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Evaluation of a Commercially Available Direct Immunofluorescent Staining Reagent for the Detection of Respiratory Syncytial Virus in Respiratory Secretions

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A commercially-available direct immunofluorescence (IF) reagent (Imagen; Boots-Celltech, Slough, Berkshire, United Kingdom) was similar in sensitivity and specificity to the conventional indirect IF test for the detection of respiratory syncytial virus in respiratory secretions. Both IF tests were more sensitive than culture, particularly for specimens transported from outside the institution.

Rapid diagnosis of respiratory syncytial virus (RSV) infection is an important clinical service because of the need to control the potential spread of this infection in a hospital. The availability of effective therapy in the near future is likely to increase the demand for this service. Methods for the detection of viral antigens in respiratory secretions by immunofluorescence (IF) are now widely accepted and have excellent sensitivity and specificity as compared with tissue culture isolation. The major disadvantage of IF methods is the time consumed in preparing the slides to read. We were thus interested in comparing a commercially available reagent (Imagen; Boots-Celltech, Slough, Berkshire, United Kingdom), one which involves the direct IF technique and is thus rapidly completed, with the traditional indirect method.

Respiratory secretions submitted to the Children's Hospital Diagnostic Virology Laboratory in January and February 1985 formed the materials for study. A portion of each secretion was inoculated onto HEp-2, human diploid lung, and primary rhesus monkey kidney monolayers for virus isolation. The cells from another portion were washed by several cycles of centrifugation at 300 × *g* and dropped onto 13-mm wells on Teflon-coated slides as previously described (4). After air drying, the slides were fixed for 10 min with cold acetone, and duplicate slides were stained by the direct and indirect methods.

The Imagen reagent consists of a pool of monoclonal antibodies directed against the fusion and nucleoproteins of RSV; the antibodies are conjugated to fluorescein isothiocyanate and provided at a working dilution with Evans blue counterstain. The preparation and selection of monoclonal antibodies for this reagent have been previously described (2). As recommended by the manufacturer, a single 15-min incubation in a moist chamber at 37°C was followed by a 5-min rinse in phosphate-buffered saline and air drying. Our standard indirect IF procedure, as previously described (4), requires two 30-min incubations (each followed by three 10-min washes), a distilled water rinse, and air drying. Bovine anti-RSV (Wellcome Diagnostics, Research Triangle Park, N.C.) is the first antibody, and fluorescein isothiocyanate-conjugated goat anti-bovine immunoglobulin G (IgG; Kierkegaard and Perry, Gaithersburg, Md.) is the second. Both were used in predetermined optimal working dilutions, and the second antibody contained 0.1% amido black.

Slides were examined under ×400 magnification with a Zeiss epifluorescence microscope and scored for the number of cells, the percentage of cells fluorescing, and the intensity of the fluorescence. The presence of even one cell with characteristic fluorescence was considered to constitute a positive result; preparations with less than 200 cells on the slide and no specific cytoplasmic fluorescence were reported as inadequate for diagnosis and omitted from this study. The percentage of positive cells was graded <1+ when one to five cells fluoresced, 1+ when more than five cells but less than 25% of the cells fluoresced, 2+ for 25 to 50% fluorescent cells, 3+ for 50 to 75% fluorescent cells, and 4+ for >75% fluorescent cells. Slides prepared by the two methods were read independently, but a second reader reviewed all discrepancies. Ninety-seven specimens, all obtained from children with acute respiratory illness, were suitable for analysis. Sensitivity and specificity were defined as the percentage of culture-positive specimens positive by IF and the percentage of culture-negative specimens negative by IF, respectively (3).

Overall agreement between the two IF methods was 94% (Table 1), with the six discrepant results from specimens containing very few antigen-bearing cells. Correlation with culture was not as good (Table 2), with a sensitivity of 94% and a specificity of 69% for the direct method and a sensitivity of 97% and a specificity of 74% for the indirect method. Because in previous years the specificity of the indirect IF test with respect to culture has been 93 to 96% in this laboratory, we examined possible reasons for this change. One consideration was that the time in transport for specimens received from institutions other than Children's Hospital may have contributed to the low yield on culture. Of the

TABLE 1. Agreement between IF tests

Indirect test result	No. of specimens producing the following direct test result:	
	Positive	Negative
Positive	47	2
Negative	4	44

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TABLE 2. Sensitivity and specificity of IF tests compared with culture

Specimen group and test	No. of specimens giving the following result:		Specificity (%)	Sensitivity (%)
	Positive	Negative		
All specimens				
Culture	33	64		
Direct IF	31	44	69	94
Indirect IF	32	47	74	97
Childrens Hospital specimens only				
Culture	14	30		
Direct IF	12	24	80	86
Indirect IF	13	26	87	93

44 specimens from Children's Hospital, 6 were antigen positive by the direct IF test and negative on culture, compared with 14 of 63 of the non-Children's Hospital specimens (chi-square = 2.4; $P = 0.10$). For the indirect IF, 4 of 44 Children's Hospital specimens and 13 of 63 specimens from other institutions were positive by the indirect IF test and negative on culture (chi-square = 3.96; $P < 0.05$). It is also possible that the culture technique was less sensitive in 1985 than in the past, because of variability in the HEp-2 cells or other unknown factors.

Strain differences among RSV isolates were examined by direct and indirect IF with monoclonal antibodies against Long and 18537 strains (1). Eighteen strains were similar to Long, 11 strains were similar to 18537, and 5 strains could not be typed. All strains were detected equally well by the two IF methods under evaluation; one nontypable strain was missed by the direct IF, and one 18537-like strain was missed by both methods. In addition, viruses other than RSV were isolated from 15 specimens. Eight specimens yielded influenza A; one was positive for RSV by the direct IF test only. Three specimens containing adenovirus, one containing both adenovirus and parainfluenza 3, and one containing rhinovirus gave negative results in both IF tests. Single specimens yielding cytomegalovirus and enterovirus but not RSV on culture were positive by both IF tests. It is impossible to be sure whether these represent false-positive results or dual infections.

The direct IF test with Imagen reagent was quicker to perform than the indirect IF test, easy to read, and similar to the indirect test in sensitivity and specificity. RSV was detected in more specimens by both IF methods than by culture techniques. We believe that these are true-positive results because of the concordance between the two IF tests despite the use of different antibodies and because of the correlation of culture negativity with specimen sources from outside Children's Hospital. Our experience in a clinical diagnostic setting confirms the results reported by the developers of this reagent (2).

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