outbreak of people with ILI, (4) was hospitalized, or (5) lived in an institutional setting.

Testing criteria were revised again on October 2, 2010 to focus on patients who were hospitalized in the intensive care unit or died and had unexplained febrile respiratory illness, ILI, pneumonia or sepsis-like syndrome. Results were included in this analysis if rRT-PCR was performed through OCPHL; both rRT-PCR and either IFA and/or RIDT testing were performed for the same patient; specimens were collected on the same day, and the patient did not have a positive test for seasonal influenza. Results of 638 specimens collected from April 27 through October 14, 2009, from 633 patients met these criteria.

Specimen types and testing methods

Specimens were received from hospitalized patients (70%), emergency room visits (17%), CDC Sentinel Provider Influenza Surveillance Program sites (10%), and other outpatient visits (3%). Specimen types were known for 600 specimens and included nasopharyngeal or nasal swabs (256; 85%) and washes (322; 54%), tracheal aspirates (18; 3%), bronchoalveolar lavage (3; 0.5%), and lung tissue (1; 0.2%). The majority (84%) of IFA specimens were nasal washes and the majority (68%) of RIDT specimens were nasopharyngeal swabs. Samples were taken at the point-of-care and initial testing was done onsite or referred to a commercial laboratory. Additionally, samples were forwarded to OCPHL for confirmation by rRT-PCR using reagents and protocol provided by CDC (CDC Swine Influenza Virus Real-time rRT-PCR Detection Panel). Each hospital supplied viral transport medium for specimens. Cool packs were used to maintain proper temperature of specimens during transport to OCPHL. Once received by OCPHL, specimens were placed in a refrigerator at 4°C$$\pm$$2°C, then frozen to $$-70^\circ$$C prior to extraction. All specimens were typed with InfA and InfB primers and probes. Influenza A positive specimens were sub-typed with seasonal H1 and H3 primers and the CDC Swine Influenza Detection Panel was used to detect swine flu A and swine H1. All IFA testing was conducted at a hospital laboratory serving two hospitals using Bartel® Viral Respiratory Screening and Identification Kit (Trinity Biotech, PLC, Co Wicklow, Ireland). RIDTs were performed at a variety of facilities and included QuickVue Influenza, which does not distinguish between A and B antigens (Quidel Corporation, San Diego, CA), QuickVue Influenza A+B, which distinguishes between A and B antigens (Quidel), and BinaxNOW Influenza A&B test (BinaxNOW; Inverness Medical, Waltham, MA).

Sensitivity and specificity were calculated using rRT-PCR for influenza A(H1N1)pdm09 virus as the reference. Test performance was determined for children (<18 years of age) and adults and for hospitalized patients and outpatients. Data was analyzed using SPSS 16 (SPSS Inc. IBM, Chicago, IL). Exact Binomial 95% confidence intervals were calculated using JavaStat (http://statpages.org/confint.html), accessed March 2011.

Results

Results were available for 394 children, 243 adults and 1 person of unknown age. Overall 245 specimens (38%) were positive for influenza A/H1N1(pdm09 (139 children/106 adults). There were 438 respiratory specimens taken from hospitalized patients, of which 131 (30%) were positive for A/H1N1(pdm09 (82 children/49 adults). Of the 200 specimens from non-hospitalized patients, 114 (57%) were positive for A/H1N1(pdm09 (57 children/57 adults). Median age of patients for whom specimens were included was 12 years (range: <1 to 93). The median age for those specimens with confirmed influenza was 15 years (range: <1 to 81).

Overall sensitivity of IFA tests and RIDTs was 71% and 69%, respectively. Very few IFA results were received on adults and on outpatient children. Figure 1 and Table 1 presents the sensitivity of RIDT and IFA tests by severity and age. Sensitivity of IFA and RIDT performed on hospitalized children was 75% and 84%, respectively. Sensitivity of RIDT for outpatient children was 76%. In comparison, sensitivity of RIDT performed on adults in outpatient settings was 75% compared to only 45% for hospitalized adults. Overall specificity was 91%. Figure 1 and Table 2 presents the specificity of RIDT and IFA tests by severity and age. Specificity of tests performed on hospitalized children was 94% for IFA and 80% for RIDT. Specificity for RIDT performed in pediatrics and adult outpatients was 91% and 90%, respectively. QuickVue Influenza A+B, the most commonly performed RIDT, had a sensitivity of 69% (CI: 60% to 77%) and a specificity of 96% (CI:92% to 98%). Due to small numbers, sensitivity and specificity of QuickVue Influenza (non-A+B) and BinaxNOW A&B RIDTs are not displayed.

Mean time from illness onset to specimen collection was similar for hospitalized adults, (2.7 days) and outpatient adults (2.2 days) and was also similar for hospitalized children (2.8 days) and outpatient children (2.1 days), $$p$$>0.05. Mean age was significantly higher among hospitalized adults (52 years) compared to outpatient adults (31 years), $$p$$<0.05, and significantly lower among hospitalized children (5 years) compared to outpatient children (9 years), $$p$$<0.05.

Discussion

To our knowledge, this is the first study to evaluate an IFA for the diagnosis of influenza A(H1N1)pdm09 in the clinical setting. There is an IFA specifically for the diagnosis of A(H1N1)pdm09 that was approved by the Food and Drug Administration on an emergency use authorization basis, but it has only been evaluated in the lab [17,18]. In hospitalized children, our IFA performed no better than RIDT. Other investigators looking at DFA and RIDT have had similar findings [9,16]. Our overall sensitivity of RIDT is comparable to previous published studies on A(H1N1)pdm09 and sensitivity of IFA is similar to what has been reported in children.
for seasonal influenza (40–90%) when compared to viral culture 
[19].

In choosing between RIDT and IFA tests, RIDT offers quicker 
results with similar sensitivity, requires less experienced personnel 
to perform and utilizes less laboratory personnel time and 
equipment. However, IFA testing is often performed as part of a 
respiratory virus panel and positive results for a different 
respiratory virus than influenza would provide useful information 
for infection control and other management decisions.

With such a low sensitivity, negative RIDT and IFA test results 
must be interpreted with caution. Since these tests produce a high 
number of false negative results, clinicians would not be able 
to rule out a diagnosis of influenza based on a negative result.

Sensitivity of RIDTs was lower in outpatient children (76%) 
compared to hospitalized children (84%) and was especially poor 
in hospitalized adults (45%) compared to outpatient adults (75%). 
Time from symptom onset to specimen collection was not 
significantly different between the various groups. We also looked 
at age as a possible factor affecting sensitivity. Hospitalized 
children were significantly younger than outpatient children, while 
hospitalized adults were significantly older than outpatient adults. 
It is well known that children shed more influenza virus and in 
greater quantities than adults. However, the effect of age on viral 
shedding in adults is less established. Clinicians should be aware 
that sensitivity of RIDTs varies greatly and may be poor in older 
adults.

The overall specificity of RIDT (91%) is similar to what has 
been reported in the literature for influenza A(H1N1)pdm09 (86% 
to 100%), however, results among certain subgroups and test types 
were much lower than expected [3,4,5,8,11,19,20]. Since RIDT 
and IFA tests were performed in the clinical setting and PCR 
testing was performed at a different facility, it is possible that some 
RIDT and IFA tests were truly positive and the specimens lost 
integrity during transport to OCPH. Given that PCR testing 
performed at OCPH was used as the gold standard for disease 
classification, this would result in more false positives then is 
accurate due to misclassification of those who had 
A(H1N1)pdm09. While our results may be due to small sample 
size or loss of specimen integrity during transport, healthcare 
providers should be aware that these tests may produce false 
positives under certain conditions.

Our study is limited by the lack of detailed information recorded 
on specimen type (i.e. nasal swab versus nasopharyngeal swab) 
restricting our ability to account for different collection methods in 
our analysis. Additionally, in a small number of patients, the 
specimen tested by IFA or RIDT may not have been the exact 
same specimen tested by rRT-PCR although all specimens were 
collected on the same day. One hospital laboratory performed all 
IFA testing, while RIDT testing was performed in a variety of 
facilities and using different methods. Finally, since IFA and RIDT 
were performed at a different facility than rRT-PCR, storage or 
transportation issues (including transport temperature) may have 
affect the integrity of the sample and are limitations of the study.

In our study, clinically based diagnostic tests for influenza 
A/H1N1)pdm09 had variable sensitivities and specificities and may 
lead to false negative and false positive results. Treatment and 
infection control decisions should not be changed or delayed based 
on negative IFA or RIDT results. Research is needed to develop 
and validate more sensitive rapid testing for influenza.

### Table 1. Comparison of Sensitivity for RIDT and IFA Tests by Severity and Age using PCR as the Gold Standard.

|                | IFA          | RIDT          |                |                  |                  |                |                  |
|----------------|--------------|---------------|----------------|------------------|------------------|----------------|------------------|
|                | Positive     | False Negative| Sensitivity (95% CI) | Positive     | False Negative| Sensitivity (95% CI) |
| Inpatients     |              |               |                  |                  |                  |                |                  |
| Children       | 43           | 14            | 75% (62 to 86)   | 21              | 4                | 84% (64 to 95)  |
| Adults         | 2            | 3             | -----------------| 20              | 24               | 45% (30 to 61)  |
| Outpatients    |              |               |                  |                  |                  |                |                  |
| Children       | 2            | 2             | -----------------| 41              | 13               | 76% (62 to 87)  |
| Adults         | --           | --            | -----------------| 43              | 14               | 75% (62 to 86)  |

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Author Contributions

Conceived and designed the experiments: SN MC FA-S HM. Performed the experiments: KK KV. Analyzed the data: SN. Contributed reagents/materials/analysis tools: KK KV. Wrote the paper: SN MC FA-S.

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Table 2. Comparison of Specificity for RIDT and IFA Tests by Severity and Age using PCR as the Gold Standard.

|       | IFA     |       | RDT     |       |
|-------|---------|-------|---------|-------|
|       | Negative| False Positive | Specificity (95% CI) | Negative| False Positive | Specificity (95% CI) |
| Inpatients |         |         |         |         |         |
| Children | 171     | 11     | 94% (89 to 97) | 24      | 6       | 80% (61 to 92) |
| Adults  | 9       | 1      |         | 80      | 4       | 95% (88 to 99) |
| Outpatients |       |         |         |         |         |
| Children | 7       | 1      |         | 31      | 3       | 91% (76 to 98) |
| Adults  |       |         |         | 38      | 5       | 88% (75 to 96) |

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