Comparison of Two New Tests for Rapid Diagnosis of Respiratory Syncytial Virus Infections by Enzyme-Linked Immunosorbent Assay and Immunofluorescence Techniques

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The sensitivity and the specificity of two new commercial reagent tests, an indirect fluorescent antibody test (FAT) with a mouse monoclonal antibody (MAb) against respiratory syncytial virus (RSV) and an enzyme-linked immunosorbent assay (ELISA) RSV antigen detection kit, were determined by a comparison of results from these tests with those of tissue culture isolation and an indirect FAT with bovine polyclonal antibody (BPA). Of 251 nasal aspirates from infants with suspected RSV infection, positive results were found for 99 (39%) by the FAT-MAb, 93 (37%) by the FAT-BPA, and 87 (35%) by the ELISA; 69 of 240 (29%) were positive by cultures. The FAT-MAb was a more sensitive technique than cultures, with 87% sensitivity for the FAT-MAb and 84% for the ELISA. It was also more sensitive than the FAT-BPA, with 97% sensitivity for the FAT-MAb and 85% for the ELISA. This could be caused only by the distinctive volume of suspended specimens used in these tests. Of 171 negative culture specimens, positive (but not false-positive) results were found for 18% by the FAT-MAb and for 12% by the ELISA. Inversely, 13% of 69 culture positive specimens were FAT-MAb negative and 16% were ELISA negative, emphasizing the importance of tissue cultures for the maximum recovery of RSV, as well as for detection of other respiratory viruses. The FAT-MAb and ELISA were easy to perform and interpret, thus facilitating wider use.

As in other countries, respiratory syncytial virus (RSV) is in France the leading cause of serious acute lower respiratory viral disease in infants, occurring regularly as winter outbreaks (6). Direct microscopic detection of RSV antigen in respiratory secretions by the indirect fluorescent antibody test (FAT) as described by Gardner and McQuillen (7) is more rapid and technically less difficult than antigen isolation in cell cultures. Mouse monoclonal antibodies (MAbs) directed against different RSV epitopes have been produced and used to detect RSV from clinical specimens by the indirect FAT (2, 5, 14, 18, 19). The enzyme-linked immunosorbent assay (ELISA) was introduced for the detection of RSV antigens in nasopharyngeal secretions in 1979 (4). The ELISA has been shown to be less or more sensitive than the indirect FAT or tissue cultures, respectively (3, 8, 9, 11, 12, 15, 16, 20, 21). Recently two new kits have become commercially available for rapid detection of RSV antigens: an indirect FAT with a mouse MAb directed against the nucleoprotein of RSV (Diagnostics Pasteur, Marnes, France) and an ELISA RSV antigen test (Abbott Laboratories, Rungis, France). The purpose of this study was to compare the results of these new tests with those of standard tissue cultures and an indirect FAT with a polyclonal antiserum for use on fresh nasal aspirates from infants with suspected RSV infection.

MATERIALS AND METHODS

Patients. The patients were infants and young children hospitalized with acute lower respiratory tract infections in the University Hospital of Caen (Normandy, France) during January and February 1986.

Specimens. Nasal secretions were collected by mechanical suction into mucus traps by hospital staff nurses and transported to the Virus Laboratory, usually within 3 h. Upon arrival, specimens were suspended in 5 ml of transport medium (Eagle minimal essential medium) (5 mg of bovine albumin per ml, 4.76 mg of HEPES [N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid] per ml, 1,500 U of penicillin per ml, 1.000 μg of streptomycin) and mixed.

FAT. Of the suspended specimens, 2 ml was used for the FAT. The cells were separated by centrifugation, washed in phosphate-buffered saline, deposited on microscope slides, and fixed in acetone. The indirect FAT was used (7). The standard test included bovine polyclonal antibody (BPA) (Wellcome Research Laboratories, Beckenham, England) and a rabbit anti-bovine fluorescein isothiocyanate conjugate (Nordic, Tilburg, The Netherlands) at dilutions of 1/10 and 1/30, respectively. The RSV-MAb is the 18B2 clone obtained from Pothier et al. (18). It came ready to be used in the kit. Anti-mouse fluorescein isothiocyanate conjugate (Diagnostics Pasteur) at a dilution of 1/10 was used in stage 2. All incubations were carried out at 37°C for 30 min, and slides were washed twice in phosphate-buffered saline. No counterstain was used. The stained slides were then examined for specific fluorescence, i.e., cytoplasmic inclusions with a granular aspect and heterogeneous sizes.

ELISA procedures. The ELISA was run by the protocol of the manufacturer. The suspended specimens (0.1 ml) were used in the test. Polystyrene beads were used for the solid phase, and goat anti-RSV antiserum was used to provide the capture antibody. Detector antibodies were from rabbit

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anti-RSV antiserum and peroxidase-conjugated goat antirabbit antiserum. The ELISA cutoff was the mean optical density of the negative controls at 492 nm plus a factor of 0.120. All specimens with an optical density within ±10% of the cutoff were considered borderline and were retested. A positive control was also used in the test.

**Virus cultures.** Cultures of MRC-5 human embryonic lung fibroblasts in 25-cm² surface flasks were inoculated with 0.2 ml of the suspended specimens and 0.2 ml of sheep antihuman alpha interferon at a dilution of 1/50, as previously described for the isolation of rhinovirus (F. Freymuth, M. Quibriac, J. Petitjean, C. Pierre, J. F. Duhamel, A. Denis, and C. Legoas, Arch. Fr. Pediatr. in press). Human alpha interferon antiserum was obtained as a gift from L. Montagnier, Institut Pasteur, Paris, France. Cultures exhibiting typical RSV cytopathic effects were confirmed by an indirect FAT-MAb. MRC-5 cells were kept 4 weeks before a culture was considered negative. Blind passages were not done routinely, and no other cell lines were used.

**RESULTS**

Nasal aspirates of 251 infants were examined. Positive results were found for 99 (39%) by the FAT-MAb, 93 (37%) by the FAT-BPA, and 87 (35%) by the ELISA. In the comparison between viral isolation (cultures) and the FAT-MAb or the FAT-BPA (Table 1), 11 specimens, including 9 with bacterial contamination and 2 with rhinovirus isolation, were excluded and could not be considered negative because we could not know whether they yielded RSV. Of the 240 samples remaining, 69 (29%) were positive by cultures. In nasal specimens of infants with suspected RSV infection, the FAT-MAb was 87% sensitive and 83% specific for the detection of RSV, as compared with the results of cultures. The FAT-MAb appeared to be more sensitive than RSV cultures in that 30 (18%) of 171 negative culture specimens were positive by this test. However, 9 (13%) of 69 positive culture specimens were negative by the FAT-MAb, and 11 (16%) were negative by the FAT-BPA, emphasizing the importance of using traditional tissue cultures to detect RSV as well as other respiratory viruses. The nasal aspirates included 99 specimens (41%) that were positive by a combination of cultures and the FAT-MAb.

Seventy-eight specimens were positive by the ELISA. Of seven borderline ELISA specimens, five were positive and 2 were negative when retested. The ELISA was 84% sensitive and 88% specific for the detection of RSV, as compared with the results of cultures. Altogether, 20 (12%) of 171 negative culture specimens were true positive for RSV by ELISA, as confirmed by the FAT-MAb and an ELISA blocking test. Also, 11 (16%) of 69 RSV-positive culture specimens were negative by the ELISA (and the FAT-MAb), 8 nasal aspirates were positive by cultures only, 10 samples were positive by the FAT-MAb only, but none was positive by the ELISA alone.

Because RSV cultures were shown in this study to be less sensitive for viral diagnosis than the indirect FAT, the two new tests, FAT-MAb and ELISA, were compared with the usual FAT-BPA performed in our laboratory for routine diagnosis of RSV infections in infants. (Table 2). One distinctive difference between the two FAT assays was that the FAT-MAb showed very distinct intracytoplasmic viral inclusions which contrasted with the surrounding darker cytoplasm, whereas we observed more background fluorescence in nasal aspirate cells with the FAT-BPA.

The FAT-MAb and the RSV ELISA were 94 and 95% specific, respectively, and 97 and 85% sensitive, respectively, as compared with the FAT-BPA. Thus, the FAT-MAb was more sensitive than the RSV ELISA. Of the 93 FAT-BPA-positive specimens, 3 were negative by the FAT-MAb and 14 were negative by the RSV ELISA.

**DISCUSSION**

The FAT-MAb proved to be a very useful diagnostic technique for rapid detection of RSV antigens in nasal aspirates. The fluorescence patterns do not require considerable experience to distinguish between viral and nonspecific background fluorescence (18), making the test easier to interpret. Its high sensitivity (87%) as compared with that of cultures has been reported by several investigators (2, 5, 14, 19). Bell et al. (2) reported 85% sensitivity with an indirect FAT with a mouse MAb directed against the nucleoprotein of RSV. Kim et al. (14) reported 93% sensitivity with two specific RSV MAbs, one directed against surface protein and the other directed against internal protein. Freke et al. (5) reported a sensitivity of 94% with a directly conjugated pool of five MAbs and 88% with an indirect FAT with the same MAb pool.

The mouse MAb of the Monofluokit RSV (Diagnostics Pasteur) is directed against the nucleoprotein of RSV. MAbs against nucleoprotein and fusion protein are the most useful means to detect RSV antigens in nasal aspirates. In the two new subtypes of RSV which have been recently described (1, 10, 17), the main antigenic difference was observed with large glycoprotein and not with the nucleoprotein and the fusion protein (17). In addition, anti-fusion protein MAbs and anti-nucleoprotein MAbs have been reported to stain cells in respiratory secretions more frequently than anti-glycoprotein MAbs do (19). The fusion protein and nucleoprotein are probably highly expressed in infected cells, and Ward et al. (24) have observed in infants an immunoglobulin G response to the nucleoprotein and the fusion protein but not to the glycoprotein.

Several reports have concluded that the FAT-MAb is more sensitive than cultures: Bell et al. (2) detected RSV in

<table>
<thead>
<tr>
<th>Specimen reaction in RSV culture</th>
<th>No. of specimens by reaction in assay*</th>
<th>FAT-MAb</th>
<th>FAT-BPA</th>
<th>RSV ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>60</td>
<td>9</td>
<td>58</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>14</td>
<td>26</td>
<td>145</td>
</tr>
</tbody>
</table>

* Relative to the sensitivity and specificity of the culture method, the respective performance values of the FAT-MAb were 87 and 83%, of the FAT-BPA were 84 and 85%, and of the RSV ELISA were 84 and 88%. Percentages were calculated by the method of Thorner and Remein (22).
19% of the negative culture specimens by the FAT-MAb, and Kim et al. (14) (as in our research) found the virus in 18% of such specimens. This could be explained by the appearance of specific antibodies with neutralizing activity in the nasal secretions of infants as early as 3 days after the onset of illness (13). In this study, this sensitivity could also be due to the use of 2 ml of the suspended specimens for the FAT-MAb and 0.2 ml for the cultures. In addition, the sensitivity of RSV isolation was probably lower than what would have been achieved if other cell lines, such as more sensitive HEp-2 cells, had been used (15). Inversely, 13% of the 69 positive culture specimens were FAT-MAb negative, in agreement with results reported by Bell et al. (15% [2]), Kim et al. (15% [14]), and Waner et al. (9% [23]). These findings emphasize the importance of traditional tissue cultures not only for isolating other respiratory viruses, but for detecting RSV in some cases in which direct antigenic detection by the FAT or ELISA is not efficient.

The reported sensitivity of the ELISA in various laboratories has ranged from 61 to 86% as compared with that of cultures (3, 4, 15, 20). In the studies that include the FAT and ELISA with polyclonal antibody as reagents, the ELISA has been found to be less sensitive than the FAT. The sensitivity of the ELISA compared with that of the indirect FAT has been measured as 85% (8), 83% (11), and 86% (16). Swenson and Kaplan (21) found that the Abbott RSV ELISA has a sensitivity and specificity of 87.5 and 95%, compared with the combination of virus isolation (cultures) and the FAT-BPA. Recently Hornsleth et al. (12) have described a biotin-avidin ELISA which is able to detect approximately 0.5 to 1.0 ng of RSV protein and is at least four times as sensitive as the conventional ELISA, which detects about 10 ng of RSV protein (8, 9). When compared with the FAT-BPA, the ELISA RSV antigen detection kit was less sensitive (85%) than the FAT-MAb (97%). In this study, this could be caused only by the distinctive volumes of suspended specimens used in the tests (2 ml each for the FAT-MAb and the FAT-BPA and 0.1 ml for the ELISA) and by the suspension of nasal aspirates in 5 ml of transport medium rather than the 2.5 ml recommended by the protocol of the manufacturer. However, the RSV ELISA has a specificity (95%) similar to that of the FAT-MAb. One other commercially available ELISA for the detection of RSV antigens with a mixture of two MAbS in the solid phase has been found to be more sensitive than the FAT-BPA (15).

The FAT-MAb and ELISA are both easy to perform and take 2 and 5 h to complete, respectively. Although we found the RSV ELISA to be less sensitive than the FAT-MAb, as compared with the FAT-BPA, the ELISA does not require either intact respiratory cells or investigators experienced with the indirect FAT and is an acceptable alternative when viral isolation and the FAT are not available.

LITERATURE CITED
21. Swenson, P. D., and M. H. Kaplan. 1986. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by a...

