

## Evaluation of R-Mix FreshCells in Shell Vials for Detection of Respiratory Viruses

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**The performance of a mixture of mink lung and A549 cell lines in shell vials (MSVs) for the detection of respiratory viruses in 159 specimens was evaluated. MSVs, conventional culture, and direct immunofluorescence assay identified 96, 85, and 67% of the influenza A virus-positive specimens, respectively. MSVs provided both a high degree of sensitivity and rapid turnaround times for the detection of influenza A virus.**

During the winter season, diagnostic virology laboratories must provide a rapid, accurate, and sensitive means of identification of respiratory viruses. Influenza viruses are the most frequently detected viral pathogens. Hence, the rapid detection and differentiation of influenza viruses from other common respiratory viruses is important, since treatment is available for both influenza A and B viral infections (12).

Direct detection of antigen in clinical specimens, including by direct immunofluorescence assays (DFAs) and enzyme immunoassays (EIAs), offers rapid turnaround times but is highly dependent on the quality of the specimen. EIAs are costly and do not allow determination of sample quality. DFAs cannot always be performed because of insufficient cell numbers in the specimen, and the interpretation of the results on DFA slides can at times be difficult and subjective. In addition, several studies have shown that these direct assays should be used in conjunction with cell culture assays (1, 2, 9).

Conventional culture (CC) for respiratory viruses typically requires the use of primary rhesus monkey kidney (RhMK) cells as well as a number of continuous cell lines such as A549, HEp-2, MRC5, and/or Madin-Darby canine kidney (MDCK) cells. CC is generally sensitive but is often too slow to provide useful clinical information. The use of spin amplified shell vial cultures in combination with pooled monoclonal antibodies was an important advance because it shortened the turnaround time and increased the sensitivity of virus detection (3, 7, 10, 11). However, multiple types of cell cultures must be used for each specimen in order to detect different respiratory viruses.

Use of a combination of several selected cell lines in a single tube or shell vial allows the simultaneous detection of multiple types of respiratory viruses and thereby eliminates the need to use different types of cell cultures separately. Mink lung cells have recently been shown to be highly sensitive to influenza A and B viruses (4, 8), and the combination of A549 and mink lung cells was found to have increased susceptibility to other respiratory viruses (4). Navarro-Mari et al. (6) have also reported on the rapid detection of respiratory viruses using a simultaneous culture of three cell lines in the same shell vial. Furthermore, the R-Mix FreshCells product, which incorporates mink lung and A549 cells in a single monolayer, is now

available commercially. Recently, the use of R-mix FreshCells for detection of respiratory viruses has been explored by several investigators, as documented by presentations at the 15th and 16th Annual Clinical Virology Symposia (1999 and 2000). However, the performance of this type of cell culture compared to those of CC and DFA has not been published, and only two of the abstracts from the symposia presented the results of such comparisons (N. Patel, R. Hartwig, I. Kauffmann, and M. R. Evans, Abstr. 15th Annual Clinical Virology Symposium, abstr. S10, 1999; C. K. Y. Fong, M. K. Lee, and B. P. Griffith, Abstr. 16th Annual Clinical Virology Symposium, abstr. S16, 2000).

In the present study, we compared the R-Mix FreshCells product in shell vials (MSVs), DFA, and CC for the detection of respiratory viruses in a total of 159 specimens. Most of these ( $n = 135$ ) were fresh specimens obtained between November 1999 and February 2000 at Veterans Affairs (VA) and non-VA hospitals. The other 24 specimens were influenza A virus-positive specimens that had been stored at  $-70^{\circ}\text{C}$ ; these specimens had been collected during the 1998–1999 influenza season. The specimens comprised the following types: nasopharyngeal swab ( $n = 132$ ), bronchoalveolar lavage ( $n = 12$ ), pleural fluid ( $n = 2$ ), sputum ( $n = 2$ ), and lung tissue ( $n = 1$ ), specimens. For 10 of the respiratory specimens, a collection site was not specified.

MSVs were obtained from Diagnostic Hybrids, Inc. (Athens, Ohio). The culture medium was removed from each MSV before inoculation with each specimen (0.2 ml per vial). The MSVs were then centrifuged at  $800 \times g$  for 40 min and refed with refeed medium, which was serum-free and which contained trypsin (Diagnostic Hybrids, Inc.). The coverslips were fixed with acetone and were stained with monoclonal antibody to influenza type A (Centers for Disease Control and Prevention reagent) at 20 to 24 h and with the respiratory virus screen IFA kit reagent (Chemicon International Inc., Temeluca, Calif.) for detection of seven respiratory viruses, including adenovirus, influenza A and B viruses, parainfluenza type 1, 2, and 3 viruses, and respiratory syncytial virus (RSV), at 40 to 44 h. The coverslips were examined with a UV-light fluorescence microscope at  $\times 200$  magnification.

For CC, specimens were inoculated into culture tubes with RhMK, A549, and MRC5 cells. Specimens submitted for influenza virus isolation were inoculated only into RhMK cells and refed with serum-free culture medium. The culture tubes were incubated at  $35^{\circ}\text{C}$  in a roller drum and were examined daily for cytopathic effect. Hemadsorption (HAD) tests were performed on days 3 and 7 after inoculation with a 0.5%

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TABLE 1. Detection of influenza A and other respiratory viruses by MSV, CC, and DFA

Method (no. of samples tested)	No. of samples positive <sup>a</sup>			No. of samples negative	No. of samples <sup>b</sup> with no result
	Influenza A virus	RSV	Parainfluenza type 3 virus		
MSV (159)	62	3	1	91	2
CC (158)	55	1	1	101	0
DFA (129)	33	7	NT <sup>c</sup>	65	24

<sup>a</sup> Overall, influenza A virus was detected in 64 samples, RSV was detected in 7 samples, and parainfluenza type 3 virus was detected in 1 specimen.

<sup>b</sup> These specimens were not evaluated because there were not enough cells in the samples or the stained slides were difficult to interpret and gave inconclusive results.

<sup>c</sup> NT, not tested.

guinea pig red blood cell suspension. HAD-positive cultures were further evaluated by immunofluorescence with monoclonal antibodies to influenza A and B viruses and parainfluenza viruses.

For DFA, specimens collected on swabs in viral transport medium were vortexed to release virus and virus-infected cells and were then centrifuged at 1,500 × g for 5 min. The cell pellets were resuspended in 0.6 to 0.8 ml of phosphate-buffered saline. For each specimen, three smears were prepared by cytocentrifugation in a cytospin apparatus. DFA slides were fixed, and the three smears were stained with the SimulFluor screen for seven respiratory viruses, SimulFluor RSV/FluA, and SimulFluor FluA/FluB (Chemicon International, Inc., Temeluca, Calif.), respectively.

Of the 159 specimens examined, 159, 158, and 129 specimens were evaluated by MSV, CC, and DFA, respectively. Seventy-two specimens were found to be virus positive (64 were positive for influenza A virus, 7 were positive for RSV, and 1 was positive for parainfluenza type 3 virus). A summary of the results obtained is shown in Table 1. Forty-eight of the influenza A virus-positive samples were evaluated by the three methods: 96, 85, and 67% were positive by MSV, CC, and DFA, respectively (data not shown). The turnaround times for DFA, MSV, and CC were 4 h, 1 day, and 2 to 5 days, respectively.

A total of 146 samples were evaluated by both CC and MSV (Table 2). MSV allowed the detection of 60 influenza A virus-positive samples, whereas CC detected only 54 influenza A virus-positive samples. Influenza A virus was detected by MSV but not by CC in six samples. CC had a sensitivity of 90% (54 of 60 samples), whereas MSV had a sensitivity of 100% (60 of 60 samples). Similarly, comparison of the results obtained for 97 samples tested by both DFA and MSV showed that MSV detected more influenza A virus-positive samples than DFA (Table 2). MSV allowed the detection of 39 influenza A virus-

TABLE 2. Comparison of MSV with CC and DFA for detection of influenza A virus

MSV result	No. of samples with the indicated result by:					
	CC			DFA		
	Positive	Negative	Total	Positive	Negative	Total
Positive	54	6	60	31	8	39
Negative	0	86	86	2 <sup>a</sup>	56	58
Total	54	92	146	33	64	97

<sup>a</sup> These two DFA-positive samples were MSV and CC negative.

positive samples, whereas DFA detected only 33 influenza A virus-positive samples. Influenza A virus was detected by MSV but not by DFA in eight samples. Only two samples were positive by DFA but negative by MSV. DFA had a sensitivity of 80% (33 of 41 samples), whereas MSV had a sensitivity of 95% (39 of 41 samples).

The use of mixtures of cell cultures for the detection of respiratory viruses has been described in two previous reports (4, 6). One study compared simultaneous cultures of HEp-2, LLC-MK2, and MDCK cells in shell vials with conventional culture and found that the mixed cell vial assay detected 95% of the viruses in 48 h, whereas CC detected 98% of viruses within an average of 6 days (6). In the present comparison of MSV, CC, and DFA, we used MSV in combination with monoclonal antibody pools and found MSV to be more sensitive than CC and DFA for the detection of influenza A virus. Similar results were obtained by Patel et al. (15th Annual Clinical Virology Symposium, 1999). It should be noted that the majority of samples evaluated in the present study were shipped from distant sites. Hence, it is possible that MSV allowed the detection of samples with low levels of virus infectivity that were missed by CC. Also, MSV may be more sensitive than DFA when specimens with insufficient numbers of cells are tested.

Conclusions regarding the results obtained for the detection of RSV must be viewed with caution in light of the small number of RSV-positive specimens detected in the present study. Nevertheless, our results with MSV appear comparable to those of previous studies with shell vials with single cell types; DFA was more sensitive than centrifugation culture for the detection of RSV, and centrifugation culture was more sensitive than CC for the detection of RSV (5, 10).

In addition to improved sensitivity, the MSV procedure described in this paper had several advantages over other established techniques. First, use of MSV in combination with antibody pools really simplified the respiratory virus isolation procedure because it allowed the detection of different virus types in a single shell vial culture. Second, the procedure was less labor intensive than CC. In addition, compared to the results on DFA slides, the results on MSV coverslips were easier to read and interpret. Third, the use of mink lung cells for the detection of influenza and parainfluenza viruses was a good and sensitive alternative to the use of RhMK cells, which are often difficult to manage because of lot-to-lot variability and the presence of latent monkey viruses. Finally, detection of all seven respiratory viruses could occur within 2 days, and the turnaround time for the detection of influenza virus was 1 day. Overall, these data demonstrate the usefulness of MSV for the laboratory detection of influenza A virus, providing a procedure that combines speed, sensitivity, and ease of performance.

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