Rapid Detection of Respiratory Syncytial Virus in Nasopharyngeal Secretions by Enzyme-Linked Immunosorbent Assay

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A new enzyme-linked immunosorbent assay (ELISA) respiratory syncytial virus antigen detection kit (Ortho Diagnostics, Inc., Raritan, N.J.) was compared with virus culture and with the indirect fluorescent antibody test (FAT) by using fresh nasal washings from children with suspected respiratory syncytial virus infection. Both uncentrifuged nasal washings and pellets from centrifuged split specimens were examined by ELISA. The ELISA was considered positive when the optical density was greater than the mean background optical density plus 0.15. Specimens positive by ELISA but negative by culture and FAT were reexamined in an ELISA blocking assay. Of 337 uncentrifuged specimens, 124 (37%) were positive by culture, 107 (32%) were positive by FAT, and 166 (49%) were positive by ELISA. Blocking assays showed that 21 of 30 (70%) of the seemingly false-positive ELISA tests were, in fact, true-positives and that the cultures and FATs were falsely negative. A patient specimen was considered positive when it was positive by virus culture, FAT, or blocking assay. The sensitivity, specificity, and positive predictive value of the ELISA test were 88, 94, and 95%, respectively. Centrifugation of nasal washings raised the sensitivity from 88 to 92% but reduced the specificity from 94 to 81%. We conclude that the Ortho ELISA test of uncentrifuged nasal washings is more sensitive than virus culture or indirect FAT and is highly specific.

Respiratory syncytial virus (RSV) is the major cause of serious acute lower respiratory tract infection in infants and young children in the United States and worldwide (29). Bronchiolitis and pneumonia are the two common clinical presentations and frequently lead to hospitalization. Rapid viral diagnosis of RSV infection aids patients management and hospital infection control decisions by distinguishing RSV infection from other causes of respiratory infection and, in the future, will be important in selecting patients for antiviral therapy (9, 21, 28). Unfortunately, traditional tissue culture diagnostic methods are slow (5 to 10 days) (15, 25), and direct microscopic detection of RSV antigen in respiratory secretions by the direct or indirect fluorescent antibody test (FAT) requires considerable laboratory expertise and is not widely available (1, 4–7, 13–17, 23, 24). Detection of RSV antigen by the enzyme-linked immunosorbent assay (ELISA) technique is more rapid than tissue culture and technically less difficult and less subjective than FAT, but earlier studies of RSV ELISA tests developed in research laboratories did not show ELISA to be sufficiently sensitive (3, 11–13, 20, 27).

The purpose of this study was to compare the results of a new ELISA RSV antigen detection test (Ortho Diagnostics, Inc., Raritan, N.J.) with standard tissue culture and with the indirect FAT by using fresh nasal wash specimens from children with suspected RSV infection and to determine whether concentration of respiratory epithelial cells in nasal wash specimens by centrifugation enhances the sensitivity of the RSV ELISA.

MATERIALS AND METHODS

Patients. The patients were infants and young children hospitalized with acute lower respiratory tract infection in Denver area hospitals during January, February, and March 1984. Because the children were suspected clinically and epidemiologically to have RSV infection, nasal washings were collected for rapid viral diagnosis and submitted to the Pediatric Diagnostic Virology Laboratory.

Specimens. Nasopharyngeal secretions were collected by hospital staff nurses with a plastic catheter, a mucus trap, and irrigation with a small volume (about 1.0 ml) of sterile saline (6, 17). The nasal washing in the mucus trap was refrigerated in the clinical laboratory of the referring hospital until it could be transported on wet ice to our laboratory, usually within 24 h. Specimens were not diluted in transport medium, and instructions were given not to freeze the specimen. Upon arrival the specimen volume was brought to 2.0 to 3.0 ml with phosphate-buffered saline (pH 7.4) to assure a sufficient volume for testing. The specimens were unselected; none were excluded because of insufficient volume or delay in transport. Blood was not obtained for RSV serological studies.

ELISA procedure. The ELISA procedure was a microtiter, double-antibody technique. The capture antibody was a mixture of two monoclonal antibodies: one to an RSV nucleoprotein antigen and one to a capsid antigen. The detector antibody was peroxidase-conjugated bovine RSV antiserum. The substrate was o-phenylenediamine.

Using split specimens, we examined both uncentrifuged nasal washings and pellets from specimens centrifuged at 1,500 rpm (450 × g) for 10 min. Each test required 100 μl of specimen. The total time required to perform the test was <5 h including 4.5 h of incubation time. All tests were done in duplicate with positive and negative controls without knowledge of the results of the virus cultures or FAT. The optical density (OD) was read at 490 nm with a Bio-Tek EIA Reader model EL 307. An ELISA test was defined as positive when the mean OD was greater than the mean background OD plus 0.15; no borderline zone of OD was used in this study.

ELISA blocking test. To help determine whether speci-
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Anti-RSV antiserum for cultures and represented positive specimens in an ELISA blocking assay. Nasal specimens were retested with undiluted rabbit anti-RSV antiserum for 2 h at 37°C and then overnight at 4°C. Specimens incubated with undiluted normal rabbit serum served as controls. The ELISA test was done as previously described, and a positive result was defined as ≥50% reduction in OD compared with that of the normal rabbit serum control. As additional controls, the original specimen was retested and the positive controls provided in the kit were tested with rabbit anti-RSV antiserum and normal rabbit serum.

Virus cultures. HEp-2 cells (two tubes) and human embryonic lung fibroblasts (one tube) were inoculated with 0.1 ml of nasal washing per tube and incubated at 37°C for up to 21 days. Cultures exhibiting typical RSV cytopathic effect were confirmed by the indirect FAT (17, 18). Blind passages were not done routinely, but selected monolayers with questionable cytopathic effect were passaged to new tubes of HEp-2 cells before final reading.

FAT. Washed respiratory epithelial cells in centrifuged nasal washings were examined for RSV antigen by the indirect FAT as previously described (17). The test used rabbit antiserum to RSV produced in our laboratory and fluorescein-conjugated antibody to rabbit globulin (Meloy Laboratories, Inc. Springfield, Va.). The antiserum was prepared by immunizing New Zealand White rabbits with the Long strain of RSV grown in HEp-2 cells. The controls were positive and negative nasal washings. The coded slides were read by one experienced technician at ×400 magnification with a Zeiss microscope equipped for incident light fluorescence. A slide was judged to be positive when ≥1 cell was seen with characteristic 2 to 4 cytoplasmic fluorescein.

Statistics. The sensitivity, specificity, and positive and negative predictive values of the RSV ELISA were calculated by standard formulas (10). For these calculations we used three different definitions of a positive specimen: positive by culture only, positive by culture or FAT, and positive by culture, FAT, or blocking assay.

RESULTS

Nasal washings from 337 children were examined. The children ranged in age from 1 week to 5 years. Their mean age was 4.8 months; 94% were less than 12 months old. Sixty percent were boys. Of the specimens, 92% were received in the virology laboratory and tested <24 h after collection. Because of specimen collection over a weekend or unexplained delays, 2% of specimens were not tested until >72 h after collection. It was not possible to determine whether any specimens had been frozen.

Of the 337 uncentrifuged split specimens, 166 (49%) were positive by ELISA, 124 (37%) were positive by culture, 107 (32%) were positive by FAT, and 157 (47%) were positive by culture or FAT (Table 1). In addition, 21 of 30 (70%) uncentrifuged specimens which were seemingly falsely positive by ELISA were successfully neutralized in the ELISA blocking assay, usually to the OD of the negative control, indicating that they were true positive specimens. All nine remaining specimens were weakly positive initially (OD < 0.4). Six were negative when retested after storage at −70°C, and three could not be blocked in our assay system.

Because neither virus culture nor FAT alone is sufficiently sensitive in detecting RSV in clinical specimens to serve as a completely reliable standard for comparison, we calculated the accuracy of the ELISA based on three different definitions of a positive specimen (Table 2). The sensitivity was similar regardless of the manner of calculation, ranging from 86 to 88%. The specificity, however, varied from 72% when a specimen was positive by culture to 94% when a positive specimen was positive by culture, FAT, or blocking assay. We believe that the latter figure most closely represents the true specificity of the ELISA test.

Of the 337 centrifuged specimens, 191 (57%) were positive by ELISA, 124 (37%) were positive by culture, 157 (47%) were positive by culture or FAT, and 172 (51%) were positive by culture, FAT, or blocking assay (Tables 3 and 4). Depending on the definition of a positive specimen, the sensitivity and specificity ranged from 91 to 92% and 63 to 81%, respectively. Thus, centrifugation of specimens did not increase the sensitivity of the test appreciably and, because of more false-positive results, reduced the specificity rather markedly.

Of 124 (59%) culture-positive specimens, RSV was isolated from 73 (59%) in both HEp-2 cells and human embryonic lung fibroblasts, from 43 (35%) in HEp-2 cells only, and from 8 (6%) in lung fibroblasts only. Virus cultures became positive 1 to 31 days after inoculation; the mean time to positivity was 8 days. Viruses other than RSV were recovered from 15 specimens, including adenovirus (7 specimens), cytomegalovirus (3 specimens), enterovirus (2 specimens), and rhinovirus (3 specimens). Three of the specimens with adenovirus and one with cytomegalovirus also grew RSV. Three of these four mixed cultures were positive by RSV ELISA: the exception was an ELISA-negative specimen that did not become positive for RSV by culture until the culture was passaged and 31 days had elapsed.

### TABLE 1. Diagnostic results of ELISA RSV antigen test compared with virus culture, FAT, and ELISA blocking assay with uncentrifuged nasal washings

<table>
<thead>
<tr>
<th>ELISA result (no.)</th>
<th>Culture</th>
<th>FAT</th>
<th>Culture or FAT</th>
<th>Culture, FAT, or blocking assay&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos. (166)</td>
<td>107</td>
<td>59</td>
<td>101</td>
<td>65</td>
</tr>
<tr>
<td>Neg. (171)</td>
<td>17</td>
<td>134</td>
<td>6</td>
<td>165</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pos., Positive; Neg., negative.

<sup>b</sup> Of 30 uncentrifuged specimens positive by ELISA only, 21 were neutralized in an ELISA blocking assay.

### TABLE 2. Accuracy of ELISA RSV antigen test compared with virus culture, FAT, and ELISA blocking assay with uncentrifuged nasal washings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Accuracy of ELISA vs:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>FAT</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>86.3</td>
<td>94.4</td>
</tr>
<tr>
<td>Specificity</td>
<td>72.3</td>
<td>71.7</td>
</tr>
<tr>
<td>Positive predictive</td>
<td>64.5</td>
<td>60.8</td>
</tr>
<tr>
<td>value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative predictive</td>
<td>90.1</td>
<td>96.5</td>
</tr>
<tr>
<td>value</td>
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<td></td>
</tr>
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</table>
DISCUSSION

Laboratory diagnosis of RSV infection is useful to the clinician for several reasons. The children most severely affected by RSV usually are very young, less than 1 year old, or suffer from underlying cardiopulmonary disease (4, 8, 19, 29). In these infants it is often difficult to distinguish acute lower respiratory tract infection due to RSV from bacterial pneumonia or other infectious and noninfectious respiratory problems. Laboratory confirmation of RSV infection may help support a decision to discontinue administration of antimicrobial agents or to permit earlier discharge from hospital. Also, severely ill infants may be candidates for antiviral therapy with ribavirin or the future other agents (9, 21, 28). Intelligent selection of patients for early antiviral therapy will require the availability of a reliable rapid viral diagnostic technique like ELISA. Finally, diagnosis of RSV infection facilitates hospital infection control measures (4, 25). During community outbreaks of RSV, nosocomial transmission of RSV on pediatric wards is a serious problem (8). Isolation precautions, hand washing, and cohorting of RSV-positive infants may help to limit transmission of RSV to other susceptible children.

Because isolation of RSV in tissue culture is slow and not widely available, there has been interest in the development of simple rapid diagnostic techniques aimed at detecting viral antigen directly in respiratory secretions. These alternative techniques include direct and indirect immunofluorescence microscopy with polyclonal antisera or monoclonal antibody, immunoperoxidase (IP), ELISA, and radioimmunoassay (26, 30).

The sensitivity of the FAT in various laboratories has been reported to be 45 to 98% compared with that of culture (1-3, 6, 13-17, 22, 23). In our laboratory in 1982, we found the FAT to be 95% sensitive and 87% specific versus virus culture (17). The sensitivity of IP and agreement between IP and FAT in a recent study was 100% (2). However, in an earlier multicenter study sponsored by the World Health Organization, the sensitivity of IP compared with that of culture was only about 60% (3). Both FAT and IP techniques require a skilled microscopist to correctly interpret the slides, and slides may be uninterpretable when an adequate number of respiratory epithelial cells are not present in the specimen. Quality control of both techniques is difficult without the availability of a tissue culture laboratory.

The reported sensitivity of ELISA and radioimmunoassay tests developed in research laboratories has ranged from 61 to 82% compared with that of culture (3, 11-13, 20, 22, 27). In the studies that included FAT as well, the ELISA was found to be no more sensitive than the FAT (3, 13, 20). Only the FAT and the Ortho ELISA are commercially available in the United States at this time.

In this study, the Ortho ELISA RSV test was highly accurate in detecting RSV antigen in uncentrifuged nasopharyngeal secretions. It was more sensitive than either virus culture or FAT alone and was also highly specific. Centrifugation of specimens to concentrate antigen-bearing respiratory epithelial cells did not enhance the sensitivity of the ELISA and led to a reduction in specificity. The ELISA was simple to perform, and each run was completed in less than 5 h.

Of special interest, 21 of 30 uncentrifuged specimens positive by ELISA but negative by culture and FAT were neutralized in an ELISA blocking assay, indicating that they were true-positive specimens. Thus, only 9 of 337 specimens ultimately were judged to be falsely positive. Similar observations about discrepancies between FAT and culture have been made by Gardner and others who have shown that cultures frequently become negative late in illness when antigen detectable by FAT, and presumably by ELISA, is still present (7, 17, 22). Freezing of specimens also may lead to discrepancies between results of culture and ELISA because RSV does not survive freezing well while RSV antigen is quite stable (10, 18, 20).

We have not yet had the opportunity to test by ELISA other types of clinical specimens such as nasopharyngeal swabs, tracheal aspirates, or lung tissue. Based on our experience with the FAT and on the experience of others, we believe that nasopharyngeal swab specimens probably will not be satisfactory unless they are sufficient in volume and numbers of respiratory epithelial cells (20). Until the appropriate studies are done, nasal washings should be considered the preferred specimen for testing by the Ortho RSV ELISA.

ACKNOWLEDGMENTS

We thank the technologists in the Pediatric Diagnostic Virology Laboratory, University of Colorado Health Sciences Center, Denver, for excellent technical assistance and Caroline N. V. Adams for typing the manuscript.

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LITERATURE CITED


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**TABLE 3.** Diagnostic results of ELISA RSV antigen test compared with virus culture, FAT, and ELISA blocking assay with centrifuged nasal washings

<table>
<thead>
<tr>
<th>ELISA result (no.)</th>
<th>Culture</th>
<th>FAT</th>
<th>Culture or FAT</th>
<th>Culture, FAT, or blocking assay*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos. (190)</td>
<td>112</td>
<td>78</td>
<td>106</td>
<td>84</td>
</tr>
<tr>
<td>Neg. (147)</td>
<td>12</td>
<td>135</td>
<td>1</td>
<td>146</td>
</tr>
</tbody>
</table>

* Pos., Positive; Neg., negative.

* Of 47 centrifuged specimens positive by ELISA only, 15 were neutralized in an ELISA blocking assay.

**TABLE 4.** Accuracy of ELISA RSV antigen test compared with virus culture, FAT, and ELISA blocking assay with centrifuged nasal washings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Accuracy of ELISA vs:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>63.4</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>58.9</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>91.8</td>
</tr>
</tbody>
</table>
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