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Rapid Diagnosis of Respiratory Viral Infections by Using a Shell Vial Assay and Monoclonal Antibody Pool

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We compared the detection of seven respiratory viruses by using a commercially available monoclonal antibody pool in a 2-day shell vial assay with that by using standard cell culture with respiratory syncytial virus (RSV) enzyme-linked immunosorbent assay (ELISA)-negative nasal secretions from hospitalized children. We found 179 respiratory virus isolates by either method in 675 specimens. Overall, the shell vial assay detected 147 of 179 (79%) of the positives after 2 days; cell culture detected 148 of 179 (80%) after a mean incubation period of 7.6 days (range, 1 to 14 days). The sensitivity of the shell vial assay was 78% for RSV, 94% for influenza B virus, 83% for adenovirus, and 80% for parainfluenza viruses. The sensitivity of the cell culture was 70% for RSV, 79% for influenza B virus, 90% for adenovirus, and 89% for parainfluenza viruses. The 2-day shell vial assay allowed the detection of respiratory viruses in a clinically relevant time frame and rapidly detected RSV in specimens lacking RSV antigen by ELISA.

Many diagnostic virology laboratories detect respiratory viruses other than respiratory syncytial virus (RSV) by inoculating cell culture tubes with respiratory secretions and observing for cytopathic effect (CPE) or hemadsorption. This method is problematic, since several days are often required for CPE to develop, making culture results available to clinicians in a time frame that may not be clinically relevant. More rapid viral culture results can be obtained by using the shell vial technique. This involves centrifugation of the specimen onto cell monolayers and viral antigen detection by immunofluorescence within 1 to 2 days after inoculation. Since it was first described for the rapid detection of herpes simplex virus (8) and cytomegalovirus (7), the shell vial technique has been successfully used for the detection of influenza viruses A and B (1, 6, 9, 12, 19) and adenovirus (5, 13). We report the first use of a commercially available monoclonal antibody pool (Baxter-Bartels, Bellevue, Wash.) for viral antigen detection in shell vials by immunofluorescence and compare its results with those of standard cell culture with fresh, RSV enzyme-linked immunosorbent assay (ELISA)-negative respiratory secretions from hospitalized children.

MATERIALS AND METHODS

Specimen collection and processing. Nasal washes-aspirates and endotracheal aspirates from children hospitalized with acute respiratory symptoms at Kosair Children's Hospital between November 1990 and August 1991 were evaluated. Between November 1990 and April 1991 (phase I), specimens were first screened for RSV antigen by ELISA (RSV Pathfinder; Kallestad, Chaska, Minn.), and only specimens lacking RSV antigen were set up for cell culture and shell vial assay. All specimens collected from April through August 1991 (phase II) were set up in shell vials and cell culture without first being screened for RSV. Respiratory secretions were placed in 2 ml of viral transport medium at the patients' bedsides and transported on ice to the virology laboratory, where the specimen vial containing glass beads was vortexed for 15 s and centrifuged for 5 min (4°C, 2,000 × *g*), and the supernatant was used as the inoculum for both the shell vials and cell culture. Specimens were processed once daily within 24 h of collection.

Shell vial assay. The specimen (0.2 ml) was centrifuged for 45 min at 35°C (700 × *g*) onto each single shell vial monolayer of primary rhesus monkey kidney cells and A549 cells (Whittaker, Walkersville, Md.). We used a shell vial of A549 cells instead of HEp-2 cells because preliminary experiments showed that RSV was detected equally well in A549 and HEp-2 cells by immunofluorescence (data not shown), and A549 cells were easier to work with. Eagle minimal essential medium (1 ml) was then added to each vial. After 40 h of incubation at 37°C, cell monolayers were washed with phosphate-buffered saline (PBS), scraped into 0.5 ml of PBS with a Pasteur pipet, and spotted onto a 2-well glass slide (for screening) and an 8-well slide (for virus identification). This is a modification of the usual shell vial procedure, in which cells on the original coverslips are stained. Slides were air dried and fixed in cold acetone for 10 min. The monoclonal antibody pool was used to screen for RSV, adenovirus, influenza viruses A and B, and parainfluenza viruses 1, 2, and 3 by adding 1 drop of the antibody pool per well and incubating at 37°C for 30 min. The monoclonal antibodies developed by Baxter-Bartels are affinity-purified antibodies from mouse ascitic fluid. After the slide was washed twice in PBS, 1 drop of fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Baxter-Bartels) per well was added for 30 min at 37°C. After the slide was washed twice in PBS, a drop of buffered glycerol mounting medium was added to each well and a coverslip (22 by 50 mm) was applied to the slide. Slides were examined by using a Leitz fluorescence microscope. When virus-specific fluorescence was noted, virus identification was performed by using the individual monoclonal antibodies on the 8-well slide. We did not do the staining procedure with the individual monoclonal antibodies when the screening-pool slide showed no fluorescence. The presence of three or more cells per well with specific apple-green fluorescence was considered a positive identification. Positive- and negative-specimen control slides (Baxter-Bartels) were run weekly and whenever a new batch of

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TABLE 1. Distribution by pathogen of virus isolates detected by shell vial assay and cell culture[a]

Virus (no. of isolates)	No. of isolates with the following result:		
	SV+ CC−	SV− CC+	SV+ CC+
RSV (44)	13	11	20
Influenza B (34)	7	2	25
Adenovirus (30)	3	5	22
Parainfluenza 1 (6)	2	0	4
Parainfluenza 2 (4)	1	1	2
Parainfluenza 3 (61)	5	13	43
Total (179)	31	32	116

[a] SV+ CC−, positive by shell vial assay and negative by cell culture; SV− CC+, negative by shell vial assay and positive by cell culture; SV+ CC+, positive by both shell vial assay and cell culture.

monoclonal antibodies was used. Nonimmune mouse antibody was used as a negative control on each slide. Nonspecific background staining was not a problem.

Cell culture. The specimen (0.2 ml) was inoculated into each culture tube of primary rhesus monkey kidney, A549, and HEp-2 cells (Whittaker) containing Eagle minimal essential medium. Tubes were incubated at 37°C and examined on alternate days for CPE. Cell monolayers were screened for hemadsorption activity with 0.4% guinea pig erythrocytes between days 5 and 7 of incubation when the shell vial assay for that specimen failed to detect a virus. For cell cultures that developed CPE or hemadsorption, the method of virus identification depended on the shell vial result for that specimen. When a virus was detected by the shell vial assay, immunofluorescence identification with cell scrapings from tubes was done only when the CPE or hemadsorption was inconsistent with the shell vial result. When a virus was not detected by the shell vial assay, immunofluorescence identification by cell culture was always done. For cell cultures that were hemadsorption negative and lacked CPE after 12 to 14 days of incubation, cell scrapings from each tube were routinely screened for viral antigen; when the cells were positive, immunofluorescence identification was done. Screening and immunofluorescence identification by cell culture were done with the same reagents and procedures used in the shell vial assay.

RESULTS

Overall comparison of shell vial assays and cell culture. We processed 675 specimens (91% nasal washes-aspirates) in both the shell vial assay and cell culture: 451 in phase I and 224 in phase II. We cultured 179 respiratory virus isolates from 174 of 675 (26%) specimens by either method. The shell vial assay detected 147 of 179 (79%) virus isolates after 2 days; cell culture detected 148 of 179 (80%) virus isolates after a mean of 7.6 days (range, 1 to 14 days). Table 1 shows the distribution of shell vial and cell culture results for these 179 virus isolates. Mixed infection was found with 5 of 174 (3%) virus-containing specimens: 3 with parainfluenza virus and adenovirus, 1 with RSV and adenovirus, and 1 with RSV and parainfluenza virus.

RSV detection. During phase I, RSV antigen was detected by ELISA in 365 of 958 (38%) specimens submitted for RSV ELISA. Of the 593 ELISA-negative specimens, 451 were available for setup in the shell vial assay and cell culture. RSV was detected by either method in 41 of 451 (9%) specimens: 32 of 41 (78%) by shell vial assay at 2 days and 28 of 41 (68%) by cell culture (CPE or blind staining) after a mean incubation period of 7.2 days (range, 2 to 14 days). By using the RSV ELISA positives plus the shell vial assay and cell culture positives during phase I as the "gold standard," the overall sensitivity of the RSV ELISA was 365 of 365 + 41 (90%). During phase II, three additional RSV isolates were detected (two by cell culture alone and one by both shell vial assay and cell culture). The medical records of the 13 children whose respiratory secretions contained RSV by shell vial assay but not by cell culture were reviewed, and each child had an illness compatible with RSV bronchiolitis or pneumonia and had no other etiologic agent identified.

Detection of other respiratory viruses. A total of 135 other respiratory virus isolates were detected (phases I and II), including 34 influenza B virus isolates, 30 adenovirus isolates, and 71 parainfluenza virus isolates. No influenza A virus strains were isolated during the study period. Assuming that the gold standard is the combination of positives detected by either shell vial assay or cell culture, the sensitivities of the 2-day shell vial assay were 32 of 34 (94%) for influenza B virus, 25 of 30 (83%) for adenovirus, and 57 of 71 (80%) for parainfluenza viruses. The mean numbers of days to virus identification by cell culture (CPE, hemadsorption, or blind staining) for influenza B virus, adenovirus, and parainfluenza viruses were 5.6, 7.8, and 9.0, respectively. CPE in cell culture was noted only by the rhesus monkey kidney cell culture for influenza B virus and the parainfluenza viruses and only in the A549 cell culture tube for adenoviruses and by HEp-2 cell culture for RSV. RSV did not cause CPE in cell cultures of A549 or rhesus monkey kidney cells.

Blind immunofluorescence staining of cell culture monolayers. Of the 148 cell culture positives, 24 (16%) were found only by blind immunofluorescence staining of cell scrapings from tubes that exhibited no CPE after 12 to 14 days of incubation. These included 16 parainfluenza virus isolates, 4 adenovirus isolates, and 4 RSV isolates.

DISCUSSION

The shell vial assay uses both a centrifugation step and immunofluorescence staining of cell monolayers for virus detection, while cell culture relies on the development of CPE or hemadsorption. Any comparison of these two assays is therefore inherently unequal. We did not attempt to compare the use of immunofluorescence staining in shell vials to the use of such staining in cell culture tubes. Instead, our purpose was to compare these assays as they are routinely performed in diagnostic virology laboratories and determine whether the shell vial assay with the Baxter-Bartels monoclonal antibodies could rapidly detect respiratory viruses without a clinician having to wait for CPE or hemadsorption to develop. This 2-day shell vial assay allowed the rapid detection of respiratory viruses for the majority of virus-containing specimens because the immunofluorescence endpoint was independent of and preceded the development of CPE or hemadsorption.

The culture of RSV ELISA-negative respiratory secretions in shell vials allowed us to make a virologic diagnosis with 79% of the virus-containing specimens in 2 days. This represents a significant savings in time over conventional cell culture. Our results are comparable to the shell vial detection of RSV, adenovirus, and influenza A and B viruses with other antibodies for immunofluorescence detection. One- or 2-day shell vial assays detect 73% of RSV isolates (10), 77 to 97% of adenovirus isolates (5, 13), and 55 to 84%

of influenza A and B virus isolates (1, 6, 9, 12, 19). This use of the Baxter-Bartels monoclonal antibody pool in a 2-day shell vial assay and its comparison to cell culture with results similar to ours has been reported in abstract form only (17, 20). Rapid viral culture results could potentially be used to shorten the duration of hospitalization, allow the discontinuation of unnecessary antibiotics, and reduce the need for other diagnostic tests, making the added expense of shell vials cost effective, although we have not done such a cost-benefit analysis.

In addition to being more rapid, the shell vial assay was more sensitive than cell culture for some specimens. Thirty-one of 179 (17%) viral isolates would have been missed if cell culture alone had been used. Although this finding raises the possibility that the shell vial assay is less than 100% specific, the shell vial assay is more sensitive for RSV detection than cell culture (10). Shell vial assays have not yet been shown to be more sensitive than cell culture for the detection of influenza virus, adenovirus, or parainfluenza viruses, but, in our study, the shell vial assay was positive and cell culture was negative for 7 of 34 (20%) influenza B virus isolates, 3 of 30 (10%) adenovirus isolates, and 7 of 71 (10%) parainfluenza virus isolates. We feel that these represent true positives and that this justifies their inclusion when defining the gold standard as cell culture plus shell vial assay positives.

The 2-day shell vial assay cannot completely replace cell culture, however, since 32 of 179 (18%) of the virus isolates in our study were found only by cell culture. Performing a second shell vial assay after 5 days of incubation may detect virus isolates missed at day 2, as has been shown for adenovirus (13) and influenza B virus (12).

The combination of shell vial assay and cell culture detected RSV in 41 of 451 (9%) specimens lacking RSV antigen by ELISA. This was not an unexpected finding, and it is consistent with other reports of 4 to 27% false-negative RSV ELISA results in comparison with those of cell culture (2–4, 10, 11, 14, 18, 21, 22). Since RSV diagnosis is important for infection control practice and for considering ribavirin therapy, we request cell culture backup of ELISA-negative specimens. The shell vial assay has advantages over standard cell culture for this purpose, because it is more sensitive and it provides results for the majority of ELISA-negative specimens in 2 days instead of the mean of 7 days that is required for CPE to develop.

Blind staining of cell scrapings from tubes that lacked CPE after 2 weeks of incubation detected 16% of our cell culture positives. Whether all 16 parainfluenza virus isolates so detected would have shown hemadsorption before 2 weeks of incubation is not known. Cell cultures of nine isolates found by the shell vial assay were not screened for hemadsorption, and none of the remaining seven parainfluenza virus isolates demonstrated hemadsorption when screened after 5 to 7 days of incubation. Hemadsorption controls run weekly with laboratory strains of influenza and parainfluenza viruses consistently demonstrated hemadsorption. The detection of hemadsorption caused by some parainfluenza viruses as late as 10 days after incubation has been reported previously (15) and may be one possible explanation for our inability to detect these parainfluenza isolates by hemadsorption after 5 to 7 days. Another possibility is that immunofluorescence is more sensitive than hemadsorption for specimens with low virus titers. Note that we saw similar results during an influenza B virus outbreak in 1989 in which 4 of 25 (16%) influenza B virus isolates detected in cell culture were hemadsorption negative and failed to develop CPE and were found only by blind immunofluorescence staining after 2 weeks of incubation (16).

Our study provides the first published data on the use of this monoclonal antibody pool for respiratory viral detection by immunofluorescence in a shell vial assay. This assay has several advantages over standard cell culture for the rapid diagnosis of respiratory virus infections in children and provides a useful adjunct to cell culture.

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