JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1988, p. 588–591
0095-1137/88/030588-04$02.00/0

Vol. 26, No. 3

Detection of Respiratory Syncytial Virus in Clinical Specimens by Viral Culture, Direct and Indirect Immunofluorescence, and Enzyme Immunoassay

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Received 9 July 1987/Accepted 25 November 1987

We evaluated prospectively the detection of respiratory syncytial virus (RSV) by culture and by direct antigen detection using an indirect immunofluorescence assay (IFA), a direct monoclonal immunofluorescence assay (DFA), and a monoclonal enzyme immunoassay (EIA). Of 221 specimens, 95 (43%) were culture positive for RSV, 4 (1.8%) contained more than one virus, and 17 (7.6%) contained a virus other than RSV. Overall, HEp-2 and Flow 6000 cells grew significantly more RSV isolates (82 and 72%, respectively) than A549 cells, which grew only 29% of the isolates. The mean time for RSV detection with HEp-2 cells was 2.9 days. This was significantly less than the mean time for RSV detection with either Flow 6000 cells (6.1 days) or A549 cells (6.4 days). Of 221 specimens, 129 were tested simultaneously by culture, IFA, and DFA. Of these 129 specimens, 62 (48%) were positive by culture, 69 (53%) were positive by IFA, and 70 (54%) were positive by DFA. For 92 specimens screened simultaneously by culture, IFA, and EIA, positive results were obtained for 33 (36%) of the specimens by both culture and IFA and for 29 (32%) of the specimens by EIA. Of 126 culture-negative specimens, 21 (17%) were positive for RSV when determined by IFA. Conversely, 14 (15%) of 95 RSV culture-positive specimens were negative by IFA, whereas DFA missed 19% of the culture-positive specimens. Compared with culture, the Kallestad EIA kit had a sensitivity and specificity of 73 and 92% respectively, but missed 9 (27%) of 33 culture-positive specimens. These data demonstrate that isolation by culture continues to be important for viral diagnosis of RSV infections and that for valid comparative studies between viral isolations and rapid detection methods, both sensitive host cells and appropriate test conditions are required.

Respiratory syncytial virus (RSV) is a common cause of acute lower-respiratory tract disease in children (23, 38) and is responsible for frequent nosocomial infections (21, 42). Methods used for the routine diagnosis of RSV infections are antigen detection by immunofluorescence (2, 3, 5, 6, 11, 13, 15–19, 32–36, 40–43, 45–47, 50, 52) and viral isolation (4, 20, 22, 36, 40, 46, 52). Peroxidase staining (9, 31) and radio- and enzyme immunoassays (EIA) (1, 7, 8, 10, 12, 14, 16, 18, 26–30, 35, 37, 39, 44, 48, 49, 51) are also used for the rapid detection of RSV in clinical specimens or in cultures. One factor that has increased the need for rapid diagnosis of RSV infections is the availability of antiviral therapy (24, 25). In this report, we describe our experience with the prospective evaluation of two different direct antigen detection methods for the rapid diagnosis of RSV infections. Fresh respiratory specimens obtained in 1985 and 1986 were used for antigen detection by direct immunofluorescence assays (DFA) with a monoclonal antibody supplied by Whittaker Bioproducts. Fresh respiratory specimens obtained in 1987 also were tested directly for RSV antigen by using the enzyme immunoassay (EIA) kits from Kallestad Diagnostics, Austin, Tex.

For this study, only specimens from patients admitted to The Columbus Children's Hospital were used. The clinical diagnosis for patients included bronchiolitis (53%), pneumonia (13%), croup (6%), upper respiratory-tract infections (14%), and other miscellaneous diagnoses (7%). Fifteen (7%) of the requests were to rule out RSV infections and did not include a diagnosis. Specimens were limited to nasal washes (77%), nasopharyngeal swabs (10%), tracheal aspirates (7%), and throat swabs (6%).

For indirect immunofluorescence assay (IFA) tests, the primary antibody used was polyclonal bovine antibody to human RSV (Burroughs Wellcome Research Laboratories, Beckenham, England). We used fluorescein isothiocyanate-conjugated rabbit anti-bovine immunoglobulin G as the secondary antibody. Fluorescein isothiocyanate-conjugated monoclonal antibody (Whittaker Bioproducts, Walkersville, Md.) was used for DFA tests. Smears of RSV-infected and noninfected HEp-2 cells were used as positive and negative antigen controls, respectively. A negative monoclonal antibody control consisted of fluorescein isothiocyanate-labeled nonimmune ascites fluid reacted with RSV-infected and noninfected HEp-2 cell culture smears. For RSV antigen detection in clinical specimens by EIA, we used the methods described by the manufacturer (Kallestad). All specimens were also simultaneously tested for RSV by standard cell culture isolation in HEp-2 cells, human diploid fibroblasts (Flow 6000 cells), and A549 cells and by IFA.

Of 221 specimens, 95 (43%) were culture positive for RSV. Of 221 specimens, 4 (1.8%) contained more than one virus and 17 (7.6%) contained viruses other than RSV.

Overall, HEp-2 cells grew 82% of the RSV isolates, whereas Flow 6000 and A549 cells grew 72% and 29%, respectively, of the RSV isolates. Significantly more RSV isolates grew in either HEp-2 or Flow 6000 cells than in the cloned line of A549 cells ($P < 0.001$, chi-square of 43.0 and 27.9, respectively). No significant differences in isolation rates were detected between HEp-2 and Flow 6000 cells. The mean time for RSV detection in HEp-2 cells was 2.9 ± 1.4 days. This was significantly less ($P < 0.001$, Student's t

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TABLE 1. Results of RSV isolations compared with DFA, IFA, and EIA

RSV culture result	No. of RSV results by:							
	DFA (1985–1986)		IFA (1985–1986)		EIA (1987)		IFA (1987)	
	+	−	+	−	+	−	+	−
Positive	50	12	53	9	24	9	28	5
Negative	20	47	16	51	5	54	5	54

test) than the mean time for RSV detection in either Flow 6000 cells (6.1 ± 2.2 days) or A549 cells (6.4 ± 3.1 days).

Of 129 specimens obtained in 1985 and 1986 and tested by culture, IFA and DFA, RSV was detected in 62 (48%) by culturing, in 69 (53%) by IFA, and in 70 (54%) by DFA. Viruses other than RSV were isolated from 6 (4.6%) of the 129 specimens. The other viruses isolated were two enteroviruses, one adenovirus, one cytomegalovirus, and two hemadsorbing viruses. In four of these six specimens, RSV was not detected in smears by either IFA or DFA. However, two of the specimens were positive for RSV by both IFA and DFA techniques.

Overall, 102 (46%) of the 221 specimens were positive by IFA versus 95 (43%) by culture ($P < 0.60$). Of 126 culture-negative specimens, 21 (17%) were positive by IFA (Table 1). Of 16 RSV culture-negative but IFA-positive specimens, 15 (94%) were also positive by DFA. Of the 129 specimens tested in 1985 and 1986, 70 (54%) were positive by DFA compared with 48% by culture ($P < 0.40$). DFA detected RSV in 20 (30%) of 67 culture-negative specimens. Of 20 specimens that were RSV culture negative but DFA positive, 15 (75%) were also positive by IFA. However, 14 (15%) of 95 culture-positive specimens were negative by IFA and 12 (19%) of 62 culture-positive specimens were negative by DFA (Table 1). A comparison of the sensitivity, specificity, positive and negative predictive values, and agreement for the detection methods mentioned above is presented in Table 2.

Of 92 specimens tested in 1987 for RSV by EIA, IFA, or isolation in cell culture, RSV was detected in 33 (36%) by culturing, in 33 (36%) by IFA, and in 29 (32%) by EIA. Viruses other than RSV were found in 11 of the 92 (12%) specimens. The other viruses isolated were eight adenoviruses, one enterovirus, and two hemadsorbing viruses. For 9 of these 11 specimens, RSV was not detected by either IFA or EIA. However, two of these specimens were positive for RSV by IFA only. With the EIA testing, 5 (8.4%) of 59 culture-negative specimens were EIA positive, and 3 of these were also positive by IFA. Of 33 culture-positive specimens, 9 (27%) were EIA negative and 5 (15%) were IFA negative (Table 1). A comparison of the sensitivity, specificity, positive and negative predictive values, and agreement for the EIA and IFA results for the 1987 specimens is also given in Table 2.

For our combined studies, HEp-2 cells grew 82% of the RSV isolates whereas human diploid fibroblasts grew 72% of the isolates. In previous studies, HEp-2 cells grew 35% (37) and 73 to 77% (4) of the RSV isolates and fibroblasts grew 6% (37) and 65 to 76% (4) of the RSV isolates.

We observed vast differences in RSV isolation between HEp-2 or Flow 6000 cells and a clone of A549 cells. The sensitivity of RSV isolation in A549 cells over our two-season study period ranged from 20% to 42%. In contrast, the HEp-2 cell sensitivity for parallel RSV isolations ranged from 76% to 91%, and the sensitivity for Flow 6000 cells was 64% to 78%. Similar differences in host cell sensitivity from one year to the next have been reported (4). Such differences are probably due to host cell differences or variability in laboratory conditions for growing and maintaining cells or detecting viral cytopathic effect. Therefore, it is important to monitor host cells to ensure that they will be adequate for viral isolations. Furthermore, appropriate and sensitive host cells are required for valid comparative studies between viral isolations and rapid detection methods.

The mean times for RSV detection were 2.9, 6.1, and 6.4 days respectively, for the HEp-2, Flow 6000, and A549 cells. Mean detection times of 5 to 6 and 6 days for HEp-2 cells (4, 49) and 9 and 10 days for fibroblasts (4) have previously been reported.

The Whittaker Bioproducts monoclonal antibody for the DFA detection of RSV in clinical specimens was found to be a sensitive and specific reagent. A sensitivity of 81% compared with cell culture isolation of RSV is similar to that previously reported for other RSV monoclonal antibodies (6, 11, 15, 16, 32, 34, 45, 47). The sensitivity and specificity of DFA compared with IFA were 90 and 87%, respectively.

Both immunofluorescence methods used in this study detected more RSV-positive specimens than were detected by cell culture isolation. Results showing specimens negative for RSV by culture but positive by one of the immunofluorescence methods were supported by the other fluorescence method in 83% of the cases. For our combined IFA studies, IFA detected RSV in 17% of the 126 culture-negative specimens. Previously, RSV was detected in 19% and 18% of the culture-negative specimens by fluorescence methods (6, 16, 34). However, 15% of the 95 culture-positive specimens were negative by IFA, and 19% were negative by DFA. From previous studies, 9 to 15% of the culture-positive specimens were negative by fluorescence (6, 16, 34, 52). This demonstrates the importance of using cell cultures to detect RSV as well as other respiratory viruses in clinical specimens. In this study, other viruses were also detected in 9.4% of the specimens. In other RSV studies 10 to 20% of the

TABLE 2. Comparison of direct antigen detection methods with viral isolation for diagnosing RSV infections

Tests compared[a]	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value	% Agreement
DFA vs culture	81 (50/62)[b]	70 (47/67)	71 (50/70)	80 (47/59)	75 (97/129)
IFA vs culture	85 (53/62)	76 (51/67)	77 (53/69)	85 (51/60)	81 (104/129)
DFA vs IFA	90 (62/69)	87 (52/60)	89 (62/70)	88 (52/59)	88 (114/129)
EIA vs culture	73 (24/33)	92 (54/59)	83 (24/29)	86 (54/63)	85 (78/92)
IFA vs culture	85 (28/33)	92 (54/59)	83 (28/33)	92 (54/59)	89 (82/92)
EIA vs IFA	79 (26/33)	95 (56/59)	90 (26/29)	89 (56/63)	89 (82/92)

[a] For test comparisons, either culture or IFA positivity was used as the reference test to determine sensitivity, specificity, positive and negative predictive values, and agreement (54).

[b] Values in parentheses are the actual numbers used for percent determinations.

specimens contained viruses other than RSV (5, 11, 12, 15, 37, 44, 46, 49, 52).

The reported sensitivity of enzyme-linked immunosorbent assay and radioimmunoassays when compared with culture or immunofluorescence methods have ranged from 61 to 96% (1, 7, 8, 10, 12, 14, 16, 18, 28, 29, 35, 37, 39, 44, 48, 49, 53). In this study, an EIA kit manufactured by Kallestad Diagnostics was found to have a sensitivity of 73% and a specificity of 92% when compared with cell culture. The differences in sensitivity between EIA versus culture (73%) and IFA versus culture (85%) are not significant for this population. The sensitivity and specificity of the Kallestad EIA were 79 and 95%, respectively, when compared with IFA. However, of 33 culture-positive specimens, 9 (27%) were EIA negative.

Since it is difficult to accurately compare results of EIA testing performed in different clinical laboratories with different specimens and under different conditions, more comparative testing within laboratories is necessary to determine which commercial EIA kits are acceptable. Comparative testing should include standard methods for specimen collecting, handling, and processing.

Certainly there are limitations associated with RSV cell culture isolations. Some of these limitations are overgrowth or loss of cells that would limit formation of RSV syncytia, decreased host cell sensitivity, extended detection times, and failure to detect virus late in an illness when antigen still may be detectable by other methods. However, it is clear that the exclusive use of a single rapid test for the detection of a single viral group can lead to a failure to detect other important viral pathogens. Thus, cell culture is still important for the detection of respiratory viruses that may be missed by direct detection methods.

We acknowledge the excellent technical assistance of Mike Reed and Annette Pagura, and we thank Laura Jo Hughes for typing this manuscript.

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