Comparison of the Denka-Seiken INFLU A·B-Quick and BD Directigen Flu A+B Kits with Direct Fluorescent-Antibody Staining and Shell Vial Culture Methods for Rapid Detection of Influenza Viruses

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The INFLU A·B-Quick and Directigen Flu A+B enzyme immunoassays were compared with direct immunofluorescence and cell culture for detection of influenza A and B viruses in a total of 255 patient specimens. Both assays identified 23 of 42 influenza A viruses (sensitivity, 54.8%; specificity, 100%; positive predictive value [PPV], 100%; negative predictive value [NPV], 91.8%). The INFLU A·B-Quick assay identified 10 of 16 influenza B viruses (sensitivity, 62.5%; specificity, 99.6%; PPV, 90.9%; NPV, 97.5%), and the Directigen Flu A+B assay detected 9 of 16 influenza B viruses (sensitivity, 56.3%; specificity, 99.6%; PPV, 90%; NPV, 97.1%).

Influenza virus infections are responsible for significant morbidity and mortality in the United States, with estimated annual costs of $3 billion to $12 billion (3). Rapid diagnosis of influenza virus infections is of medical importance for the initiation of antiviral therapies, reductions in the lengths of patient hospital stays, and discontinuation of the use of unnecessary antibiotics (1, 12). Rapid tests for the detection of influenza virus are amenable for use in physicians’ offices or small laboratories that lack more complex viral diagnostic capabilities. In addition, those assays that differentiate influenza A and B viruses provide physicians with valuable information regarding possible treatment and prophylaxis. Anti-influenza virus medications are most effective when they are given within 48 h of the onset of symptoms (7). Several of these drugs are active only against influenza A virus, while the newer neuraminidase inhibitors appear to be efficacious against both types of influenza virus (5, 9).

The Directigen Flu A+B (Becton Dickinson, Cockeysville, Md.) rapid assay is approved for the detection and differentiation of influenza A and B viruses. We compared this assay to a new test (INFLU A·B-Quick; Denka-Seiken, Tokyo, Japan) that also allows the differential identification of influenza A and B viruses and, at the time of this writing, is under review for Food and Drug Administration approval. In a prospective study of 255 patient specimens submitted during the 2002 respiratory virus season, individual samples were subjected to testing by four methods: direct fluorescent-antibody (DFA) staining, cell culture, the INFLU A·B-Quick assay, and the Directigen Flu A+B assay.

Specimens were submitted in either sterile containers or M4 viral transport medium (MicroTest, Inc., Lilburn, Ga.) at 4°C. Aliquots for rapid testing were processed according to the instructions of the manufacturers. The Directigen Flu A+B package insert recommends the following sample types for testing: nasopharyngeal (NP) washes, bronchoalveolar lavage (BAL) samples, NP aspirates, throat swabs with or without transport medium, and NP and lower nasal passage swabs. The INFLU A·B-Quick package insert recommends testing of NP aspirates and washes and throat and NP swabs without transport medium. While testing of specimens in transport medium was not expressly approved by the manufacturer, an evaluation was undertaken whereby samples submitted in M4 medium were processed by addition of 1 ml of specimen to a detergent-containing vial prior to testing by the INFLU A·B-Quick assay. Sample aliquots for testing by DFA staining were brought to a total volume of 4 to 5 ml with phosphate-buffered saline (PBS) and centrifuged, and the pellets were resuspended in the residual fluid. Following a second wash with PBS, the pellets were spotted onto eight-well slides. The dried and fixed slides were then stained and examined according to the instructions of the manufacturer (Bartels, Inc., division of Trinity Biotech, Wicklow, Ireland). A minimum of 20 cells per spot was considered an adequate sample, and the presence of one or more characteristically staining respiratory tract cells was considered a positive result. Cell culture was performed with approximately 0.2 ml of prepared clinical samples inoculated into BGM, A549, and MRC-5 cell (all from ARUP Cell Culture Laboratory, Salt Lake City, Utah) shell vials, a PRMK cell (ViroMed Laboratories, Minneapolis, Minn.) shell vial, and two R-Mix cell (Diagnostic Hybrids, Inc., Athens, Ohio) shell vials. The vials were centrifuged at 3,000 × g for 15 min at 20°C and incubated at 35°C in 5% CO2. One R-Mix shell vial was stained at 20 to 24 h postinoculation by using the respiratory virus antibody screen (Bartels, Inc.). Positive screens were further identified by using the second R-mix shell vial. The contents of the coverslips were scraped off and spotted onto eight-well slides. Dried and fixed slides were then stained with virus-specific monoclonal antibodies (Bartels, Inc.). The remaining vials were observed for cytopathic effects for up to 10 days, at
TABLE 1. Performances of rapid assays for influenza virus compared to those of cell culture and DFA staining for samples submitted in either sterile containers or M4 viral transport medium

<table>
<thead>
<tr>
<th>Assay or transport condition</th>
<th>No. of specimens</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>No. of specimens</th>
<th>Sensitivity (%)</th>
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<th>No. of specimens</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFA staining and culture</td>
<td>100</td>
<td>99.6</td>
<td>100</td>
<td>110</td>
<td>99.6</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
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<td>100</td>
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<tr>
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<td>1</td>
<td>100</td>
<td>0</td>
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<td>100</td>
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<tr>
<td>Sterile container</td>
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<td>1</td>
<td>100</td>
<td>0</td>
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<td>100</td>
<td>0</td>
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*Data not shown for M4 medium*
which time hemadsorption was performed on the PRMK shell vial.

Viruses were identified in 110 of the 255 specimens by DFA staining and/or cell culture (42 specimens had influenza A viruses, 16 specimens had influenza B viruses, 33 specimens had respiratory syncytial viruses [RSVs], 8 specimens had adenoviruses, 6 specimens had enteroviruses, 4 specimens had parainfluenza type 3 viruses, 2 specimens had cytomegaloviruses, and 1 specimen had a parainfluenza type 1 virus). Two patient specimens revealed dual infections: one with RSV and influenza A virus and the other with adenovirus and enterovirus. For the purpose of this study, specimens were considered positive for influenza A or B viruses if either DFA staining or culture was positive.

Of the 42 specimens positive for influenza A virus, viruses were identified in 28 and 40 specimens by DFA staining and cell culture, respectively. Twenty-three of these influenza A virus-containing specimens were positive by both the INFLU A-B-Quick and the Directigen Flu A+B enzyme immunoassays for an overall sensitivity of 54.8% (Table 1). The positive predictive value (PPV) and the negative predictive value (NPV) for influenza A virus by both assays were 100 and 91.8%, respectively. Compared to DFA staining or cell culture individually, both assays displayed comparable sensitivities (Table 1).

Of the 16 specimens positive for influenza B virus, viruses were identified in 12 and 15 specimens by DFA and cell culture, respectively. The INFLU A-B-Quick and Directigen Flu A+B assays correctly identified influenza B virus in 10 (62.5%) and 9 (56.3%) samples, respectively (Table 1). For the INFLU A-B-Quick and Directigen Flu A+B assays, PPVs for influenza B virus were 90.9 and 90%, respectively, and NPVs for influenza B virus were 97.5 and 97.1%, respectively. Similar sensitivities were achieved when the results of the assays were compared to those of cell culture alone. However, when the results of the INFLU A-B-Quick and Directigen Flu A+B assays were compared to those of DFA staining alone, the sensitivities were 83.3 and 75%, respectively (Table 1). Each assay reported a single false-positive reaction for influenza B virus with separate samples (specificities, 99.6%) (Table 1).

Neither rapid assay method resulted in false-positive reactions for the remaining 52 samples in which viruses other than influenza viruses were identified or the remaining virus-negative specimens. However, with the INFLU A-B-Quick device, correctly identified positive reactions with four specimens positive for influenza A virus and one specimen positive for influenza B virus were noted to occur several minutes beyond the manufacturer’s prescribed reading time of 10 min. This issue is under investigation by the manufacturer.

The type of transport system in which samples were submitted had a significant impact on the sensitivities of the rapid testing methods. Seventeen nasal wash specimens submitted in sterile containers without viral transport medium were positive for influenza A virus by DFA staining and/or culture. Of these, 12 (70.6%) and 14 (82.4%) were identified by the INFLU A-B-Quick and Directigen Flu A+B assays, respectively (Table 1). Only 44% (11 of 25) and 36% (9 of 25) of influenza A viruses identified by DFA and/or cell culture in specimens containing M4 medium (20 nasal wash specimens, 4 NP swab specimens, 1 sputum specimen) were positive by the INFLU A-B-Quick and Directigen Flu A+B assays, respectively. Similarly, influenza B viruses were identified in 80% of the samples submitted in sterile containers (eight nasal wash specimens, one sputum specimen, one BAL specimen) but in only 33.3% of the specimens submitted in M4 medium (five NP swab specimens, one nasal wash specimen) for the INFLU A-B-Quick assay, and influenza B viruses were identified in 70% of sterile containers but in only 33.3% of the specimens submitted in M4 medium for the Directigen Flu A+B assay (Table 1). No influenza viruses were identified by any method in other specimen types tested (seven throat swab specimens, five tissue specimens, one tracheal aspirate specimen, one pleural fluid specimen).

The sensitivities of the different rapid assays for influenza virus available at present vary from 54 to 96% among different studies, depending on the reference methods used (2, 4, 6, 8, 10). Generally, positive results by the rapid methods correlate well with actual infection. However, due to the typically low sensitivities and NPVs, confirmatory testing by traditional methods is recommended. The use of more sensitive culture methods such as those with R-Mix cells has enhanced the ability to recover influenza viruses (11; J. J. Dunn, R. D. Woolstenhulme, J. Langer, and K. C. Carroll, submitted for publication) and may skew the analytical data for these rapid methods. St. George et al. (11) demonstrated that methods that use R-Mix cells can detect 4 to 5 log fewer influenza A virus particles than two commercial rapid assays for influenza virus and have 100-fold greater sensitivity than conventional cell culture methods. Other studies evaluated Directigen Flu A+B with only Madin-Darby canine kidney cells for culture confirmation and found the rapid test to be 82.9 to 96% and 51.5 to 87.5% sensitive for influenza A and B viruses, respectively (2, 10). Compared to DFA staining and shell vial culture with R-Mix cells, our data demonstrate that the new INFLU A-B-Quick test is comparable to the Directigen Flu A+B assay for the rapid detection and differentiation of influenza viruses. The INFLU A-B-Quick assay requires minimal sample manipulation and produces sharp, easy-to-read results.

REFERENCES