

Evaluation of a Rapid Enzyme Immunoassay for Detection of Influenza A Virus

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The Directigen FLU-A enzyme immunoassay for the detection of influenza A virus was compared with direct smear and culture in 211 clinical specimens. The FLU-A enzyme immunoassay proved to be a reliable, rapid screen for influenza A from symptomatic patients and was less dependent on technical expertise for interpretation than were direct smears.

The availability of amantadine hydrochloride for prophylactic and symptomatic treatment of influenza A infections has created a demand for rapid, specific, and sensitive tests for this virus. The standard method of detection is isolation of the virus in primary rhesus monkey kidney (RMK) cells, which can take 1 to 10 days (1, 2). Monoclonal antibody reagents which can detect influenza virus in direct smears in less than 0.5 h are also commercially available (3, 4). However, the sensitivity of the direct smear technique is highly dependent upon the quality of the specimen (1, 5).

The Directigen FLU-A (Becton Dickinson Microbiology Systems, Cockeysville, Md.) test is a membrane enzyme immunoassay (EIA) which can directly detect influenza A in clinical specimens in less than 15 min. An evaluation of the Directigen FLU-A EIA test for the rapid detection of influenza A was made in comparison with direct smear and culture.

A total of 211 specimens were collected from individual patients presenting influenza symptoms. Nasopharyngeal and pharyngeal swabs were collected, with one swab each for the EIA, culture, and direct smear. These swabs were used randomly on the three tests. The swab used to make the direct smear was placed with the culture swab in viral transport medium after the application of the specimen to the microscope slide.

All swabs were placed in viral transport medium consisting of veal infusion broth and antibiotics. Specimens were transported to the laboratory immediately upon collection and stored at 4°C until inoculated into two RMK, one HEP-2, one A549, and one HF cell culture tube. Inoculated cells were placed in an incubator at 33 to 35°C and rotated. The cultures were observed for cytopathic effect every 2 days and tested for hemagglutinin activity by hemagglutination with guinea pig erythrocytes twice a week and hemadsorption once before being discarded at 2 weeks (3). Cells displaying positive hemagglutination or hemadsorption results or cytopathic effect for any virus were scraped and confirmed by using type-specific monoclonal-antibody-staining reagents.

An individual swab collected for direct smear was transported to the laboratory and rolled onto two 8-mm wells of a microscope slide. The slide was allowed to air dry, fixed with acetone for 10 to 15 min, and stained with influenza A- and B-specific monoclonal antibody reagents (Bartels Immuno-

diagnostics, Bellevue, Wash.) as described in the manufacturer's protocol.

The FLU-A EIA was performed by following the manufacturer's protocol. A separate nasopharyngeal or pharyngeal swab was collected for the EIA and processed immediately upon collection. The swab was placed in a glass tube (12 by 75 mm) with 1 to 2 ml of transport medium or saline and vortexed. Excess fluid was then extracted from the swab, if possible, and the swab was discarded.

Approximately 125 μ l each of specimen and extraction reagent was added to the flexible plastic DispensTube provided in the kit. The sample was dropped into the ColorPac test well, a triangle-shaped, self-contained unit. Any influenza A antigen in the specimen was nonspecifically bound to the membrane of the unit. Detector enzyme-conjugated monoclonal antibodies specific for the influenza A nucleoprotein antigen were then dropped through the ColorPac unit where they were bound to any trapped antigen. Another sequence of two substrate additions with a 5-min incubation period completed the reaction in less than 15 min. A positive result was indicated by a purple triangle of any intensity with a purple dot in the center. A negative test was indicated by a purple dot and no triangle. Controls were built into the control dot, although positive and negative controls included in the kit were run once when each kit was put into routine use.

Initially, 126 specimens were collected for direct smear, culture, and FLU-A EIA. Of these, 18 specimens were positive for influenza A by the EIA, 20 were positive by culture, and 12 were positive by direct smear. There were three parainfluenza 1 virus, three respiratory syncytial virus, one herpes simplex type 1, and four influenza B isolates from these specimens, all of which were EIA negative.

In comparison with the direct smears, the EIA had a sensitivity of 100%, a specificity of 95%, and positive and negative predictive values of 67 and 100%. There were four negative direct smears which were positive by both culture and EIA.

An additional 85 specimens were evaluated by EIA and cell culture only. Thirty were positive by culture. With the 85 additional samples, the EIA in comparison with cell culture had a sensitivity of 62%, a specificity of 94%, positive and negative predictive values of 76 and 89%, and agreement of 86%.

When the case histories of the four culture-negative and EIA-positive specimens were examined, it was found that they were all obtained from patients 6 weeks after the local influenza A season had ended. These patients presented with

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respiratory symptoms not necessarily associated with an influenza infection. Although our laboratory could not demonstrate that these isolates were missed by culture or prove that these were false-positive EIA results, this finding does underscore the fact that the FLU-A EIA is only recommended by the manufacturer's protocols for use on symptomatic patients.

During the study, 20 cell culture supernatants from respiratory specimens were also tested with the EIA. Supernatants from inoculated cell cultures were treated as direct patient specimens and tested by the EIA 2 to 5 days after specimen inoculation. The EIA correctly identified influenza A in these cell culture supernatants. Although this is a more expensive means of confirming influenza A in culture than scraping and staining with a monoclonal antibody, it was more rapid.

This evaluation confirmed the 100% sensitivity of the FLU-A EIA compared with that of direct smears as reported by Waner et al. (6). We could not confirm the 100% sensitivity of the EIA compared with that of culture, although the specificity of the EIA in our laboratory was slightly higher than this previous report. The isolation of respiratory viruses other than influenza A and the isolation of influenza A from EIA-negative specimens did support the need to perform supplementary cell culture isolations. Culture and subsequent typing of influenza A also served the purpose of providing epidemiologic information necessary for vaccine formulation in future years.

Overall, the FLU-A EIA proved to be an accurate and rapid test in our laboratory. Backup cultures served an essential role in detecting viruses other than influenza A and

in enhancing the low sensitivity and positive predictive value of the EIA in our population. Unlike direct smears, this EIA could be run around the clock by personnel not specially trained in immunofluorescent work and did not require specialized equipment. The EIA could also be performed in less time than the direct smear or cell culture isolation and was also less dependent on the quality of the specimen.

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