Impact of Sample Type on Rapid Detection of Influenza Virus A by Cytospin-Enhanced Immunofluorescence and Membrane Enzyme-Linked Immunosorbent Assay

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Influenza virus A is a major cause of morbidity and mortality, especially in the elderly and in those with chronic cardiac or pulmonary conditions. The impact of influenza virus and other respiratory viruses on immunocompromised hosts has been increasingly recognized (9). Rapid diagnosis benefits patient management, helps to prevent nosocomial transmission, and allows early institution of amantidine or rimantidine treatment, as well as newer antiviral therapies (3).

Rapid membrane enzyme-linked immunosorbent assay (ELISA) tests can be completed within 15 min and do not require extensive technical expertise or equipment. This test can be implemented in doctors’ offices or in laboratories without virology expertise. However, reported studies have shown a wide variation in sensitivity of detection, ranging from 67 to 100% (6–8). Direct fluorescent-antibody assay (DFA) can provide excellent results, but it requires greater technical expertise, is more labor intensive, and requires adequate numbers of ciliated epithelial cells.

In this study, rapid membrane ELISA was compared with cytospin-enhanced DFA by using routine samples submitted to the clinical virology laboratory by a variety of clinicians. The impact of sample type on test results was examined.

Sixty nasopharyngeal (NP) or throat swabs, 30 NP aspirates, and two bronchoalveolar lavage specimens were submitted to the clinical virology laboratory for diagnosis of influenza virus A. All 30 NP aspirates were obtained from children, and 59 of the 60 swabs were from adults.

Samples were divided into three aliquots: one aliquot was applied to slides by cytocentrifugation for DFA, one aliquot was tested with the Directigen Flu A ELISA (Becton Dickinson, Cockeysville, Md.), and one aliquot was cultured in rhesus monkey kidney cell cultures for 25 of the antigen-negative samples (4). After the addition of 5 ml of phosphate-buffered saline (PBS), specimens for DFA were centrifuged at 700 × g for 5 min. Each cell pellet was resuspended in 3 parts PBS, then 200 μl was cytocentrifuged (Cytospin 3; Shandon, Inc., Pittsburgh, Pa.) for 4 min at 800 rpm. Excess cell suspensions were stored for 2 to 3 days at 4°C. After air drying, slides were fixed in cold acetone, then stained with SimulFluor Influenza A/B reagent (Chemicon International, Temecula, Calif.), and were examined with an epifluorescence microscope. Influenza virus A-positive respiratory epithelial cells exhibited characteristic apple-green, granular nuclear, and/or cytoplasmic staining. For Directigen Flu A ELISA, specimens were tested according to the manufacturer’s instructions.

Influenza virus A was detected in 15 NP aspirates by both DFA and ELISA (100% agreement). In contrast, only 24 of 38 positive swabs were detected by both DFA and ELISA. Ten swabs were positive by DFA only, one was positive by ELISA only, and three were positive by culture only. Thus, DFA detected 89.5% and ELISA detected 66% of influenza virus A-positive swabs, a significant difference (P = 0.007, McNemar’s test). When results for all specimen types were combined (Table 1), the overall sensitivity was 92.5% for cytospin-enhanced DFA and was 75.5% for ELISA (P = 0.007, McNemar’s test).

Compared with culture, membrane ELISA tests offer a rapid diagnosis in a simple format and are thus useful to clinicians, especially in doctors’ offices. Published studies of the Directigen Flu A ELISA have reported widely varying sensitivities, ranging from 67 to 100% (6–8). The study by Waner et al. reported a sensitivity of Directigen Flu A of 100%, compared to the results of isolation in cell culture and DFA. NP washes from children less than 10 years old comprised over 90% of samples tested (8). Similarly, all 15 positive NP aspirates obtained from children in our study were detected by Directigen Flu A ELISA. NP aspirates and washes are more reliable than swabs (1), and young children generally shed higher titers of virus than do adults.

Leonardi et al., who investigated geriatric patients, reported a sensitivity of 86.8% for Directigen Flu A ELISA. For the purpose of the study, however, staff members were specifically trained in specimen collection. NP and throat swabs were vigorously collected early in illness and were combined in one specimen vial for testing (6). In contrast, Steed et al. tested samples submitted to a reference laboratory from patients ranging in age from 2 weeks to 84 years. Sensitivity was 67% for ELISA and only 47% for DFA compared with culture (7).

DFA enables the technologist to assess sample quality microscopically. If the laboratory is on site, the clinician can be informed of poor sample quality and another sample can be requested or the test report can be modified. Nevertheless, if a substantial proportion of tested samples are deemed inadequate for DFA, much time and reagents are wasted. The use of a cytospin to prepare slides for DFA reduces inadequate
smears, improves cell morphology, and enhances the accuracy of interpretation (2, 5).

Sample collection designed to maximize the amount of virus available for detection will improve test results for both ELISA and DFA. In many diagnostic laboratories, however, control over sample collection is minimal, and the results obtained may differ substantially from studies using optimal sample collection.

In this study, we demonstrated that, overall, cytospin-enhanced DFA detected significantly more influenza virus A-positive samples than did the rapid membrane ELISA Directigen FLU-A. Sample type, and possibly patient age, had a major impact on test sensitivity, especially for the ELISA, and could explain discrepancies among previous studies. The difference in detection rate between DFA and ELISA was seen exclusively in swabs obtained from adults and not in NP aspirates from children, presumably due to the higher titers of virus in the latter samples. These problems may be reduced, but probably not eliminated, by greater efforts to educate and train clinicians to optimize sample collection.

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