

## Rapid Detection of Respiratory Viruses by Shell Vial Culture and Direct Staining by Using Pooled and Individual Monoclonal Antibodies

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The Bartels respiratory virus panel detection kit is an indirect fluorescent-antibody (IFA) method that uses pooled and individual antisera for tissue culture confirmation of seven respiratory viruses. We evaluated these reagents for detecting viral antigen in shell vial cultures and by direct staining of cells from respiratory specimens. The isolation from 254 specimens of respiratory viruses in shell vial cultures compared with standard tube cultures was highly sensitive (94%) and specific (97.3%). The numbers of viral isolates detected in three consecutive years of testing with shell vial cultures were 68 of 254 (26.8%), 101 of 381 (26.5%), and 122 of 430 (28.4%). IFA direct staining of all 1,065 specimens resulted in 183 (17.2%) being uninterpretable because of inadequate numbers of cells or interfering fluorescence. The sensitivity and specificity of the interpretable IFA direct stains in comparison with shell vial cultures were 85.9 and 87.1%, respectively. For detection of 881 adequate specimens, Bartels respiratory syncytial virus IFA direct staining compared with an Ortho Diagnostics Systems direct fluorescent-antibody test for respiratory syncytial virus RSV was highly sensitive (95.5%) and specific (97%). Shell vial cultures combined with Bartels IFA reagents are a rapid alternative to standard tube cultures. Bartels IFA direct staining with individual antisera provides useful same-day screening of respiratory specimens, but the antiserum pool was not effective in screening for positive specimens because of excessive amounts of nonspecific fluorescence.

Respiratory viruses cause a variety of human infections, ranging from the common cold to life-threatening pneumonia (10, 14, 15). Over 200 strains of virus can cause respiratory disease, and the differential diagnosis of respiratory viruses has often been considered impractical and of limited value for patient care. The development of clinically effective antiviral agents has changed this perspective and has stimulated the development of rapid diagnostic tests for several viruses, with particular emphasis on respiratory syncytial virus (RSV) and influenza A virus (1, 2, 7-9, 11-13). The most common commercially available rapid diagnostic tests use immunofluorescence or enzyme immunoassay methods with monoclonal antibodies (1, 3, 7-9).

Cultivation in tissue cultures remains the standard for the detection of most viruses, although this procedure necessitates days to weeks for isolation and identification. The use of centrifugation-enhanced shell vial cultures combined with fluorescein-labelled monoclonal antibodies has significantly shortened turnaround times (2, 5, 6, 9, 12). Most shell vial techniques and rapid test kits usually detect only one virus, but rapid diagnosis of respiratory virus infections could be enhanced by testing for several viruses simultaneously. A commercially available fluorescent-antibody kit manufactured by Bartels (Bellevue, Wash.) was designed to confirm seven different viruses in tissue cultures. The kit contains individual monoclonal antisera to adenovirus, influenza A and B viruses, parainfluenza viruses 1, 2, and 3, RSV and an antiserum pool containing monoclonal antibodies to all seven viruses. This two-part study evaluated the effectiveness of Bartels individual and pooled reagents for detecting

respiratory viruses directly from respiratory specimens and in shell vial tissue cultures.

### MATERIALS AND METHODS

**Specimens.** Respiratory tract specimens from patients at the University of Iowa Hospitals and Clinics were collected by physicians, nurses, and respiratory therapists. Respiratory specimens from area community hospitals were collected by respiratory therapists and laboratory personnel. Nasopharyngeal wash, throat wash, and tracheal aspirate specimens were obtained by instilling normal saline into the appropriate area and aspirating the specimen into a mucus trap. Bronchial wash and bronchoalveolar lavage specimens were collected in sterile containers by physicians. Lung biopsy specimens were obtained by physicians and placed in sterile containers with 10 to 15 ml of Eagle's minimum essential medium with Earle's salts (MEM) containing 10% fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, Mo.), amphotericin B, and gentamicin. Specimens were transported on ice and processed within 1 h of receipt in the laboratory. Processed specimens which could not be inoculated into tissue cultures within 24 h were mixed with equal amounts of MEM containing 20% FBS and stored at -70°C. Seventy-nine specimens collected from November 1987 through April 1988 were stored for up to 12 months and tested along with 175 specimens collected from November 1988 through April 1989. Five of these 175 specimens were frozen prior to inoculation into cell cultures. Three hundred eighty-one specimens were collected from November 1989 through April 1990, and 60 of these were frozen prior to inoculation into cell cultures. Four hundred thirty specimens were collected from December 1990 through April 1991, and

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all were inoculated into cell cultures within 5 h of receipt in the laboratory.

**Specimen processing.** Specimens were processed as follows. (i) Nasopharyngeal wash, throat wash, and tracheal aspirate specimens were split into equal parts for culturing and direct staining. Specimens for culturing were brought to a 2.0-ml volume with phosphate-buffered saline (PBS), sterile glass beads were added, and the mixture was vortexed for 30 to 60 s. The specimens were centrifuged at  $1,200 \times g$  for 10 min, and the supernatant fluid was used to inoculate tissue cultures. Specimens for direct staining were also brought to 2.0 ml with PBS and vortexed, and 6.0 ml of PBS was added. The specimens were centrifuged at  $400 \times g$  for 10 min, the supernatant fluid was decanted, and the wash step was repeated one or two more times to remove excess mucus. The cell pellet was resuspended in 0.5 ml of PBS, and approximately 50  $\mu$ l of the cell suspension was dotted onto two-well and eight-well acetone-cleaned slides (Baxter Scientific Products, McGaw Park, Ill.). (ii) Bronchial wash and bronchoalveolar lavage specimens were inoculated into tissue cultures and dotted onto two-well and eight-well slides without additional processing. (iii) Lung biopsy specimens were divided with sterile scissors, and the freshly cut surface was touched directly onto two-well and eight-well slides. The specimens were homogenized to a 5 to 10% suspension in MEM containing 10% FBS, amphotericin B, and gentamicin in a stomacher blender (Tekmar Co., Cincinnati, Ohio). The suspensions were centrifuged at  $1,500 \times g$  for 10 min, and the supernatant fluid was filtered through a 0.45- $\mu$ m-pore-size filter (Millipore Corp., Bedford, Mass.). The filtered fluid was used to inoculate cell cultures.

Slides for direct staining were air dried, fixed in acetone, and stored at  $-70^{\circ}\text{C}$  when staining was not done within 2 h.

**Direct staining.** Direct staining of respiratory tract cells from patient specimens was done by indirect fluorescent-antibody (IFA) staining with reagents provided by Bartels Immunodiagnostic Supplies. The two-well slide was stained with 50  $\mu$ l of the antiserum pool containing monoclonal antibodies to adenovirus, influenza A and B viruses, parainfluenza viruses 1, 2, and 3, and RSV. The eight-well slide was stained with approximately 50  $\mu$ l of individual antisera for each of the seven viruses. A negative control was included on both the two-well and the eight-well slides. The slides were incubated for 30 min at  $36^{\circ}\text{C}$ , washed in Bartels PBS for 5 min, and blotted dry, and fluorescein-labelled anti-mouse conjugate was added to each well. The slides were incubated for 30 min at  $36^{\circ}\text{C}$ , washed in Bartels PBS for 5 min, and air dried, and mounting medium and coverslips were added.

Direct fluorescent-monooclonal-antibody staining for RSV (Ortho RSV staining; Ortho Diagnostics Systems, Raritan, N.J.) was performed on a two-well slide prepared for each respiratory specimen. Approximately 50  $\mu$ l of reagent was placed in each well, the slides were incubated at room temperature for 30 min, rinsed with PBS, and air dried, and mounting fluid and coverslips were added.

Slides were examined at  $\times 100$  and  $\times 400$  magnifications with an Olympus epifluorescence microscope (Leeds Precision Instruments, Minneapolis, Minn.). Positive direct stains had two or more fluorescent columnar epithelial cells per well, in accordance with the manufacturer's specifications for both the Bartels IFA and the Ortho RSV procedures. Fluorescence was nuclear, cytoplasmic, or both, depending on the specific reagent. Slides were considered inadequate when there were fewer than 30 intact cells per well and no positive cells were visualized. Preparations were considered

uninterpretable when nonspecific fluorescence obscured the cell layer of any well or when more than one well on the eight-well slide had specific fluorescence. Inadequate or uninterpretable eight-well slide IFA direct stains were tested again for the last 430 specimens only. Repeat testing was done with the same cell suspensions stored at  $4^{\circ}\text{C}$ . An additional wash step was performed with some specimens in an attempt to eliminate nonspecific fluorescence due to the presence of mucus.

**Tube culturing.** Tube culturing was done for the first 254 specimens only. Rhesus monkey kidney (RMK) and A549 tissue culture cells were provided by Bartels Immunodiagnostic Supplies. The maintenance medium was aspirated from each tube, and the cell monolayers were washed with PBS containing calcium and magnesium. One tube each of RMK and A549 cells was inoculated with 0.2 ml of specimen, incubated at  $36^{\circ}\text{C}$  for 30 to 60 min to allow viral adsorption, fed with 2.0 ml of MEM containing 2% FBS, amphotericin B, and penicillin-streptomycin, and incubated in stationary racks at  $36^{\circ}\text{C}$ . The culture tubes were examined daily, and slides were prepared when cytopathic effects were evident or at 7 to 10 days in the absence of cytopathic effects.

**Shell vial culturing.** RMK and A549 shell vial tissue cultures were provided by Bartels Immunodiagnostic Supplies for the first 254 specimens and purchased from Bartels or Whittaker Bioproducts (Walkersville, Md.) for the remaining specimens. Shell vials were prepared in the laboratory from RMK and A549 tube cultures purchased from Whittaker Bioproducts when commercial shell vials were unavailable. Shell vials were prepared by washing confluent tube monolayers with PBS and treating the cells with 0.25% trypsin-EDTA. The cells were suspended in and diluted approximately 1:4 with MEM containing 10% FBS, amphotericin B, and penicillin-streptomycin. One milliliter of the cell suspension was seeded into sterile shell vials (Ortho Diagnostics Systems), incubated at  $36^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere, and used for viral cultures after 2 to 6 days of incubation.

The medium was removed from the shell vials, and the cell monolayers were washed with PBS containing calcium and magnesium. One shell vial each of RMK and A549 cells was inoculated with 0.2 ml of specimen. The vials were centrifuged at  $700 \times g$  for 30 min at room temperature, fed with 1.0 ml of MEM containing 2% FBS, amphotericin B, and penicillin-streptomycin, and incubated at  $36^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere. Slides were prepared after 48 to 72 h of incubation.

**Culture staining.** One two-well slide and two eight-well slides were prepared from each tube and shell vial culture by washing the monolayers with PBS and scraping the cells into 0.5 ml of PBS. Approximately 50  $\mu$ l of the cell suspension was dotted onto each well of the two-well and eight-well slides. The slides were air dried and fixed in acetone. Slides that could not be stained within 2 h were stored at  $-70^{\circ}\text{C}$ .

The two-well slides were stained with Bartels IFA antiserum pool. The RMK cell eight-well slides were stained with Bartels IFA individual reagents as described for direct staining. The A549 cell eight-well slides were stained only when the A549 cell two-well slides were positive and the RMK slides were negative.

The second set of eight-well slides prepared from 80 tube cultures and 64 shell vial cultures were stained with reagents from the Centers for Disease Control (CDC), Atlanta, Ga., to confirm Bartels staining results. The CDC reagents were individual monoclonal antibodies to adenovirus, influenza A

TABLE 1. Distribution of viral isolates in tube and shell vial cultures<sup>a</sup>

Virus	Viral distribution of the first 254 specimens in:		Viral distribution of 811 additional specimens in shell vial cultures	Viral distribution of all 1,065 specimens in shell vial cultures
	Tube cultures	Shell vial cultures		
RSV	50 (25) <sup>a</sup>	52 (26)	152 (11)	204 (37)
Influenza A	9 (1)	10 (3)	11 (2)	21 (5)
Influenza B	5	3	21	24
Parainfluenza 1	1	1	10	11
Parainfluenza 2	0	0	5	5
Parainfluenza 3	2 (1)	2 (1)	17 (1)	19 (2)
Adenovirus	0	0	7	7
Total	67 (27)	68 (30)	223 (14)	291 (44)

<sup>a</sup> Numbers in parentheses are numbers of specimens frozen prior to inoculation into cultures.

and B viruses, and parainfluenza viruses 1, 2, and 3. Slides were stained as described for Bartels IFA direct staining.

### RESULTS

Two hundred fifty-four specimens were used to compare shell vial cultures with standard tube cultures (Table 1). Sixty-three specimens were positive for a respiratory virus in both tube and shell vial cultures. Four isolates were detected in tube cultures only, and five isolates were detected in shell vial cultures only, and all nine of these isolates were confirmed by CDC culture confirmation reagents or Ortho RSV staining. The high sensitivity (94%) and specificity (97.3%) of shell vial culturing versus tube culturing led us to adopt shell vial culturing only for testing all subsequent specimens.

Table 1 shows the results for 811 additional specimens and the individual viruses identified for all 1,065 specimens. The incidences of positive cultures in shell vials in three consecutive years of testing were 68 of 254 (26.8%) positive in the first year, 101 of 381 (26.5%) positive in the second year, and 122 of 430 (28.4%) positive in the third year.

Results of Bartels pooled and individual antiserum staining of cultures were in agreement, except for one specimen which showed positive A549 cell pooled antiserum staining but no staining on the eight-well slide. The specimen was retested, and the pooled and individual stains were negative.

Ten RSV, 1 influenza B virus, 1 parainfluenza virus 3, and 3 adenovirus isolates were detected in A549 cell cultures only. All of the remaining isolates were detected by staining of the RMK cell eight-well slide. Two dual infections due to RSV and parainfluenza virus 3 were detected by shell vial culturing. CDC reagent staining confirmed all tube culture positive results and all shell vial culture positive results, except one influenza A virus isolate and one parainfluenza virus 3 isolate which the CDC reagents did not detect in the shell vial cultures but did detect in the tube cultures.

The second aspect of this study evaluated the effectiveness of Bartels IFA pooled and individual antisera for detecting the seven respiratory viruses in direct staining of patient specimens. The antiserum pool was used only for the first 254 specimens, since direct staining with the pool produced slides with squamous epithelial cells, bacteria, mucus, and cellular debris showing significantly increased nonspecific fluorescence, which made interpretation very

TABLE 2. Distribution of positive results for shell vial culturing and IFA direct staining

Virus	No. of specimens that were			
	Culture positive and IFA direct stain positive	Culture positive and IFA direct stain negative	Culture positive and IFA direct stain uninterpretable <sup>a</sup>	Culture negative and IFA direct stain positive
RSV	175	10	19	72
Influenza A	9	10	2	3
Influenza B	15	4	5	1
Parainfluenza 1	6	5	0	1
Parainfluenza 2	3	0	2	1
Parainfluenza 3	15	3	1	2
Adenovirus	2	5	0	0

<sup>a</sup> Inadequate numbers of cells or interfering fluorescence.

difficult. Results were therefore based on individual antiserum staining.

The number of specimens tested by Bartels IFA direct staining was 1,065. Two hundred twenty-five specimens were IFA direct stain and shell vial culture positive. Five hundred forty specimens were IFA direct stain and shell vial culture negative. One culture result was uninterpretable because multiple wells showed specific staining. Thirty-seven specimens were IFA direct stain negative but shell vial culture positive, and 80 specimens were IFA direct stain positive but shell vial culture negative. One hundred eighty-two specimens had inadequate numbers of cells or uninterpretable results in Bartels IFA direct staining (29 were culture positive, and 153 were culture negative). The majority of the slides considered inadequate were so designated because the cells washed off during the staining procedure. Sixty-seven inadequate specimens were tested again; 30 had adequate numbers of cells on repeat testing, but 37 still had cells in one or more wells of the eight-well slide wash off during staining. Uninterpretable IFA direct staining results were due to specific fluorescence in more than one well of the eight-well slide; such slides were not tested again, but the stain apparently carried over from one positive well to surrounding wells, making interpretation difficult. For 882 interpretable results, the sensitivity and specificity of Bartels IFA direct staining versus isolation in shell vial cultures were 85.9 and 87.1%, respectively.

A comparison of Bartels IFA direct stain and shell vial culture results by virus is shown in Table 2. Bartels IFA direct staining detected 175 of 204 (85.8%) RSV isolates, 9 of 21 (42.9%) influenza A virus isolates, 15 of 24 (62.5%) influenza B virus isolates, 24 of 35 (68.6%) parainfluenza virus isolates, and 2 of 7 (28.6%) adenovirus isolates.

Bartels IFA direct staining detected 80 additional positive specimens which were not detected by shell vial culturing. Bartels IFA direct staining detected 72 RSV antigen-positive specimens which were shell vial culture negative. Fifty-nine of these 72 were confirmed positive by Ortho RSV staining. Since RSV was the predominant isolate in this study, Bartels IFA direct staining for RSV was compared with Ortho RSV staining with all 1,065 specimens. Ortho RSV staining detected 11 RSV antigen-positive specimens which were found negative by Bartels IFA direct staining; 3 of these 11 were confirmed positive by RSV isolation in shell vial cultures. Bartels IFA direct staining detected 19 antigen-positive specimens which were found negative by Ortho RSV staining; 6 of these 19 were confirmed positive by shell vial

culturing. Seven of 618 specimens which were found negative by both antigen detection methods were found positive for RSV in shell vial cultures. One hundred eighty-two specimens were uninterpretable by Bartels IFA direct staining because of inadequate numbers of cells or interfering fluorescence, and 26 of these specimens also had inadequate numbers of cells on the Ortho RSV stain slides. Ortho RSV stain specimens which were inadequate were generally due to poor specimen quality and not to cells washing off the slides. Two specimens which had inadequate numbers of cells for Ortho RSV staining were acceptable for Bartels IFA direct staining. The sensitivity, specificity, positive predictive value, negative predictive value, and overall agreement of Bartels IFA direct staining versus Ortho RSV staining for 881 interpretable specimens were 95.5, 97, 92.5, 98.3, and 96.6%, respectively.

### DISCUSSION

Rapid diagnosis of respiratory viral infections can have an impact on patient care by indicating appropriate antiviral therapy, eliminating unnecessary antibacterial therapy, and determining patient isolation requirements (10, 14, 15). This study evaluated rapid diagnosis of respiratory viruses by shell vial culturing and direct staining of cells in respiratory tract specimens. Shell vial cultures combined with Bartels IFA reagents were shown to be equivalent to standard tube cultures in the first phase of this study. Shell vial cultures were negative for four standard tube culture-positive isolates but detected five isolates which were not detected by standard tube cultures. The significant advantage of using shell vial cultures is the decreased turnaround time, from 7 to 10 days to 2 to 3 days. Technologist time is also decreased with shell vial cultures, since the vials are not examined daily for cytopathic effect development. Since both methods rely on immunofluorescence staining for a definitive endpoint, no increases in costs were realized in the application of shell vials and Bartels IFA reagents. The antiserum pool was shown to be effective in screening for positive specimens in both tube and shell vial cultures.

Comparable shell vial and tube culture results in the first group of specimens tested allowed the use of shell vials only for the remaining specimens. Shell vial cultures showed essentially the same isolation rates for all 3 years of testing. As this study demonstrates, the primary respiratory virus isolated at our tertiary-care center is RSV, and the limited number of influenza A and B virus, parainfluenza virus, and adenovirus isolates necessitates further study to support shell vial culturing and Bartels IFA staining for all seven viruses.

The second aspect of this study evaluated Bartels IFA reagents for direct staining of patient specimens. The sensitivity of Bartels IFA direct staining with the individual reagents versus shell vial culturing was 85.9% for the adequate specimens. This result is consistent with those obtained by other direct antigen detection methods (7, 9, 11). Direct staining with the antiserum pool was not effective in screening for positive specimens because of the problems in interpretation caused by all the extraneous fluorescence.

Since RSV was the predominant isolate in this study, it was of particular interest to compare Bartels IFA direct staining for RSV with Ortho RSV staining, which has been our standard test for several years. This study showed that the two methods were comparable and that no single method detected all RSV-positive specimens. Fifty nine specimens (5.5%) were found positive by both RSV antigen detection

methods but were shell vial culture negative. These results were attributed to delays in the inoculation of cultures due to staffing limitations or tissue culture cell shortages and to problems in the delivery of specimens from outpatient clinics or outside health care facilities. Also, nonviable RSV has been detected by immunofluorescence in the late stages of infection (4).

In this study, the sensitivity of direct staining decreased from 85.9 to 77.3% when the uninterpretable results which were culture positive were included. The large number of specimens with inadequate numbers of cells for interpretation is a major problem with Bartels IFA direct staining. The problem is due to cells washing off one or more wells during staining. The washing procedure for Bartels IFA direct staining requires rinsing of the slides with a gentle stream of PBS directed away from the cell wells and soaking of the slides in PBS for 5 min. Ortho RSV staining does not require soaking of the slides, and we did not experience significant problems with cells washing off with Ortho RSV staining. However, when we tried to minimize the effects of washing with Bartels IFA direct staining by elimination of the soaking step or by more gentle manipulation of the PBS rinse, there were more problems with nonspecific fluorescence and with specific fluorescence that seemed to carry over from one positive well into adjacent wells of the eight-well slide. The presence of excess mucus in a specimen also appeared to contribute to cells washing off and to increased nonspecific fluorescence, so the majority of the last 430 specimens tested were washed a minimum of three times prior to slide preparation; however, the problem with cells washing off the slides persisted. Repeat testing of inadequate specimens was less than 50% effective in preventing the cells from washing off the slides.

The need for repeating Bartels IFA direct staining and the inability to use the antiserum pool as a screening test because of interpretation difficulties proved extremely costly. The Bartels kit has sufficient antisera to screen 100 specimens with the antiserum pool but only enough individual reagents to stain 25 slides. Eliminating the pooled antiserum screen quadruples the cost to almost \$32 per test, since only 25 tests per kit can be completed instead of 100 tests. The cost of Bartels IFA direct staining would have to be weighed against the benefits of having same-day test results for the seven respiratory viruses. We believe that Bartels IFA direct staining could be an extremely effective same-day test if the problems with inadequate numbers of cells could be eliminated, and efforts will be made to overcome these problems.

Even with the problems encountered, IFA direct staining provides a useful screen for our laboratory, which serves a 904-bed tertiary-care hospital with a large number of immunocompromised pediatric and adult patients. Our protocol for respiratory virus testing, based on the viral prevalence and test performance results of this study, will be to screen specimens for RSV (Ortho RSV staining) and then test all Ortho RSV stain-negative specimens by Bartels IFA individual reagent direct staining and shell vial culturing.

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### REFERENCES

1. Ahluwalia, G., J. Embree, P. McNicol, B. Law, and G. W. Hammond. 1987. Comparison of nasopharyngeal aspirate and

- nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **25**:763-767.
2. **Bartholoma, N. Y., and B. A. Forbes.** 1989. Successful use of shell vial centrifugation and 16 to 18-hour immunofluorescent staining for the detection of influenza A and B in clinical specimens. *Am. J. Clin. Pathol.* **92**:487-490.
  3. **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.
  4. **Gardner, P. S., J. McQuillin, and R. McGucken.** 1970. The late detection of respiratory syncytial virus in cells of respiratory tract by immunofluorescence. *J. Hyg.* **78**:575-580.
  5. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1985. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J. Clin. Microbiol.* **21**:217-221.
  6. **Gleaves, C. A., D. J. Wilson, A. D. Wold, and T. F. Smith.** 1985. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h postinoculation. *J. Clin. Microbiol.* **21**:29-32.
  7. **Halstead, D. C., S. Todd, and G. Fritch.** 1990. Evaluation of five methods for respiratory syncytial virus detection. *J. Clin. Microbiol.* **28**:1021-1025.
  8. **Hughes, J. H., D. R. Mann, and V. V. Hamparian.** 1988. Detection of respiratory syncytial virus in clinical specimens by viral culture, direct and indirect immunofluorescence, and enzyme immunoassay. *J. Clin. Microbiol.* **26**:588-591.
  9. **Johnston, S. L., 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.
  10. **Palumbo, P. E., and R. G. Douglas, Jr.** 1986. Respiratory tract infections, p. 263-282. *In* S. Spector and G. J. Lancz (ed.), *Clinical virology manual*. Elsevier Science Publishing, Inc., New York.
  11. **Ray, C. G., and L. L. Minnich.** 1987. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. *J. Clin. Microbiol.* **25**:355-357.
  12. **Smith, M. C., C. Creutz, and Y. T. Huang.** 1991. Detection of respiratory syncytial virus in nasopharyngeal secretions by shell vial technique. *J. Clin. Microbiol.* **29**:463-465.
  13. **Stout, C., M. D. Murphy, S. Lawrence, and S. Julian.** 1989. Evaluation of a monoclonal antibody pool for rapid diagnosis of respiratory viral infections. *J. Clin. Microbiol.* **27**:448-452.
  14. **Sullivan, C. J., and M. C. Jordan.** 1988. Diagnosis of viral pneumonia. *Semin. Respir. Infect.* **3**:148-161.
  15. **Welliver, R. C.** 1988. Detection, pathogenesis, and therapy of respiratory syncytial virus infections. *Clin. Microbiol. Rev.* **1**:27-39.