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# Use of Nasal and Pharyngeal Swabs for Rapid Detection of Respiratory Syncytial Virus and Adenovirus Antigens by Enzyme-Linked Immunosorbent Assay

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**Nasal and pharyngeal swabs from 134 children with acute respiratory diseases were examined for the presence of respiratory syncytial (RS) virus and adenovirus antigens by enzyme-linked immunosorbent assay (ELISA). The results were compared with those obtained by virus isolation and serology. Altogether, 56 RS virus-positive (prospective study), 51 adenovirus-positive (retrospective study), and 27 negative (prospective study) samples were examined. The sensitivities of ELISA were 96 and 98% for RS virus and adenovirus antigen detection, respectively. No false-positive results were observed. It is concluded that the joint eluate from a nasal and a pharyngeal swab, properly taken and handled, serves well for the detection of RS virus and adenovirus acute respiratory infections by ELISA.**

The quality of specimens is of basic importance for the success of viral diagnosis. This applies to traditional virus isolation techniques as well as to the techniques for rapid viral diagnosis.

For diagnosis of acute viral respiratory infections, nasopharyngeal secretions have been shown to yield higher isolation rates of virus than cough and nasal swabs do. Nasopharyngeal specimen collection by suction is also generally recommended for diagnosis by immunofluorescence (5) and by enzyme-linked immunosorbent assay (ELISA [16]), but this collection method is rather difficult in field conditions.

The aim of the present study was to investigate whether clinical material collected by swabbing could be used for diagnosis of respiratory syncytial (RS) virus and adenovirus by ELISA. Results obtained by virus isolation and serological investigation were compared with those obtained by ELISA on joint eluates of the nasal and pharyngeal swabs.

## MATERIALS AND METHODS

**Specimens.** Nasal and pharyngeal swabs collected from patients with acute respiratory disease were investigated by ELISA for the presence of RS virus or adenovirus antigens or both. Most specimens were from children ranging in age from 6 months to 7 years. Two series of specimens were included in the study: (i) 83 consecutive specimens which arrived in the laboratory during an outbreak of RS virus infections (prospective study) and (ii) 51 specimens which were stored frozen at the laboratory and which were obtained from children with adenovirus infections proven by either virus isolation or serological investigation.

The secretions were collected by vigorous swabbing with cotton swabs attached to nontoxic wooden sticks. Both nasal and pharyngeal swabs were taken from the patients. Veal infusion supplemented with 0.5% bovine albumin and antibiotics was used as transport medium. Immediately after sampling, the swabs from both nares and from the pharynx were immersed in one common tube containing transport medium and were further processed as one specimen.

**Virus isolation.** The freshly collected unfrozen specimens were inoculated in tissue cultures of HEp-2 and L-132 cells grown in minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2% fetal calf serum. The morphology of the cells was observed, and the appearance of cytopathic effect was noted. In case of negative results, three blind passages were performed at 10- to 14-day intervals for RS virus and adenovirus isolation. Virus identification was performed by neutralization tests in microplates and by demonstration of viral antigens in complement fixation tests. Attempts were made to isolate influenza, parainfluenza, and rhinoviruses from the same specimens in different cell culture systems and in embryonated eggs as part of the routine virus watch program performed in the Prague laboratory.

**Serology.** Paired sera were taken at 2- to 3-week intervals after the onset of disease and stored at −20°C until examined. Complement fixation tests were performed by standard procedures in microplates with RS virus and adenovirus antigens. A fourfold or greater antibody titer rise was considered positive.

**ELISA for antigen detection.** The procedure of ELISA for RS virus and adenovirus diagnosis as well as that of production of reagents and treatment of specimens has been described (6). The double-antibody sandwich technique was performed in microdilution, flat-bottom, 96-well plates (M29AR; Dynatech Industries, Inc., McLean, Va.) with 100 μl of each reagent. Each specimen was tested in duplicate. Separate wells were coated with guinea pig antisera to RS virus and adenovirus antigens. Secretions eluted from swabs in veal infusion were diluted 1:2 in phosphate-buffered saline, applied to the wells, and incubated overnight at 37°C. After repeated washings, rabbit antisera to RS virus and adenovirus were added to the appropriate wells and incubated for 1 h at 37°C. Subsequently, after new washings, alkaline phosphatase-conjugated (Orion Diagnostica, Espoo, Finland) or horseradish peroxidase-conjugated (USOL, Prague, Czechoslovakia) anti-rabbit immunoglobulin G pro-

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TABLE 1. Detection by ELISA of RS virus and adenovirus antigens in eluates from swabs (nasal and pharyngeal) compared with results by virus isolation and serology

| Virus and diagnosis | No. of ELISA results | |
|---|---|---|
| | + | − |
| RS-virus[a] | | |
| Isolation, + | 49 | 2 |
| Isolation, −; serology, + | 5 | |
| Isolation, −; serology, − | | 27 |
| Adenovirus infection | | 21 |
| Adenovirus[b] | | |
| Isolation, + | 39 | 1 |
| Isolation, −; serology, + | 11 | |
| Isolation, −; serology, − | | 27 |
| RS virus infection | | 12 |

[a] Sensitivity, 54 (96%) of 56; specificity, 100%.
[b] Sensitivity, 50 (98%) of 51; specificity, 100%.

duced in swine was added, and the plate was incubated again for 1 h at 37°C. *para*-Nitrophenyl-phosphate (Sigma Chemical Co., St. Louis, Mo.) was used as substrate for the alkaline phosphatase, and 5-aminosalicylic acid was used for the horseradish peroxidase.

**Calculation of ELISA results.** A spectrophotometer (microplate reader MR580; Dynatech) was used to register adsorption values at 405 or 450 nm. An ELISA result was defined as positive when the mean optical density of the specimen exceeded by three times the background activity. The cutoff values were usually within the 0.15 to 0.25 optical density range.

## RESULTS

The eluates from the nasal and pharyngeal swabs from a total of 134 patients were examined. The examined patients included 56 with positive RS virus isolation and/or serology, 51 with positive adenovirus isolation and/or serology, and 27 patients with negative virus isolation and serology.

The ELISA results were compared with those obtained by virus isolation and by serology (Table 1). In 54 (96%) of 56 patients with RS virus infection, RS virus antigen was detected by ELISA in the clinical specimens. ELISA failed to detect RS virus antigen in 2 of 51 isolation-positive specimens (both of them were diluted 1:10 because of the small volumes of material). On the other hand, ELISA detected RS virus antigen in five isolation-negative specimens for which diagnosis was confirmed only by rise of the antibody titer. The specificity of ELISA was tested by examination of RS virus isolation-negative specimens from patients with negative serology (27 samples) and of 21 adenovirus-positive specimens. No false RS virus-positive results were obtained.

The sensitivity of ELISA for adenovirus detection was 98% compared with results obtained by virus isolation and serology (Table 1). One specimen, shown by virus isolation to contain adenovirus type 5, failed to give a positive ELISA result. On the other hand, virus isolation-negative specimens from 11 patients with serologically diagnosed adenovirus infections were positive by ELISA. The reagents used reacted with the adenovirus hexon group antigen. Positive ELISA results were obtained for specimens yielding adenovirus types 1, 2, 3, 4, 5, and 7 by isolation and neutralization tests. No false-positive results were obtained for 27 adenovirus isolation- and serology-negative specimens or for the RS virus ELISA-positive specimens.

## DISCUSSION

The rapid etiological diagnosis of respiratory viral infections is of great importance (5). New antiviral drugs appear continuously, and proper selection of patients for early antiviral therapy based on a reliable rapid viral diagnosis is required. Today, e.g., the introduction of therapy with ribavarin for infants with severe RS virus infections (7, 17) requires rapid establishment of the etiologic diagnosis. Moreover, nosocomial spread of respiratory viral infections in pediatric wards is a serious problem worldwide (2, 4, 5, 15). Control measures, such as isolating and grouping infants infected with different viruses, may help to limit the spread of these agents to other susceptible children.

Several techniques for rapid diagnosis are currently performed in virological laboratories; the most common are fluorescent antibody tests (1, 5, 9, 10, 14) and enzyme immunoassays (3, 6, 8, 11–13, 18). Data from various laboratories on the sensitivities of both tests are not in complete agreement, mainly because of the varying quality of the clinical specimen, a factor which is of basic importance in rapid viral diagnosis.

In the present study, cotton swabs were used instead of the commonly recommended suction method for collection of nasal and pharyngeal specimens in order to detect respiratory virus infections. Swabbing is generally used for isolation of viral and bacterial agents in respiratory infections, and it is performed by a number of physicians and nurses as a simple and less traumatic method for children than suction. After elution of both swabs in a common tube containing transport medium, the material was suitable for detection of viral antigens by ELISA as well as for the isolation of the respiratory viruses. However, for the diagnosis by fluorescent-antibody test, this method was less suitable, as the material did not contain sufficient amounts of undamaged epithelial cells for proper evaluation. The high sensitivities of RS virus and adenovirus antigen detection by ELISA—96 and 98%, respectively, compared with virus isolation and serology—showed that the quality of specimens collected on swabs for this study was satisfactory.

The fact that two RS virus isolation-positive specimens were negative by ELISA could be explained by higher dilution of samples because of the insufficient amount of material. RS virus lability resulting in loss of infectivity explains the negative RS virus isolation results for five ELISA-positive specimens from patients with serologically verified infections.

ELISA failed to detect viral antigens in one adenovirus isolation-positive specimen (adenovirus type 5). However, it detected adenovirus antigen in 11 virus isolation-negative specimens from patients with positive adenovirus serology. This was surprising and could result from suboptimal conditions for the virus isolation attempts. The types of adenovirus infection in the 11 specimens were not assessed.

Investigation of viral respiratory infections by ELISA facilitates the transportation and handling of specimens, as the presence of infectious virus is not a prerequisite for diagnosis. Virus isolation is, however, a necessity during periods when antigen detection methods are being introduced in the laboratory. Moreover, typing of certain virus isolates, such as adenovirus, and antigenic analyses of currently circulating influenza virus strains are important epidemiological tasks. Although no comparison was made between diagnostic results for nasopharyngeal secretions collected by suction and those for specimens collected by swabbing, the high frequency of positive results (96 and 98%

for RS virus and adenovirus, respectively) indicates that the quality of specimens obtained by swabs was good enough for laboratory diagnosis of RS virus and adenovirus acute respiratory infections by ELISA. The examination of nasal and pharyngeal swabs as one common sample increases the amount of virus in the specimen and easily explains the higher sensitivity of detection of viral antigen in the present study in comparison with some recently published results (11).

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