Rapid Viral Diagnosis of Acute Respiratory Infections: Comparison of Enzyme-Linked Immunosorbent Assay and the Immunofluorescence Technique for Detection of Viral Antigens in Nasopharyngeal Secretions

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Nasopharyngeal secretions from adults and children were obtained in Stockholm, Sweden, for routine diagnosis of influenza A virus, influenza B virus, respiratory syncytial (RS) virus, parainfluenza type 3 virus, and adenovirus infections by demonstration of viral antigens directly in the specimens. The cells in nasopharyngeal secretions were isolated by centrifugation for preparation of cell deposits for diagnosis by the immunofluorescence technique (IF) in London, England, and in Stockholm, whereas the supernatants were used to diagnose infection by the enzyme-linked immunosorbent assay (ELISA) in Stockholm. Titration of the various purified viruses showed that ELISA could detect viral antigens in amounts corresponding to 1 to 10 ng of virus protein per test well. In a series of 73 specimens tested for influenza A, RS, and parainfluenza type 3 viruses by IF in London and by ELISA in Stockholm, 15 of 18 RS, 14 of 15 influenza A, and 2 of 2 parainfluenza type 3 viral infections were diagnosed by ELISA as compared with IF, giving sensitivities for RS and influenza A viral diagnosis of 93% and 83%, respectively, and a specificity of 100%. In another series of specimens from 35 patients tested for influenza B virus and adenovirus, five influenza B virus and four adenovirus infections were diagnosed by both methods; one additional adenovirus infection was detected only by IF and another only by ELISA. Comparisons of diagnostic results between the two methods performed in Stockholm gave nonagreement of results for 37 of 1,593 tests (2.5%) for the five viruses. The conclusion reached was that the described ELISA, although a satisfactory test, had somewhat less sensitivity than did IF for the detection of respiratory viral infections. This could possibly be explained by unnecessary dilutions of specimens at the time of collection; transportation, processing, and storage of specimens were less complicated than for IF.

The value of rapid viral diagnosis of respiratory infections has been pointed out in many publications (3, 12). Specific virus diagnosis obtained a few hours after the admittance of the patient to the hospital may lead to antiviral treatment (4) in children seriously ill with respiratory syncytial (RS) virus infections or to the omission of unnecessary antibiotic treatment. It is also an important aid in combatting hospital cross-infections.

The immunofluorescence (IF) technique has, until the last few years, been the only good, rapid technique available for detection of viral antigen in clinical specimens. The method has been recommended by the World Health Organization (12), which has also included the technique in their Manual for Rapid Laboratory Viral Diagnosis (11). The European Group for Rapid Viral Diagnosis used the indirect IF and quality-controlled reagents for a study in children on the epidemiology of RS, influenza A, and parainfluenza types 1 and 3 viruses in several European countries (8). Using the same IF reagents as those used in Stockholm, Sweden, in the present study, we earlier compared the results of diagnosis of influenza A and RS viruses in specimens from 139 children by IF and by virus isolation. The agreement of IF between the two centers (2) was 95% for the 62 samples positive for RS virus infections and 94% for the 18 samples positive for influenza A infections. One additional RS virus result, but no additional influenza A, was found by virus isolation. The sensitivity of IF as compared with virus isolation could be calculated from the results of the two centers to be 98 and 95% for RS virus diagnosis and 100 and 94% for influenza A diagnosis.

The enzyme-linked immunosorbent assay (ELISA) was introduced for the diagnosis of RS virus in nasopharyngeal specimens (NPS) in 1979 (1). This was followed by a series of reports from Finland on immunoassays for diagnosis of different respiratory viruses (9, 10), which showed the usefulness of immunoassays for large-scale diagnosis and pointed out the advantage of the automatic reading of results with a spectrophotometer. For adenovirus diagnosis, a higher diagnostic rate, 85%, was obtained by antigen detection in NPS by radioimmunoassay than by determination of serum antibody responses by ELISA or complement fixation tests (7). In other reports ELISA has been shown to be somewhat less sensitive for RS virus diagnosis than are the IF technique (5) and virus culture (6).

The present study compared ELISA with the IF technique for the routine detection of RS virus, influenza A and B viruses, parainfluenza type 3 virus, and adenovirus in clinical specimens.

MATERIALS AND METHODS

Design of the study. Routine diagnosis was performed in Stockholm by IF as well as by ELISA. Duplicate cell smears were examined by IF in London, England. As several examinations were performed in the two centers with each

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TABLE 1. ELISA (Stockholm)-IF (London) comparison of results for diagnosis of respiratory infections in NPS

<table>
<thead>
<tr>
<th>Specimen examined for</th>
<th>No. of specimens&lt;sup&gt;a&lt;/sup&gt;</th>
<th>E+/IF+</th>
<th>E-/IF-</th>
<th>E-/IF+</th>
<th>E+/IF-</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS virus</td>
<td>15</td>
<td>15</td>
<td>55</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>14</td>
<td>14</td>
<td>58</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza type 3</td>
<td>2</td>
<td>2</td>
<td>71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>4</td>
<td>4</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>5</td>
<td>5</td>
<td>28</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> NPS were from 73 patients investigated for RS, influenza A, and parainfluenza type 3 viruses and from 35 patients investigated for influenza B virus and adenovirus infections.

<sup>b</sup> E. ELISA: +, positive; −, negative.

NPS, and the amount of secretion was limited, a choice of etiological agents to be investigated was necessary. The choice was based on the epidemiological situation and the age of the patient. (i) Comparisons of IF results from London and ELISA results from Stockholm were done on 73 specimens tested for RS, influenza A, and parainfluenza type 3 viruses and on 35 specimens tested for influenza B virus and adenovirus. (ii) For tests performed by IF and ELISA only in Stockholm, the clinical diagnosis was considered, which resulted in various numbers of tests being performed for the five different viruses. Altogether, 1,593 tests were performed using both ELISA and IF.

**Specimens.** NPS from patients with acute respiratory infections were collected in Stockholm by suction of secretions into mucus traps as described by Gardner and McQuillan (3). Upon arrival in the Stockholm laboratory, the cells were separated by centrifugation, washed and deposited on microscope slides, and fixed in acetone for subsequent immunofluorescent staining. Duplicate cell smears were sent by air mail to London, where they were received 2 to 4 days later. The supernatant fluid was used for diagnosis by ELISA.

**IF test.** Cell smears were prepared on microscope slides with three smears on each slide and then fixed in acetone. The smears were stained for influenza A, RS, and parainfluenza type 3 viruses; in certain cases the smears were stained for influenza B virus and adenovirus. The indirect IF technique was used (3, 12). The reagents used for IF in Stockholm were produced either by Wellcome Diagnostics in bovines (influenza A, RS, and parainfluenza type 3 viruses) or by the Stockholm laboratory in rabbits (adenovirus). Influenza B antiserum (rabbit) was obtained as a gift from J. McQuillan, Royal Victoria Infirmary, Newcastle upon Tyne, England. The reagents had been monitored by quality by the European Group for Rapid Viral Diagnosis and used by the authors in a previous study (2) on IF for RS and influenza A diagnosis.

In London the same bovine RS virus antiserum (Wellcome) was used for immunofluorescence as that used in Stockholm, whereas for diagnosis of the other virus infections, specific egg-globulins (Wellcome) were used.

**ELISA.** ELISA was performed in microtitre plates (M29AR; Dynatech Industries, Inc.). The wells were coated with 100 µl of virus antiserum produced either in guinea pigs (influenza A and B viruses, adenovirus, and parainfluenza type 3 virus) or in bovines (RS virus), using dilutions ranging from 1:300 to 1:1,000. After incubation for 3 h at 37°C, the wells were washed four times in saline buffer plus 0.05% Tween 20. Each specimen (100 µl) treated as described below was added to duplicate wells for each virus investigated and incubated overnight at 37°C. The wells were washed, 100 µl of virus-specific antiserum (rabbit, dilution 1:1,000) was added to each well, and the plates were incubated for 1 h at 37°C. After repeated washing, 100 µl of alkaline phosphatase-conjugated antirabbit immunoglobulin G (Orion Diagnostica, ESPO, Finland) was added at a dilution of 1:100. Incubation for 1 h at 37°C was followed by another wash and by the addition of the substrate paranitrophenylphosphate at 1 mg/ml (Sigma Chemical Co.).

The results were recorded by measuring absorbance at 405 nm in a Titertec spectrophotometer (Flow Laboratories, Inc.). A cut-off valve for positivity was estimated for each experiment as three times background activity, which corresponded to absorbance values of ±0.1.

All reagents for ELISA were produced in the Stockholm laboratory, with the exception of the bovine RS virus antiserum (Wellcome). The guinea pig sera were produced by intranasal infection, followed by intradermal immunization as described by Sarkkinen et al. (9). The rabbit sera were obtained after immunization with purified virus and immunization with incomplete adjuvant.

Specimens from London and ELISA were usually diluted two to three times in saline. The specimens were devoid of most cells, which had been pelleted for IF. They were sonicated in a Branson sonifier cell disruptor (B15) for 1 to 3 min and subsequently diluted 1:2 in dilution buffer (20% fetal bovine serum, 2% Tween 20, 0.02% NaN3).

**RESULTS**

**ELISA.** The ELISA results could easily be read by eye but were always also measured by spectrophotometer. The sensitivity obtained varied somewhat: 10, 2, 10, 10, and 1 ng of virus protein per test well (0.1 ml) could be detected of purified RS virus, influenza A virus, parainfluenza 3 virus, influenza B virus, and adenovirus, respectively. The two ELISAs developed for influenza A and B viruses showed reactions with egg material, but this had no influence on tests of tissue culture-grown virus or on clinical material.

**IF (London)-ELISA (Stockholm) study.** Specimens from 73 patients were tested for RS, influenza A, and parainfluenza type 3 viruses. ELISA gave 31 positive results: 15 RS, 14 influenza A, and 2 parainfluenza type 3 infections (Table 1). IF detected three additional RS virus infections and one influenza A (Table 1). The sensitivity for ELISA as compared with IF was 83 and 93% for detection of RS and influenza A virus, respectively, while the specificity was 100%. The two parainfluenza type 3 infections were detected by both methods. Two of three RS virus IF-positive, ELISA-negative specimens, as well as the discrepant influenza A result, were also confirmed as positives by IF in Stockholm.

In a second series of comparison, specimens from 35 patients were investigated for adenovirus and influenza B infections. Adenovirus was diagnosed in four patients by both methods, and influenza B virus was diagnosed in five patients by both methods, while IF and ELISA each diagnosed one additional positive influenza B infection. The IF-positive, ELISA-negative influenza B specimen was also confirmed by positive IF results in Stockholm, while the ELISA-positive, IF-negative specimen was not confirmed.

Altogether, in the two series of patients investigated by IF in London and ELISA in Stockholm, a viral infection was diagnosed in 40 of 108 patients by both methods and an additional positive diagnosis was obtained in 5 patients by
the IF technique and an additional one was obtained by ELISA.

**IF-ELISA (Stockholm) study.** Table 2 shows the results of the IF and ELISA routine diagnosis in the Stockholm laboratory. Of 1,593 tests, there was disagreement in the results of 37 (2.5%).

**RS virus.** From 563 specimens, RS virus was detected in 88 by both methods, IF detected 11 additional positive specimens, and ELISA detected 3 others which were not positive by IF. Sensitivity and Specificity were 89 and 99%, respectively, as compared with those of IF.

**Influenza A virus.** In 328 specimens, 36 were positive by the two methods. IF detected four additional positives, and ELISA detected seven others. ELISA sensitivity and specificity were 90 and 98%, respectively, as compared with those of IF.

**Parainfluenza type 3 virus.** There was total agreement between the results by the two methods for diagnosis of the six parainfluenza type 3 infections detected in 376 tests.

**Adenovirus.** There was total agreement between the results by the two methods for diagnosis of four adenovirus infections found in 112 specimens.

In 214 tests, 20 influenza B infections were diagnosed by both methods, another 8 were detected by IF, and 4 others were detected by ELISA, resulting in 71% sensitivity and 98% specificity.

**Additional test.** Seven ELISA-negative, IF-positive RS virus specimens were selected for additional testing. By the addition before retesting of part of the cell fraction to a portion of the NPS fluid used for ELISA, the result of one specimen changed to positive, showing a slight increase of the absorbance values.

**DISCUSSION**

The described ELISA could detect viral antigens in amounts corresponding to 1 to 10 ng of virus protein per test well. The sensitivities for diagnosis in clinical specimens were approximately 90 and 85% for influenza A and RS viruses, respectively, as compared with IF results. The same sensitivity was earlier reported for RS virus diagnosis by Hornsleth et al. comparing ELISA with IF (5) and by McIntosh et al. comparing ELISA with virus isolation (6).

In the present study the IF technique was chosen as the reference method since we earlier established the accuracy of IF diagnosis of influenza A and RS virus infections (3) by using the same IF reagents as those used in Stockholm for the present study (2). The sensitivity compared to virus isolation had been found to be 95 and 94% for RS and influenza A virus diagnosis, respectively (2).

The quality of reagents for immunodiagnosis is of paramount importance. In ELISA only a certain amount of antibody can be adsorbed to the solid phase. It is clear that antibodies in a pure state directed only against the relevant antigen would improve the sensitivity. With purified immunoglobulin fractions, Sarkkinen and co-workers constructed immunoassays detecting 1 to 3 ng (RS virus) and about 0.1 ng (influenza A virus) of virus proteins per test. The sensitivity of radioimmunoassay as compared with IF for RS virus detection in clinical specimens was 95% (9) and for influenza A was 100% (10), and the authors noted that their ELISA gave results identical with those of radioimmunoassay.

In addition, the quality of the test specimens may contribute to the discrepancy of results between IF and ELISA. At collection the NPS may be unnecessarily diluted in buffer used to wash out secretions adhering to the walls of the collection tube. Such dilution is of no significance for the IF investigation in which antigens to be detected are packed in the nasopharyngeal cells and the cells are concentrated by centrifugation before testing. ELISA, in contrast, tests the diluted secretion, and for the reported study, all secretions with any visible mucus material were investigated.

One disadvantage in the present study was that ELISA was performed on secretions in which the cell fraction was already removed (for IF). Although some cells contained large amounts of viral antigens visible in the IF investigation, the presence of cells in the samples may not have been crucial as the addition of cells to the secretion only negligibly increased the optical density readings in ELISA. However, one of seven specimens changed from negative to positive by the addition of cells to the secretions.

In the present study ELISA was found to be a good test method for the detection of RS virus, influenza A virus, parainfluenza type 3 virus, and adenovirus, although with a slightly decreased sensitivity as compared with IF. The main advantage over the IF technique was the automation in recording results, which was much less time-consuming and required less proficiency than the reading of results in a microscope. Furthermore, transportation and storage of specimens were facilitated as the viral antigens to be detected by the ELISA were not affected by 2 or 3 days of transportation or by freezing.

**LITERATURE CITED**


