

Comparison of Direct and Indirect Immunofluorescence Staining of Clinical Specimens for Detection of Respiratory Syncytial Virus Antigen

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Immunofluorescence staining methods for respiratory syncytial virus antigen detection were compared. Of 50 specimens originally positive for respiratory syncytial virus by direct immunofluorescence and culture, 49 were positive by repeat direct immunofluorescence and 32 were positive by indirect immunofluorescence. Additional results obtained on specimens originally negative for respiratory syncytial virus by direct immunofluorescence, culture, or both indicate that direct immunofluorescence staining for respiratory syncytial virus antigen was more sensitive than was indirect immunofluorescence.

Detection of respiratory syncytial virus (RSV) antigen in clinical samples has been described for the direct (DFA) and indirect (IFA) immunofluorescence staining methods (1, 2, 4-6). The purpose of this study was to evaluate the sensitivity and specificity of DFA and IFA procedures for RSV antigen detection in epithelial cells obtained from nasopharyngeal-throat swabs. The results were compared with those previously obtained by DFA and culture at the time specimens were originally submitted to our laboratory.

Fluorescein isothiocyanate-conjugated rabbit antisera to RSV (Flow Laboratories Inc.) were diluted 1:10 in phosphate-buffered saline (6). The lot in the original study was R830002F, and lot 45637005 was used in the repeat study. Bovine anti-RSV serum lot K5730 (Burroughs Wellcome Co.) was used at the dilution received, and fluorescein isothiocyanate-conjugated rabbit anti-bovine serum lot K6649 was diluted 1:10 in phosphate-buffered saline as recommended by the supplier (Burroughs Wellcome Co.). The optimal dilutions were determined as previously described on RSV-infected cell cultures and uninfected control cells (6). The antisera were also evaluated for cross-reactivity to parainfluenza virus types 1, 2, and 3, influenza A and B viruses, adenovirus, and mumps virus in infected cell cultures. No cross-reactions were detected. Antisera were dispensed in working volumes and stored at -70°C until used.

A bank of slides containing nasopharyngeal-throat epithelial cells from 80 patients with syndromes clinically compatible with RSV infections was selected. These represented a portion

of a larger group which was previously reported in a comparative study of DFA versus diagnosis by virus isolation (6). A total of 50 patients were originally culture- and DFA-positive for RSV, 10 were DFA-positive and culture-negative, and 20 were DFA-negative and culture-negative.

Specimens were collected and processed as previously described (6). Briefly, nasopharyngeal-throat swabs were obtained and placed in viral transport media. Upon receipt by the laboratory, specimens were vigorously blended in a Vortex mixer, and the swabs were removed. We then added 1 ml of additional antibiotics in Hanks balanced salt solution to the specimens, which were then centrifuged at 3,500 rpm for 20 to 30 min. A 0.2-ml sample of supernatant was inoculated onto duplicate tube cultures of primary cynomolgus monkey kidney, HL (a heteroploid cell line), and human embryonic tonsil diploid fibroblasts. Cultures were observed daily for cytopathic effects.

Sediment from centrifuged clinical specimens was suspended in just enough phosphate-buffered saline to yield a slightly turbid suspension and spotted onto slides as previously described (2, 3, 6), allowed to air dry, and fixed in cold acetone (-20°C) for 10 min. Slides were stored at -20°C until the time of study.

Slides were removed from the freezer and warmed to ambient temperature. These were labeled with patient numbers only, and no indication was made as to previous results. Each slide was divided, and one-half of each was overlaid with bovine anti-RSV serum. Slides were incubated for 30 to 60 min at 35°C in humidified chambers and then washed twice for 5 min in phosphate-buffered saline. The side of

TABLE 1. Specimens positive by DFA and IFA after repeat studies vs. original results

Original results ^a	No. of specimens	Repeat study	
		DFA+	IFA+
Culture+, DFA+	50	49	32
Culture-, DFA+	10	10	6
Culture-, DFA-	20	1	0

^a +, Positive; -, negative.

the slide to which bovine anti-RSV had been applied was then overlaid with fluorescein isothiocyanate-conjugated goat anti-bovine serum. The other half of each slide was overlaid with fluorescein isothiocyanate-conjugated rabbit anti-RSV serum (6), incubated and washed as above, counterstained with 1% Evans blue in saline, and mounted with buffered glycerol (pH 8.0) and a cover slip. Slides were read by one observer who had no knowledge of either the randomized assignment of slides for DFA or IFA methods or of previous results of fluorescence staining or culture methods. Observation of stained slides was done on a Zeiss halogen-source fluorescence microscope.

The results are summarized in Table 1. Of the 50 specimens originally culture- and DFA-positive for RSV antigen, 49 were positive by the DFA method and 32 were positive by the IFA method. All 10 of the originally DFA-positive, culture-negative specimens were positive by DFA on repeat testing, but only 6 were positive by IFA. Of the 20 specimens originally reported as negative by both DFA and culture, 1 was

positive by DFA on repeat study, whereas none were positive by IFA.

Granular fluorescence within the cytoplasm was required before a specimen was determined to be positive for RSV antigen. Cellular morphology was more easily seen in the wells stained with the direct conjugate. The direct conjugate also showed fluorescence with yeast cells in clinical specimens, but this did not create a problem because of the criteria established. This did not occur with the IFA system, but more low-level homogeneous, nonspecific staining of epithelial cells was observed. The data presented here suggest that direct immunofluorescence staining of clinical samples for RSV antigen may be considerably more sensitive than IFA.

LITERATURE CITED

1. D'Alessio, D., S. Williams, and E. Dick. 1970. Rapid detection and identification of respiratory viruses by direct immunofluorescence. *Appl. Microbiol.* 20:233-239.
2. Fulton, R. E., and P. J. Middleton. 1974. Comparison of immunofluorescence and isolation techniques in the diagnoses of respiratory viral infections of children. *Infect. Immun.* 10:92-101.
3. Goldman, M. 1968. Fluorescent antibody methods, p. 148-149. Academic Press, Inc., New York.
4. Knight, V., F. Brasier, S. Greenberg, and D. Jones. 1975. Immunofluorescent diagnosis of acute viral infections. *South. Med. J.* 68:764-766.
5. McQuillin, J., and P. S. Gardner. 1968. Rapid diagnosis of respiratory syncytial virus infection by immunofluorescent antibody techniques. *Br. Med. J.* 1:602-605.
6. Minnich, L., and C. G. Ray. 1980. Comparison of direct immunofluorescent staining of clinical specimens for respiratory virus antigens with conventional isolation techniques. *J. Clin. Microbiol.* 12:391-394.