JOURNAL OF CLINICAL MICROBIOLOGY, July 1998, p. 2112–2114 Vol. 36, No. 7
0095-1137/98/$04.00+0

Use of Fluorescent-Antibody Staining of Cytocentrifuge-Prepared Smears in Combination with Cell Culture for Direct Detection of Respiratory Viruses

KIRK M. DOING,[1,2]* MARY ANN JERKOFSKY,[1] ELAINE G. DOW,[2] AND JO ANN JELLISON[2]

Department of Microbiology, Biochemistry and Molecular Biology, University of Maine, Orono, Maine 04469,[1] and Affiliated Laboratory, Inc., Bangor, Maine 04401[2]

Received 9 December 1997/Returned for modification 2 February 1998/Accepted 30 March 1998

Over a 3-year period, 1,003 respiratory samples were collected and examined for selected respiratory viruses with cytocentrifuged prepared smears stained with fluorescently labeled antibodies (IFA) in conjunction with cell culture. IFA results were compared with results obtained by cell culture. Viruses were isolated or detected by direct means in 401 samples. Agreement between culture and IFA was 90%.

Cell culture (CC) is still the only comprehensive method readily available to detect the wide range of viruses that may cause respiratory tract infection. Enzyme immunoassays (EIAs) in various testing formats are available commercially and allow for direct detection of selected viral agents. Most of these assays require limited technical expertise and have been reported, overall, to have reasonable sensitivity and specificity (2, 10, 11). However, most studies have concluded that these test kits should be used in conjunction with, not replace, cell culture (1, 4, 14). In addition, most EIAs are costly, detect only a single viral agent, and do not allow assessment of sample quality (3).

While fluorescent-antibody staining has been used effectively for direct detection of respiratory viruses (RV), problems have been reported, including the need for highly trained staff to prepare and read the smears, inconsistencies and poor reproducibility in preparing smears, and difficulty in consistently obtaining high-quality samples containing an adequate amount of material to examine with little or no mucus contamination (3, 4, 7, 9, 11–13). Here, we report the use of standardized collection procedures coupled with cytocentrifuge-prepared smears for the direct detection of RV.

The laboratory assembled and distributed collection kits containing all necessary supplies and instructions. The requested specimen included a nasopharyngeal (NP) swab and a throat swab, placed into a single 15.0-ml conical tube containing 3.0-mm glass beads and 2.5 ml of viral transport medium (minimal essential medium with 5% fetal calf serum and antibiotics). On occasion, either a single throat swab or a NP swab was submitted. Specimens were transported on wet ice or at refrigeration temperature. When received in the laboratory, samples were held at 2 to 8°C and processed within 24 h.

Each sample was mixed by vortexing for 5 to 10 s; excess fluid was expressed from the swabs, and they were discarded. Conventional CC tubes containing MRC-5, primary rhesus monkey kidney (PMK), or HEp-2 cells (Bartels Inc., Issaquah, Wash., and Viromed Laboratories Inc., Minneapolis, Minn.) were inoculated with 0.2 ml of sample by standard procedures (5). All CC tubes were incubated at 33 to 35°C on a roller drum and examined regularly for cytopathic effect (CPE) over 10 days. Blind hemadsorptions were performed on PMK tubes after 3 days of incubation and again between days 7 and 10 if tubes were still negative for CPE (8). If CPE was noted or if a hemadsorption test was positive, cells were scraped and indirect fluorescent-antibody (IFA) staining was completed to identify the virus.

The remainder of the patient sample (approximately 1.5 ml) was transferred to a second 15.0-ml polypropylene tube and centrifuged at 700 × *g* for 10 min. The supernatant was removed, and cells were resuspended with gentle aspiration in 2 to 4 ml of sterile phosphate-buffered saline, followed by a second 5-min centrifugation. The supernatant was discarded, and the cell pellet was resuspended in 1.0 ml of phosphate-buffered saline. Five cytospin slides were prepared by placing 4 to 6 drops from a Pasteur pipette of the cell suspensions into a reusable cytofunnel. Cytocentrifugation was performed at 2,000 rpm for 5 min (Cytospin 3, Shandon Inc., Pittsburgh, Pa.). The slides were air dried and chemically fixed in cold acetone for 10 min. Cytofunnels were disinfected in a solution containing 10% bleach, rinsed in water, air dried, and reused.

Separate cytospin smears were stained for influenza A virus, influenza B virus, respiratory syncytial virus (RSV), parainfluenza virus type 3, and adenovirus. In all staining procedures, commercially prepared monoclonal antibodies were used (Bartels Inc.). The manufacturer's instructions as outlined in the package insert were followed, with the following modifications. Due to the small but well-defined area containing cells on each cytospin smear, only 15 μl of antibody or conjugate was needed to cover the cell spot completely. In addition, cytocentrifugation achieves a monolayer of cells and, based on staining pro-

TABLE 1. Number and types of viruses recovered

Virus	No. isolated
Influenza A virus	108
Influenza B virus	28
Parainfluenza virus type 1	2
Parainfluenza virus type 2	5
Parainfluenza virus type 3	6
RSV	206
Adenovirus	14
Herpes simplex virus	16
Cytomegalovirus	3
Rhinovirus	7
Enterovirus, NOS	6
Total no.	401

* Corresponding author. Mailing address: Affiliated Laboratory, Inc., 925 Union Street, Suite 4, Bangor, ME 04401. Phone: (207) 973-6900. Fax: (207) 947-2838. E-mail: doing@maine.maine.edu.

TABLE 2. Statistical parameters of IFA direct exam compared with CC

Virus	No. of specimens[a]:			Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	% Agreement
	IFA positive and CC positive	IFA positive and CC negative	IFA negative and CC positive					
Influenza A virus	65	11	21	80.4	98.7	85.5	97.6	96.6
Influenza B virus	16	0	12	57.1	100.0	100.0	98.8	98.7
Parainfluenza 3 virus	4	0	2	66.7	100.0	100.0	99.8	99.8
RSV	118	16	26	81.9	98.0	88.1	96.8	95.6
Adenovirus	5	0	4	55.6	100.0	100.0	99.6	99.6
Summary for all viruses	208	27	65	76.2	96.0	88.5	90.9	90.3

[a] A total of 946 specimens met criteria for direct IFA examination.

cedures previously verified in our laboratory (data not shown), incubation times for staining were shortened to 15 min without compromising results.

Slides were examined at ×100 and ×400 magnification under a Leitz Laborlux 11 fluorescent microscope fitted with appropriate filters to view fluorescein isothiocyanate. In order for a smear to be valid for interpretation, at least 3 intact cells per ×100 field had to be present. The majority of the 57 samples rejected for direct examination consisted of a single throat swab with no NP sample submitted. Reading and interpretation of the direct IFA smears was clear and easy. Positive samples were readily detected with the low-power (×10) objective. Minimal background or nonspecific staining was noted.

Virus was detected by CC, IFA, or both in 401 (40%) of the 1,003 samples evaluated (Table 1). Influenza A or B virus and RSV were the predominant isolates, accounting for 342 (85%) of all viruses recovered. The distribution of viruses isolated reflects the population studied, primarily hospitalized adult and pediatric patients admitted for respiratory illness.

Overall, 946 (94%) samples met the criteria for IFA examination with 300 yielding one of the five primary RV. Of these samples, 208 were positive by IFA and CC, 27 were IFA positive and CC negative, and 65 viruses were recovered only by CC. In four instances, the IFA direct staining method was positive but the CC tube became contaminated with yeast from the patient sample. These samples were considered IFA positive and CC negative. Two coinfections were noted. The first involved RSV and influenza A virus, with both viruses detected on direct examination as well as by CC. In the second instance, the direct examination was positive for influenza A virus, which was isolated by CC along with an enterovirus. Comparison of samples examined by direct testing and CC is summarized in Table 2.

With CC as a reference method, samples positive by IFA but negative by CC were considered false-positives. However, if IFA-positive, CC-negative samples were considered true positives, and viability of virus was lost during transport, the overall sensitivity of the IFA direct examination procedure would be more correctly calculated as 86%. The sensitivity of 80% that we report for direct detection of influenza A virus is notably higher than the results obtained and reported by others (6, 11). We attribute this increase in sensitivity solely to the use of standardized sample collection methods and the use of the cytocentrifuge for smear preparation, as reagents and staining protocols were similar to those used and reported by others. RSV detection by our method was similar to but slightly lower than that indicated in some reports from other studies in which a cell pellet was used in making smears (1, 4, 6, 11). This may be explained partially by the cytocentrifugation process itself. RSV-infected cells tend to become enlarged and the cytoplasm tends to become fragile, and these cells may rupture during cytocentrifugation, leaving fewer intact cells for staining. We are currently in the process of evaluating this by looking at centrifugation time and speed. It was not possible to draw conclusions regarding the effectiveness for direct detection of adenovirus or parainfluenza virus due to the small numbers recovered during the study period.

While some consider nasal washings the specimen of choice for RV recovery, excess mucus often noted in these samples may interfere with fluorescent antibody staining. Neither problem was apparent in our study, and virus yield was high, indicating that the dual sample collection method yields an acceptable sample. The standardized collection procedure allowed for sufficient quantity of sample to complete all necessary testing, was well accepted by practitioners collecting the samples, and appeared beneficial in maintaining consistency among specimens.

Immunofluorescence procedures continue to offer a rapid and cost-effective approach for direct detection of RV from clinical samples. Use of the cytocentrifuge, coupled with high-quality antibody reagents available from commercial sources and standardization of sample collection, enhances the sensitivity of these procedures. Smears prepared by cytocentrifugation exhibit uniformity, resulting in smears that are easy to interpret even by those without extensive training. The small quantity of reagent and decreased incubation times needed for staining are additional cost-saving benefits.

While direct testing plays an important role in RV infection diagnosis, it is still evident that all of these procedures should be used in conjunction with CC and not replace it. The culturing of samples negative by direct assays not only increases the yield of viruses for which direct testing is targeted but also allows for the recovery of agents not included in direct testing protocols.

REFERENCES

1. **Blanding, J. G., M. G. Hoshiko, and H. R. Stutman.** 1989. Routine viral culture for pediatric respiratory specimens submitted for direct immunofluorescence testing. J. Clin. Microbiol. **27:**1438–1440.
2. **Bromberg, K., G. Tannis, B. Daidone, L. Clark, and M. Sierra.** 1987. Comparison of HEp-2 cell culture and Abbott respiratory syncytial virus enzyme immunoassay. J. Clin. Microbiol. **25:**434–436.
3. **Dominguez, E. A., L. H. Taber, and R. B. Couch.** 1993. Comparison of rapid diagnostic techniques for respiratory syncytial and influenza A virus respiratory infections in young children. J. Clin. Microbiol. **31:**2286–2290.
4. **Johnston, S. L. G., and C. S. Siegel.** 1990. Evaluation of direct immunofluorescence, enzyme immunoassay, centrifugation culture, and conventional culture for the detection of respiratory syncytial virus. J. Clin. Microbiol. **28:** 2394–2397.
5. **Landry, M. L., and G. D. Hsiung.** 1986. Primary isolation of viruses, p. 31–50. *In* S. Specter and G. S. Lancz (ed.), Clinical virology manual. Elsevier Publishing, New York, N.Y.
6. **Matthey, S., D. N. Nicholson, S. Ruhs, B. Alden, M. Knock, K. Schultz, and A. Schmuecker.** 1992. Rapid detection of respiratory viruses by shell vial culture and direct staining by using pooled and individual monoclonal antibodies. J. Clin. Microbiol. **30:**540–544.

7. **McQuillan, J. R., R. C. Madelay, and A. P. Kendal.** 1985. Monoclonal antibodies for the rapid diagnosis of influenza A and B virus infections by immunofluorescence. Lancet **ii:**911–914.
8. **Minnich, L. L., and C. G. Ray.** 1987. Early testing of cell cultures for detection of hemadsorbing viruses. J. Clin. Microbiol. **25:**421–422.
9. **Ray, C. G., and L. L. Minnich.** 1987. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. J. Clin. Microbiol. **25:**355–357.
10. **Sarkkinen, H. K., P. E. Halonen, P. P. Arstila, and A. A. Salmi.** 1981. Detection of respiratory syncytial, parainfluenza type 2, and adenovirus antigens by radioimmunoassay and enzyme immunoassay on nasopharyngeal specimens from children with acute respiratory disease. J. Clin. Microbiol. **13:**258–265.
11. **Takimoto, S., M. Grandien, M. A. Ishida, M. S. Pereira, T. M. Paiva, T. Ishimaru, E. M. Makita, and C. H. O. Martinez.** 1991. Comparison of enzyme-linked immunosorbent assay, indirect immunofluorescence assay, and virus isolation for detection of respiratory viruses in nasopharyngeal secretions. J. Clin. Microbiol. **29:**470–474.
12. **Thomas, E. E., and L. E. Book.** 1991. Comparison of two rapid methods for detection of respiratory syncytial virus with direct immunofluorescence and virus isolation for the diagnosis of pediatric RSV infection. J. Clin. Microbiol. **29:**632–635.
13. **Waner, J. L.** 1990. Comparison of Directigen RSV with viral isolation and direct immunofluorescence for the identification of respiratory syncytial virus. J. Clin. Microbiol. **28:**480–483.
14. **Waner, J. L., N. J. Whitehurst, S. Jonas, L. Wall, and H. Shalby.** 1986. Isolation of viruses from specimens submitted for direct immunofluorescence test for respiratory syncytial virus. J. Pediatr. **108:**249–250.