Laboratory Diagnosis of Respiratory Virus Infections in 24 Hours by Utilizing Shell Vial Cultures

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Immunofluorescence staining of centrifugation-enhanced shell vial (SV) cultures for respiratory viruses (RV) after 24 h of incubation, rather than the more commonly prescribed times of 48 h and 5 days, allowed for the detection of 77% of the RV-positive specimens that would ordinarily not have been detected as positive until 48 h. Staining SVs at 24 h also permitted earlier detection of viruses that were missed by rapid antigen detection methods.

Respiratory viruses (RV) can cause a wide variety of human disease. Detection by the diagnostic virology laboratory of these viruses in clinical specimens may impact on the medical management of the patient and can be of epidemiological importance. Many virology laboratories detect RV by inoculating conventional cell culture tubes with respiratory samples and then examining for cytopathic effect and/or hemadsorption. This procedure, however, can require many days or even weeks for viral detection and identification, providing culture results to clinicians in a period of time that may not be clinically useful.

Laboratory diagnosis of RV infections has become easier and more rapid with the use of centrifugation-enhanced shell vials (SV) as the culture method. Centrifugation of the clinical specimen onto cell monolayers followed by immunofluorescence detection and identification of viral antigens in SV cultures allows a much earlier diagnosis of infection. Since it was first described for the rapid detection of cytomegalovirus (2), the SV method has been successfully used to rapidly detect RV infections (6, 7). However, what the optimal times are to stain SV cultures for RV is unclear. Many laboratories have felt, as we have, that staining at 48 h, and if negative, again at 5 days, would allow the most sensitive and cost-effective early detection of an RV. However, pressure for an even earlier detection is often placed upon the laboratory by clinicians, most especially when immunocompromised patients are involved, so that clinically relevant decisions can be made. This study assessed the value of immunofluorescence staining of SV cultures for RV at 18 to 24 h compared with 48 h and 5 days to determine whether such a procedure would allow a more rapid detection and reporting of positive clinical specimens.

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Over a 24-month period, from February 1995 through January 1997, a total of 603 clinical specimens from mainly immunocompromised patients at the Clinical Center of the National Institutes of Health were submitted to the Microbiology Service for RV culture. These specimens included bronchoalveolar lavages (BAL) (n = 297), nasopharyngeal washes or swabs (NP) (n = 188), throat swabs (THR) (n = 52), sputum samples (n = 4), biopsies (n = 2), and pleural fluids (n = 2). Specimens from nonrespiratory sources included urine samples (n = 43), eye swabs (n = 13), and samples of cerebrospinal fluid (n = 2). Urine samples and eye swabs were cultured specifically for adenovirus (AD), which can be a cause of hemorrhagic cystitis and conjunctivitis, while samples of cerebrospinal fluid were cultured for AD and influenza, which are rare causes of meningitis and encephalitis. Specimens were processed either on the day of receipt in the laboratory or after overnight storage at 4°C. All specimen samples were vortexed for 30 s, sonicated for 1 min in a Sonicator Ultrasonic Processor (model XL-2020; Heat Systems Ultrasound, Plainview, N.Y.) equipped with a cup-horn apparatus, and then centrifuged at 500 × g for 5 min to obtain a cell-free supernatant for SV inoculation.

Four SV containing human lung carcinoma cells (A549) and four SV containing primary rhesus monkey kidney cells (Bio-Whittaker, Walkersville, Md.) were inoculated for each specimen with 0.2 ml of cell-free specimen supernatant for the recovery of the following seven RV: AD, influenza A (FLU-A), influenza B (FLU-B), parainfluenza 1 (PARA-1), parainfluenza 2 (PARA-2), parainfluenza 3 (PARA-3), and respiratory syncytial virus (RSV). SV were centrifuged at 3,500 × g for 15 min at 25°C, as previously described (1), in a Centra GP8R refrigerated centrifuge (International Equipment Co., Needham Heights, Mass.). Following centrifugation, SV monolayers were rinsed with sterile phosphate-buffered saline before 1 ml of tissue culture refeeding medium (Bartels, Inc., Issaquah, Wash.) was added to the A549 SV or 1 ml of low serum medium (Bartels) was added to the rhesus monkey kidney SV. SV were then incubated at 35°C.

SV coverslips monolayers were fixed in acetone and stained at 18 to 24 h, 48 h, and 5 days with a mouse monoclonal antiviral primary antibody followed by a fluorescein-labelled anti-mouse secondary antibody (Viral Respiratory Screening and Identification Kit, Bartels). Coverslips were mounted on glass slides and examined for apple green fluorescence with a Zeiss fluorescence microscope. Monolayers of each of the two cell lines were first stained with a pool of monoclonal antibodies directed against all seven of the RV, and if positive, the cell monolayer of another inoculated SV was scraped and spotted onto an eight-well glass slide. Each well was then stained with a monoclonal antibody directed against a specific virus for final identification. If the amount of positive staining seen with the
pool was judged to be too light to scrape, scraping of another SV culture for final virus identification was postponed until the next working day. In these instances, a presumptive identification of the virus was attempted based on the immunofluorescence staining pattern of virus-infected cells observed on the antibody pool-stained coverslips.

Rapid direct detection of RSV viral antigen was performed on all NP with the TestPack RSV test (Abbott Laboratories, N. Chicago, Ill.) and on all BAL by direct fluorescent antibody staining (RSV Direct Fluorescent Antibody test, Bartels) of cytocentrifuge-prepared smears, according to the manufacturer's directions. Rapid direct detection of FLU-A viral antigen was performed on all NP and THR with the Directigen Flu A Test (Becton Dickinson, Cockeysville, Md.), according to the manufacturer's directions.

Of 603 clinical specimens submitted for RV culture, 65 (11%) were positive for one of the RV. By 24 h, RV in 50 of the 65 (77%) positive specimens were detected with the monoclonal antibody pool (Table 1), and 38 (76%) of these were able to be identified at that time. After further SV incubation to obtain growth sufficient for scraping the cell monolayer, four additional virus isolates (one FLU-A, one PARA-3, and two RSV isolates) that were detected at 24 h were identified the next day, and the other eight (one AD, two FLU-A, one PARA-2, two PARA-3, and two RSV isolates) were identified 2 to 5 days later. SV cultures stained with the pool at 48 h detected an additional nine (14%) positive specimens, four with RV (1 AD, 1 FLU-A, and 2 RSV isolates) which were identified the same day and three with RV (2 AD and one PARA-1 isolate) that were identified 3 to 4 days later. There were two specimens positive for an RV with the antibody pool at 48 h that never grew adequately enough for them to be identified, even after several passages. SV cultures stained at 5 days detected an additional six (9%) positive specimens, five of which contained RV (three AD, one FLU-B, and one RSV isolate) that were identified the same day.

Of 11 specimens in which RSV was detected at 24 h, 3 NP were negative by the TestPack RSV test and 1 BAL was negative by direct fluorescent antibody staining for RSV. Of nine specimens in which FLU-A was detected at 24 h, three NP and two THR were negative by the Directigen Flu A test.

We have shown that staining SV at 18 to 24 h is of significant value for making a more rapid laboratory diagnosis of infection due to RV. Seventy-seven percent of the specimens positive for an RV that would normally not have been detected as positive until 48 h were detected within a 24-h period, with final identification of the infecting viruses made the same day in 76% of these specimens. In those instances when same-day identification of a detected virus was not possible because of scant staining with the antibody pool, and for which there was an urgent clinical need for identification of the virus, we were able to report a presumptive identification (or at least narrow down the possibilities) based on which of the cell lines stained positive and, since the different groups of RV (i.e., AD, FLU, PARA, and RSV) give unique immunofluorescence staining patterns (i.e., cytoplasmic and/or nuclear fluorescence staining), based on the observed staining pattern of the virus-infected cells. This more rapid detection of an RV infection impacted on patient care by permitting earlier intervention by the hospital infection control service and implementation of appropriate infection control measures, such as the cohorting of known infected patients and placing these patients in respiratory isolation or the use of seasonal-infection precautions. Additional advantages included earlier administration of available antiviral chemotherapy and/or prophylaxis, if warranted, and elimination of unnecessary antibacterial therapy.

Most virology laboratories perform rapid diagnostic procedures for the direct detection of viral antigens in clinical specimens. This is done primarily for RSV and FLU-A, RV which have a significant impact on morbidity and mortality and on duration of hospitalization and for which antiviral chemotherapeutic agents are available. This testing can take the form of immunofluorescence staining of specimen smears and/or enzyme immunoassay techniques, with results obtained within 10 to 60 min. The sensitivity of these assays in comparison to culture can vary but most are in the range of 70 to 95% (3–5, 8). We found another advantage of staining SV as early as 18 to 24 h to be the more rapid detection of those positive specimens that were falsely negative by viral antigen detection assays. Thirty-six percent (4 of 11) of our specimens found to be positive for RSV at 24 h and 56% (5 of 9) of specimens positive for FLU-A at 24 h had negative results by our rapid antigen detection methods.

For those laboratories using both SV and conventional tube cultures, we suggest that SV cultures for RV be stained at 24 h, with two tubes containing cell lines of choice inoculated as a backup to detect those RV not detected in the SV at 24 h. For those laboratories using SV alone, we recommend that staining be performed at 24 h and on at least one other day. Each laboratory needs to make a decision as to what is clinically appropriate and cost-effective for its own institution. Further investigation will shortly begin to examine if staining SV at 3 or 4 days is sensitive enough to replace the 5-day stain, so that staining could be done at 24 h and then again at either 3 or 4 days.

REFERENCES

