Utility of a Respiratory Virus Panel Containing a Monoclonal Antibody Pool for Screening of Respiratory Specimens in Nonpeak Respiratory Syncytial Virus Season

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An indirect immunofluorescence respiratory virus panel containing monoclonal antibodies directed against respiratory syncytial virus, parainfluenza virus types 1, 2, and 3, adenovirus, and influenza viruses A and B was used to screen specimens in the nonpeak respiratory syncytial virus seasons in 1989 and 1990. The results indicate that the respiratory virus panel is fairly sensitive (79%) and very specific (99%) for the detection of respiratory viruses directly in clinical specimens during these time periods.

Respiratory viruses including respiratory syncytial virus (RSV), parainfluenza virus types 1, 2, and 3, adenovirus, and influenza viruses A and B are a significant cause of morbidity and mortality in pediatric populations. The introduction of antiviral therapy in the past decade has made treatment of some of these infections in high-risk patients possible and has led to a decrease in the use of unnecessary antibiotics.

During peak RSV seasons, a direct RSV immunofluorescence assay on clinical samples has been shown to be both sensitive and specific for the detection of RSV (1, 4, 5). However, in the nonpeak RSV season, when other respiratory viruses cause significant respiratory illness in children, a simple, accurate, and rapid test is necessary to detect these viruses both for therapeutic and for infection control purposes.

We evaluated a respiratory virus panel containing a pool of monoclonal antibodies directed against RSV, parainfluenza virus types 1, 2, and 3, adenovirus, and influenza viruses A and B for direct screening of respiratory specimens submitted to our laboratory in the nonpeak RSV seasons.

Clinical specimens were collected from patients at the Montreal Children's Hospital during two nonpeak RSV seasons (October to the end of December 1989 and April to the end of May 1990). Nasopharyngeal aspirates were obtained from all patients by using a French catheter inserted into the nasopharynx. Material was aspirated into a suction trap containing viral transport medium (Hanks balanced salt solution base with HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] buffer supplemented with 5% fetal calf serum—penicillin [250 U/ml]; gentamicin [20 μg/ml]; amphotericin B [25 μg/ml]; Fungizone; E. R. Squibb and Sons, Princeton, N.J.]. Specimens were transported immediately to the virology laboratory and were held at 4°C until they were processed. The average holding time was about 4 h.

Specimens for immunofluorescence were washed twice with phosphate-buffered saline (PBS; pH 7.2) and centrifuged at 600 × g (1,000 rpm) for 10 min. The pellet was resuspended in 250 μl of PBS, and then the solution was spotted onto glass slides with a drop from a Pasteur pipette. Seven smears were made from each sample. The slides were air dried and fixed in cold acetone at 4°C for 10 min. After drying, the smears were processed for immunofluorescence with individual reagents. Approximately two-thirds of the sample was used to prepare smears and one-third was reserved for tissue culture.

An indirect immunofluorescence procedure was used with the respiratory virus panel, parainfluenza virus panel, and adenovirus reagents. The respiratory virus panel that was used contained mouse monoclonal antibodies directed against RSV, adenovirus, parainfluenza virus types 1, 2, and 3, and influenza viruses A and B and was manufactured by Bartels Immunodiagnostics, Bellvue, Wash. The parainfluenza virus panel contained monoclonal antibodies directed against parainfluenza virus types 1, 2, and 3 (Chemicon International Inc., Temecula, Calif.). Adenovirus was detected by using a monoclonal antibody from Cambridge Bioscience, Worcester, Mass., which contained a group reagent for detection of all 41 serotypes. All indirect immunofluorescence assays (IFAs; including the respiratory virus panel) used fluorescein-labelled goat anti-mouse conjugate (Cappel, Worthington Biochemical, Malvern, Pa.) for the second stage.

RSV, influenza viruses A and B, and individual parainfluenza virus types 1, 2, and 3 were detected by direct immunofluorescence. The reagents used for the direct immunofluorescence procedures were a fluorescein-conjugated RSV monoclonal antibody (Ortho Diagnostics Systems Inc., Raritan, N.J.), fluorescein isothiocyanate (FITC)-conjugated influenza virus A and B monoclonal antibodies (Boots-Celltech Diagnostics Ltd., Slough, United Kingdom), and individual parainfluenza virus type 1, 2, and 3 FITC-conjugated monoclonal antibodies (Bartels Immunodiagnostics).

Smears prepared from clinical specimens were initially tested by using the respiratory virus panel. Specimens that were positive by the respiratory virus panel were then retested by specific individual viral immunofluorescence. All non-RSV-positive specimens were then inoculated onto cell culture for confirmation. All panel-negative specimens were inoculated onto tissue culture.

Antibiotic-treated specimens were inoculated into the following cell lines: primary rhesus monkey kidney (PRMK) cells (Viomed, Minnetonka, Minn.), human embryonic lung fibroblasts (prepared in-house), and human lung carcinoma cells (A549; Viromed). The cultures were incubated at 37°C.

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in stationary racks and were examined for a cytopathic effect every other day for a total of 17 days. Hemadsorption was performed with washed guinea pig erythrocytes (0.5% in PBS) in the PRMK cell tubes on days 3 and 6 after initial inoculation. Blind passages were made at day 9 or 10 on all three cell lines. Hemadsorption was repeated at days 3 and 7 following passage. Viruses producing a cytopathic effect were identified by direct or indirect immunofluorescence by using the monoclonal antibodies for specific viruses applied to cells scraped from the culture tubes. The sensitivity, specificity, positive and negative predictive values, and confidence intervals were calculated by standard formulas (3).

During these two time intervals, 348 nasopharyngeal specimens were received for evaluation by respiratory pool IFA. Of these specimens, 3 were judged to be inadequate because of an insufficient amount of cells, leaving 345 specimens for analysis.

A total of 65 (19%) of the samples were positive by the respiratory virus panel. Of these, 44 (67%) were positive for a non-RSV virus when tested by specific individual IFA. These included 4 parainfluenza virus type 1 isolates, 30 parainfluenza virus type 3 isolates, 6 adenovirus isolates, and 4 influenza virus A isolates. Twenty samples were positive for RSV. One specimen appeared to be respiratory virus panel positive; however, individual specific immunofluorescence assays for each respiratory virus were negative and the specimen did not grow a virus in tissue culture. This specimen was considered to be a false positive. All other panel-positive specimens that were not RSV positive were confirmed in tissue culture. Identification of the panel-positive specimens by specific viral immunofluorescence proved to be very specific, and there was no difficulty in interpretation of these smears.

Of the 345 specimens received in these periods, 280 (81%) tested negative by the respiratory virus panel. There was sufficient sample present in 240 specimens to put the specimen in tissue culture. Of these panel-negative, 17 (7%) were positive for a respiratory virus in tissue culture including 3 with RSV, 2 with parainfluenza virus type 2, 2 with parainfluenza virus type 3, 2 with adenovirus, and 8 with influenza virus A. Thus, the majority of these panel-negative but tissue culture-positive specimens were influenza virus A.

Fourteen (6%) of the panel-negative specimens grew a non-respiratory virus in tissue culture. Enterovirus grew in eight samples, herpes simplex virus type 1 grew in three samples, and cytomegalovirus grew in three samples.

The efficiency with which the respiratory virus panel could detect individual viruses is shown in Table 1. Specificity was excellent for all viruses. Sensitivity was very good for RSV and parainfluenza viruses, reasonably good for adenovirus, and less good for influenza viruses. This same low detection rate for influenza virus by IFA directly on the sample has been reported by others (8, 9, 10, 11). The sensitivity for influenza virus detection can be increased by enhancement by centrifugation (2), suggesting that viral titers may be too low to be picked up directly on nasopharyngeal aspirate specimens.

Overall, the sensitivity of the respiratory panel for the detection of a respiratory virus in a clinical specimen was 79% (95% confidence interval [CI], 0.69 to 0.87). When combined with specific viral immunofluorescence, the specificity was 99% (95% CI, 0.97 to 1.0). The positive predictive value of the panel was 98% (95% CI, 0.92 to 1), and the negative predictive value was 93% (95% CI, 0.89 to 0.96).

Stout et al. (10) also used this monoclonal antibody panel directly on clinical specimens as an initial screen and reported a sensitivity of 69% and a specificity of 97%. Mathay et al. (6) reported some difficulty in interpretation of the panel because of nonspecific fluorescence when used directly on clinical specimens. We did not find this to be a problem in our study.

The monoclonal antibody pool has also been shown to be useful for the detection of respiratory viruses in shell vials and has been proven to be a useful adjunct for detection of viral antigens in cell culture (6, 7).

The monoclonal antibody pool allowed us to make a virologic diagnosis in 90% of the respiratory virus-containing specimens within hours of receipt of the specimen in the laboratory, representing a significant time saving over conventional culture or shell vial techniques. Also, because the presence of a respiratory virus in a sample could be reliably predicted by a pool immunofluorescence, it precluded the necessity of performing multiple immunofluorescence assays on samples that did not contain a respiratory virus. This translated to considerable cost and time savings for the clinical laboratory.

It would be too costly to use the panel for all respiratory specimens throughout the year. In peak RSV seasons, RSV immunofluorescence should be the first test performed on clinical specimens. However, in our hands, the panel was very useful as an initial screen in nonpeak RSV seasons and allowed us to provide the treating physician with useful information quickly without the cost of performing individual immunofluorescences for all viruses in the samples received.

REFERENCES