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Comparison of Virus Culturing and Immunofluorescence for Rapid Detection of Respiratory Syncytial Virus in Nasopharyngeal Secretions: Sensitivity and Specificity

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I compared the fluorescent antibody test for respiratory syncytial virus in nasal secretions to virus cultures (N = 310). The sensitivity, specificity, and predictive value (+) were 95.1%, 86.5%, and 88.5%, respectively.

An indirect fluorescent antibody test (FAT) for the rapid diagnosis of respiratory syncytial (RS) virus in respiratory secretions was described by McQuillin and Gardner in 1968 (11). Gardner and others have reported a high degree of accuracy with the FAT, but the reports have generally come from workers actively engaged in RS virus research, not from clinical laboratories, and not all investigators have had such success (1, 3, 7, 10, 12).

During a large epidemic of RS virus infection, I had the opportunity to evaluate the accuracy of the FAT as a routine diagnostic test compared with virus cultures. The test was reliable in this laboratory.

The specimens were from infants and young children with acute bronchiolitis or pneumonia admitted to Denver-area hospitals during a 4-month period in the winter and spring of 1981. The FATs were ordered solely for diagnostic purposes by the children's physicians. Consequently, children tended to be selected who were either very young or severely ill, and often they were late in their illness.

Nasopharyngeal secretions were collected by the child's nurse or physician by manual aspiration, using a small catheter and saline irrigation (3). Early in the epidemic, some specimens were collected by the bulb suction technique described by Hall and Douglas (5). I subsequently recommended collection by catheter suction, which provided more respiratory epithelial cells. Specimens were transported to the laboratory on ice. Swabs of the nasopharynx or throat were not accepted for the FAT.

HEp-2 cells shown to be sensitive to RS virus (Flow Laboratories, Bethesda, Md.) were inoculated with nasopharyngeal secretions and were examined for the typical cytopathic effect of RS virus (9).

Nasopharyngeal secretions were washed once in 2 to 4 ml of phosphate-buffered saline (PBS), pH 7.4, and centrifuged at 1,500 rpm for 10 min; the supernatant was removed, and the pellet was resuspended in approximately 0.5 ml of PBS. Slides were spotted with approximately 10 μl, air dried, and fixed in acetone at 4°C for 10 min (3, 9).

The procedure was an indirect FAT that employed rabbit antiserum to RS virus produced in our laboratory and fluorescein-conjugated antibody to rabbit globulin (Meloy, Springfield, Va.). The antiserum was prepared by immunizing New Zealand white rabbits with the Long strain of RS virus grown in HEp-2 cells. The rabbits were inoculated intramuscularly at four sites with RS virus in complete Freund adjuvant and also intravenously and intraperitoneally with undiluted virus. They were reimmunized intravenously and intraperitoneally on days 7 and 14 and bled on day 21 (3). The antiserum was absorbed twice to remove nonspecific fluorescence by mixing equal volumes of antiserum and packed HEp-2 cells for 1.5 h at 37°C and then 48 h at 4°C and then centrifuging at 10,000 rpm for 0.5 h. Antiserum was titrated by the FAT and then frozen at −20°C in 1- to 2-ml portions. The counterstain was 0.1% amido black. HEp-2 cells infected with RS virus and uninfected cells served as positive and negative controls, respectively.

Duplicate smears were screened by the acridine orange technique before being examined by the FAT to assure that a sufficient number of respiratory epithelial cells, at least one per field, was present for satisfactory examination (8). FATs were examined by a single technologist who, although familiar with fluorescence microscopy, had no previous experience with the FAT. I used a Zeiss microscope equipped with a mercury lamp for incident light fluorescence and the following filters: BP 450-490, FT 510, and LP 520. Slides were read at ×400 magnification with an oil immersion objective. A slide was consid-

TABLE 1. Comparison of FAT and virus culture for detection of respiratory syncytial virus in nasal secretions from 310 children

Results of FAT[a]	Results of virus cultures (no. of isolates)		
	Positive	Negative	Total
Positive	154	20	174
Negative	8	128	136
Total	162	148	310

[a] Sensitivity, 95.1%; specificity, 86.5%; predictive value (+), 88.5%.

ered positive if ≥2 respiratory epithelial cells were seen that fluoresced with 2+ to 4+ intensity in the cytoplasm.

The sensitivity, specificity, and predictive value of the FAT were calculated by standard formulas (6).

A total of 399 nasal wash specimens were submitted. Fifty-eight tissue cultures were destroyed by bacterial overgrowth, and 25 specimens contained insufficient numbers of respiratory epithelial cells to perform the FAT, leaving 310 split samples that could be analyzed (Table 1). A total of 174 FATs were positive; the sensitivity and specificity were 95.1% and 86.5%, respectively.

Twenty specimens were positive by the FAT but negative by culture and were judged to be false-positives. It is likely that at least some of these 20 false-positive FATs were correct, and the isolation attempts were falsely negative. Gardner et al. have shown that late in RS virus infection the FAT may remain positive, whereas infectious virus cannot be recovered (4).

Gardner and co-workers, who have written extensively about the FAT, have reported a sensitivity similar to our results of 92% to 98% (3, 10). By using essentially the same methods, others have reported a lower sensitivity; 90% (10), 76% to 87% (1), 65% (7), and as low as 45% (12). Differences in reagents, specimen collection, slide interpretation, or other technical problems presumably account for these varying results.

The enzyme-linked immunosorbent assay (ELISA), inhibition of ELISA, and the immunoperoxidase method for the detection of RS virus antigen also have been studied (1, 2, 7); none of these methods has been shown to be significantly more sensitive than the FAT.

I conclude that the FAT for RS virus is a highly sensitive and specific test, if performed correctly, and that it can be done reliably in a clinical laboratory. I attribute the success with the FAT to the availability of high-quality antiserum to RS virus, to the rapidly acquired skill in slide interpretation of the microscopist, and to the requirement that only fresh nasal washes with adequate numbers of respiratory epithelial cells were acceptable for testing. To assure good quality control, it is important that sufficient numbers of positive and negative specimens are processed to maintain the skill of the microscopist and that viral cultures are available to periodically compare results.

The FAT for RS virus is now offered routinely through the Diagnostic Virology Laboratory as soon as the presence of RS virus in the community is detected by culture. The test is withdrawn when RS virus activity ceases.

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