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Efficiency of Immunofluorescence for Rapid Detection of Common Respiratory Viruses

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Rapid immunofluorescence (FA) methods for the detection of common respiratory viruses were compared with culture results over a 3-year period to assess the relative efficiency of FA in a clinical laboratory setting. For respiratory syncytial virus, efficiencies were high (sensitivity, 90 to 95%; specificity, 92 to 95%). The sensitivity of FA for detection of parainfluenza virus type 1, parainfluenza virus type 3, influenza A virus, and adenoviruses ranged from 28 to 63%, but specificities for these viruses were uniformly 98 to 100%. The observations form a basis for consideration of selective reduction of routine culture procedures for specimens with initial positive rapid FA results; however, the possibility of dual viral infection in some situations must also be considered.

The need for rapid, efficient methods of viral detection has grown in importance, primarily for two reasons: (i) the increasing availability of specific antiviral agents, all of which are most effective if initiated early in the course of infection; and (ii) economic pressures which mandate efforts to effect cost savings without compromising patient care.

In this study, we analyzed the efficiency of rapid immunofluorescence (FA) diagnosis of common respiratory viruses during 3 years of experience in a clinical laboratory setting. The results indicate the relative strengths and weaknesses of FA methods as applied to different viruses and can serve as a basis for future selective use of specific diagnostic approaches.

MATERIALS AND METHODS

Specimens. The period of study was from 1 January 1983 through 31 December 1985. Pooled nasopharyngeal and throat swab specimens were obtained from all patients who were referred for diagnostic studies and whose primary complaint was respiratory illness. The specimens analyzed in this report include only those in which the requesting physician also ordered rapid FA studies for one or more respiratory viral agents or in which the referral diagnosis was compatible with a specific etiology, including respiratory syncytial virus (RSV), influenza A (Flu A), influenza B (Flu B), parainfluenza virus type 1 (Para 1), parainfluenza virus type 3 (Para 3), or adenovirus. The selection of which antigens would be sought by FA testing in each instance was based on clinical and epidemiologic data available to the laboratory.

The methods of specimen collection and processing for culture as well as the preparation of cells eluted from nasopharyngeal-throat swab specimens were those previously described (5, 6).

Conjugated antisera. All conjugated antisera were labeled with fluorescein isothiocyanate.

The RSV directly conjugated reagents and sources used in 1983 and 1984 were obtained from Flow Laboratories, Inc. (McLean, Va.). Indirect reagents for RSV detection were used throughout 1985. The primary mouse antiserum was supplied by Burroughs Wellcome Co. (Research Triangle Park, N.C.), and the conjugated goat anti-mouse sera were provided by Cooper Biomedical, Inc. (West Chester, Pa.).

Indirect detection methods were used for Para 1, Para 3, adenoviruses, Flu A, and Flu B. Chicken antisera (Burroughs Wellcome Co.) and conjugated goat anti-chicken sera (Cooper Biomedical) were used. In 1985, pooled mouse monoclonal antibodies (kindly provided by Alan Kendal, Centers for Disease Control, Atlanta, Ga.) were used in an indirect assay for Flu A and Flu B antigens (4), utilizing conjugated goat anti-mouse sera (Cooper Biomedical).

The standardization of reagents, methods of indirect and direct FA, and criteria for positivity were those described previously (5, 6), except that incubation times were increased from a minimum of 30 to 60 min to 45 to 60 min for the primary antisera, and a mercury-vapor light source was used instead of a halogen source.

Data analysis. The relative efficiencies of FA were determined by using culture positivity as the arbitrary standard. Those sample pairs which were both culture positive and FA positive were considered true positives (TP). Culture-negative and FA-positive pairs were called false-positives (FP); conversely, culture-positive and FA-negative pairs were false-negatives (FN). Specimen pairs which were negative both by FA and culture made up the true negative (TN) category. The formulas applied were as follows: sensitivity = (TP/TP + FN) × 100; specificity = (TN/TN + FP) × 100; positive predictive value = (TP/TP + FP) × 100; and negative predictive value = (TN/TN + FN) × 100.

RESULTS

During the 3 years, 54 (10%) culture-positive and 38 (2%) culture-negative specimens did not yield adequate numbers of cells for FA testing. These were excluded from further analysis.

Table 1 summarizes the comparative FA and culture data for the remaining samples. The analysis of RSV data has been divided into two periods (1983 to 1984 and 1985) to compare the two different methods utilized.

The overall efficiencies of FA with respect to specific viruses are summarized in Table 2. Since only two Flu B infections were detected (both by culture and FA), no conclusions could be made with respect to FA efficiency for this virus. All 1985 specimens tested by mouse monoclonal l antibodies for Flu A did show a trend toward significant

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TABLE 1. Relationship between FA and culture results

Virus	No. that were:			
	Culture+, FA+	Culture+, FA−	Culture−, FA+	Culture− FA−
RSV (1983–1984)[a]	243	26	37	398
RSV (1985)[a]	125	7	17	299
Para 1	19	11	4	186
Para 3	5	11	0	390
Flu A	10	13	0	210
Flu B	2	0	0	74
Adenovirus	5	13	0	262

[a] Different FA methods and reagents were used for RSV in each period (see text).

improvement by switching from polyclonal antibodies, although only 32 FA and culture pairs were examined (data not shown separately in the tables). The only Flu A serotype identified in 1985 was H3N2; eight pairs were FA positive and culture positive, two were FA negative and culture positive, and none were FA positive and culture negative.

Heterologous viruses were simultaneously detected in specimens which were FA positive. Among the 368 specimens found to be both FA positive and culture positive for RSV, 35 (9.5%) also yielded heterologous viruses on culture. These included eight adenoviruses, six cytomegaloviruses, six enteroviruses, five Para 3, four Flu A, three rhinoviruses, two parainfluenza type 2, and one Flu B virus. There was one specimen which was FA positive and culture positive for both Flu A (H1N1) and RSV, and another similarly FA positive and culture positive for both RSV and adenovirus. Of the 54 FA-positive, culture-negative specimens for RSV, 10 yielded other viruses on culture, including six enteroviruses, two herpes simplex viruses, and one each of parainfluenza type 2 and adenovirus.

DISCUSSION

If a laboratory is equipped with an adequate fluorescence microscope, the use of FA for rapid diagnosis is often convenient, time saving, and potentially cost saving. The data presented here and elsewhere support its routine use as a possible alternative to culture for RSV. The specificity for certain other respiratory viruses such as Para 1 and Para 3 (9), Flu A and Flu B (4), and adenoviruses appears sufficiently great to allow a confident presumptive diagnosis by FA and consideration of a decision not to proceed with expensive routine culture in many situations. Clearly, sensitivity for viruses other than RSV could be improved. The increasing availability of monoclonal antibodies should help in this regard for some, if not all, of the common agents. In fact, even greater improvement in RSV efficiency can be expected with the use of monoclonal instead of polyclonal antibodies. Recent experience reported by others (3) and our own observations (unpublished data) suggest that not only is efficiency enhanced, but the specific localization of antibody is more easily discerned visually with pooled, RSV-specific monoclonal antibodies. It is also probable that these newer reagents will be more sensitive than the usual culture methods.

TABLE 2. Efficiency, FA versus culture

Virus	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
RSV				
1983–1984	90	92	87	94
1985	95	95	88	98
Others (all years)				
Para 1	63	98	83	94
Para 3	31	100	100	97
Flu A	43	100	100	94
Adenovirus	28	100	100	95

In this present study, we were unable to demonstrate any significant difference in results between the use of direct or indirect FA methods with respect to RSV detection. This observation is in contrast to our previous report (6) suggesting that the quality of reagents and methodology are of primary importance in enhancing efficiency.

We do not believe that these data suggest that methods other than FA for rapid respiratory viral diagnosis should be ignored. Approaches such as enzyme-linked immunosorbent assays (ELISA) (1), radioimmunoassays (7), and molecular probes (2) should all be considered, and their relative costs, convenience, and efficiencies should be analyzed carefully. On the basis of our observations, we would anticipate that one or more of these latter methods might be more efficient than FA for detection of adenoviruses in respiratory samples (2, 7) and perhaps some other viruses as well.

With respect to cost and convenience of FA, we believe there are distinct advantages for its selective use to avert routine cultures in many cases. Using a workunit system to estimate minutes of technologist time required for specific tests, we found that the usual hands-on time required for an individual FA, including collection, recording, and reporting, is 18.3 min. This compares with routine cell culture methods, including cell preparation, hemadsorption, medium changes, and serotyping, which average 82.1 min per specimen. Thus, a positive FA result, particularly with the high levels of specificity observed for the agents studied here, can often reduce or eliminate the need for routine culture, saving nearly 64 min per specimen. This time savings is particularly apparent during periods of high respiratory viral transmission in the community, when overall diagnostic rates can exceed 40%. For example, between 15 October 1985 and 1 March 1986, we found that 164 of 174 culture-positive specimens were also positive by FA. If the culture had been eliminated from the workup in each of these 164 specimens, approximately 174 h of technologist time could have been saved.

Reagent costs for FA currently range between $1.70 and $2.30 per test, including conjugate, slide, and rinse. This compares favorably with ELISA costs, in which many systems are designed for batch testing. Even with breakaway plates or single-bead options, ELISA testing requires three or more controls per run, which can be costly if only one or a few specimens are tested at a time. On the other hand, each FA reagent lot can be quality controlled before being put into use, and daily repeat control testing is not routinely required. Finally, the total time of testing, including incubations, is 45 min for direct FA and 90 min for indirect FA. With ELISA procedures, the times usually range between 2 and 3 h.

There is one potential pitfall to consider if a decision is made not to proceed with cultures based on rapid detection results. We found that 9.5% of our FA-positive and culture-positive (TP) specimens for RSV yielded other viral agents on culture. Similar observations have been reported by Waner et al. (8). We agree with their suggestion that selective culturing or inclusion of a broader routine battery of

rapid antigen detection tests or both are options that should be considered, at least in some clinical circumstances.

LITERATURE CITED

1. **Hendry, R. M., L. T. Pierik, and K. McIntosh.** 1986. Comparison of washed nasopharyngeal cells and whole nasal secretions for detection of respiratory syncytial virus antigen by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **23**:383–384.
2. **Hyypiä, T.** 1985. Detection of adenovirus in nasopharyngeal specimens by radioactive and nonradioactive DNA probes. J. Clin. Microbiol. **21**:730–733.
3. **Kao, C. L., K. McIntosh, B. Fernie, A. Talis, L. Pierik, and L. Anderson.** 1984. Monoclonal antibodies for the rapid diagnosis of respiratory syncytial virus by immunofluorescence. Diagn. Microbiol. Infect. Dis. **2**:199–206.
4. **McQuillian, J., C. R. Madeley, and A. P. Kendal.** 1985. Monoclonal antibodies for the rapid diagnosis of influenza A and B virus infections by immunofluorescence. Lancet **ii**:911–914.
5. **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.
6. **Minnich, L. L., and C. G. Ray.** 1982. Comparison of direct and indirect immunofluorescence staining of clinical specimens for detection of respiratory syncytial virus antigen. J. Clin. Microbiol. **15**:969–970.
7. **Vesikari, T., A.-L. Kuusela, H. K. Sarkkinen, and P. E. Halonen.** 1982. Clinical evaluation of radioimmunoassay of nasopharyngeal secretions and serology for diagnosis of viral infections in children hospitalized for respiratory infections. Pediatr. Infect. Dis. **1**:391–394.
8. **Waner, J. L., N. J. Whitehurst, S. Jonas, L. Wall, and H. Shalaby.** 1986. Isolation of viruses from specimens submitted for direct immunofluorescence test for respiratory syncytial virus. J. Pediatr. **108**:249–250.
9. **Wong, D. T., R. T. Welliver, K. R. Riddlesberger, M. S. Sun, and P. L. Ogra.** 1982. Rapid diagnosis of parainfluenza virus infection in children. J. Clin. Microbiol. **16**:164–167.