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Rapid Immunoperoxidase Assay for Detection of Respiratory Syncytial Virus in Nasopharyngeal Secretions

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Samples of nasopharyngeal secretions obtained from 70 infants and young children with acute respiratory disease were examined for the presence of respiratory syncytial virus by immunoperoxidase assay (IPA). The IPA was compared with the immunofluorescence assay and with cell culture isolation. Respiratory syncytial virus antigen-positive cells were detected by both IPA and immunofluorescence assay in 28 specimens; 25 samples were positive in cell culture. The agreement between virus isolation and IPA and IFA was 89%. The applicability of IPA to rapid viral diagnosis of respiratory syncytial virus infection is discussed.

The diagnosis of respiratory syncytial (RS) virus infection in young children is usually based on virus isolation from throat washings. However, virus isolation is time consuming and expensive, and rapid methods of virus detection not requiring replication in cell culture are becoming more popular.

The development of the immunfluorescence assay (IFA) (4) has greatly improved the rapid diagnosis of viral respiratory infections. More recently, radioimmunoassay and enzyme immunoassay have also been developed for the detection of viral antigens in nasopharyngeal secretions (NPS) (2, 11).

In the present study an immunoperoxidase assay (IPA) was developed for the detection of RS virus in NPS and was compared with the fluorescent antibody technique and virus isolation in cell culture.

MATERIALS AND METHODS

Specimens. NPS from 70 infants and young children hospitalized with acute respiratory disease were collected by suction and transported to the laboratory on ice. Of these specimens, 54 were collected within 7 days of the onset of symptoms, and 16 samples were collected within 14 days of onset. The specimens were processed by the technique of Gardner and McQuillin (4). Briefly, the specimens were diluted with 2 ml of sterile phosphate-buffered saline (PBS) (pH 7.3), and the mucus was broken with a Pasteur pipette. The specimens were centrifuged at 1,500 rpm for 10 min at 4°C. The supernatant fluid was discarded and the pellet was suspended in 0.3 to 0.6 ml of PBS. For each patient, duplicate eight-well multitest slides (Flow Laboratories, Irvine, United Kingdom) were spotted with 20 μl of cell suspension. The drops were air dried and fixed in cold (4°C) acetone for 7 min. One slide was used for the IFA and the other for the IPA.

IFA. An indirect IFA was used. Bovine anti-RS virus serum (Wellcome Research Laboratories, Beckenham, United Kingdom) was employed. As a second antibody, fluorescein-conjugated rabbit anti-bovine immunoglobulins (Wellcome) was used.

IPA. Since multitest slides were used, it was possible to perform both the detection of RS virus antigens and the control assays simultaneously on the same slide.

RS virus antigen detection. Three wells were treated for 30 min at 37°C with a 1:10 dilution of hyperimmune bovine RS virus serum. After incubation, the slides were washed for 30 min in PBS and then incubated for 30 min with peroxidase-labeled, affinity-purified goat antibody to bovine immunoglobulin G (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1:30 in PBS.

After washing, the enzymatic activity was detected by modification of the method of Graham and Karnowski (6), as previously described (1). Briefly, the freshly prepared substrate solution was made of 4 mg of Benzidine (Fluka AG, Buchs, Switzerland) and dissolved in 0.5 ml of acetone–9.5 ml of PBS–10 μl of hydrogen peroxide from 33% stock solution. The substrate was added for 5 min at room temperature; this was followed by washing in PBS.

Control assays. Five wells were processed for control purposes as follows. One well was treated with a viral antiserum other than RS virus (measles-specific bovine serum, Wellcome), one well was treated with normal bovine serum (Wellcome), one well was treated with peroxidase conjugate without immune serum, and two wells were treated with enzyme substrate only (test for endogenous peroxidase).

Samples stained by immunofluorescence were examined by one of us (D.M.) who is familiar with the IFA as applied to rapid viral diagnosis, and another (R.C.) examined IPA preparations. Neither knew the results of the other tests.

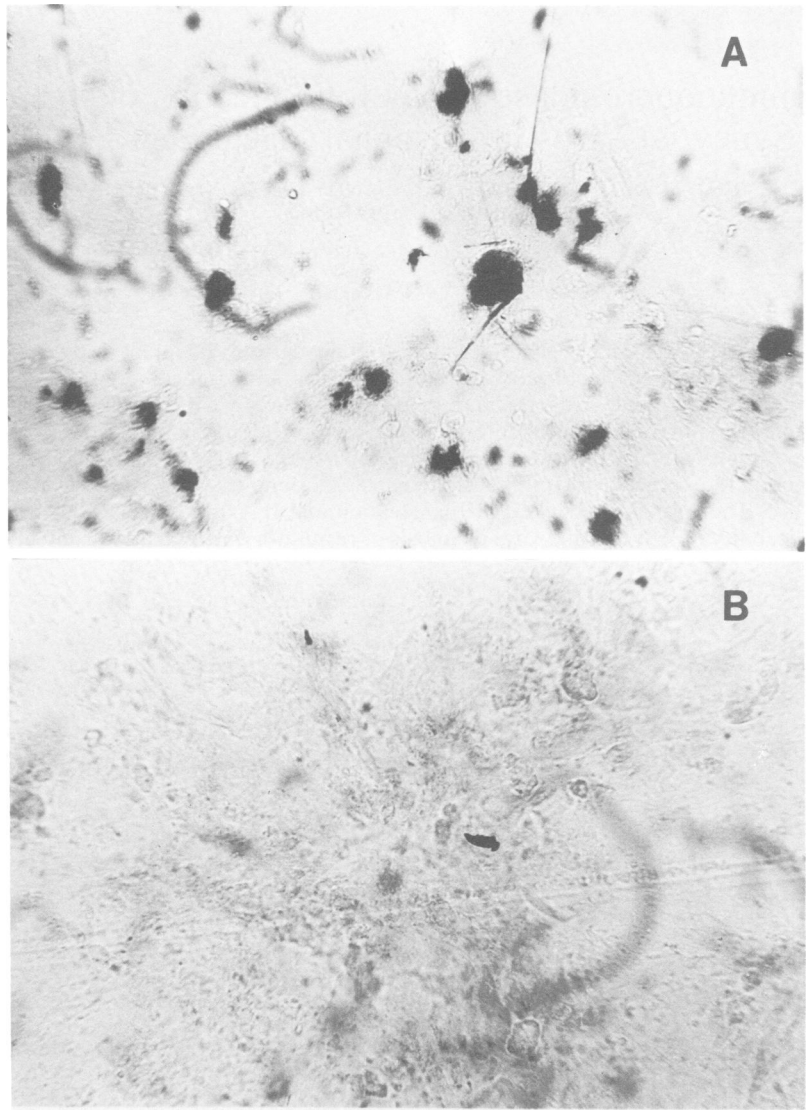

FIG. 1. (A) Typical staining of RS virus-infected cells of a nasopharyngeal secretion given by peroxidase reaction. (B) RS virus-positive sample treated with anti-bovine peroxidase conjugate without the pretreatment with specific bovine antiserum.

Virus isolation. HEp-2 cells sensitive to RS virus (Flow Laboratories) were inoculated with NPS and examined for the typical cytopathic effect of RS virus (10).

RESULTS

Of the 70 NPS examined, 28 were found positive for RS virus by IFA, and the same samples were also positive by IPA. Only 25 specimens were positive by cell culture isolation. Thus, the agreement between virus isolation and IPA and IFA was 89%.

Figure 1 shows the typical dark blue staining obtained by the peroxidase reaction on RS virus-infected cells. If RS virus-positive samples were treated with viral antiserum other than RS virus-specific serum or with normal bovine serum, the staining given by the peroxidase reaction was absent.

Omitting the RS virus-specific antiserum before incubation with anti-bovine peroxidase conjugate prevented any staining. The enzyme substrate alone did not produce any reaction which could interfere with the interpretation of the results. Very rarely, nonspecific staining of a few cells was observed, both in RS virus-positive and in RS virus-negative specimens. In these cases, the staining reaction was very weak

and clearly nonspecific. In fact, the whole cells were uniformly faintly brownish yellow.

The addition of the affinity-purified conjugate neither increased the intensity of this reaction nor increased the number of samples with this nonspecific reaction as observed in the wells not treated with RS virus-specific antiserum before incubation with conjugate. Therefore, it was not necessary to destroy any endogenous peroxidase activity before performing the IPA.

DISCUSSION

The development of a sensitive and specific IFA for the detection of viral antigens in the cells of NPS has greatly improved the diagnosis of viral respiratory infections. Other techniques such as enzyme-linked immunosorbent assay (2), inhibition enzyme-linked immunosorbent assay (9), and radioimmunoassay (11) have also been applied in the rapid detection of RS virus antigens.

Recently, the reliability of the indirect IPA has been carefully evaluated in different laboratories in comparison with other techniques for antibody detection in serum samples (1, 7, 8, 12). Complete correlation was found between the various techniques and the IPA. In addition, good evidence has been found in our laboratory of the reliability of the IPA for the detection of rotavirus and enteric adenovirus antigens in cell culture (R. Cevenini et al., J. Infect., in press).

To our knowledge, only one reported study has dealt with direct RS virus antigen detection by the IPA from clinical specimens of children with respiratory disease (3). Unsatisfactory results were obtained in this study, mainly because of the presence of endogenous peroxidase. The removal of endogenous peroxidase also destroyed the virus antigen.

In the present study, endogenous peroxidase activity was found below a level which interfered with interpretation of our staining results. Therefore, it was not necessary to destroy any endogenous peroxidase before the application of the IPA. Differences in the reagents employed (especially with respect to the peroxidase conjugate and the substrate) may explain the discrepancies between the two studies.

The distribution of RS virus antigen in the cytoplasm of infected cells was more characteristic with the IFA than with the IPA. However, positive cells were clearly and easily detected by both techniques.

The IPA described was as sensitive as the IFA since the number of positive samples detected by both techniques was identical. Three specimens were positive by IPA and IFA but negative by culture; they were from patients with a history of illness lasting longer than 7 days. The isolation attempts were likely false-negative since Gardner et al. (5) have shown that late in RS virus infection the IFA may remain positive although virus cannot be isolated.

The IPA described in the present study seems to be suitable for RS virus antigen detection in clinical specimens. The results are easily read with a low-power light microscope. Since the technique is rapid, simple, and sensitive it has potential for widespread use. This IPA seems to be well suited to clinical laboratories because the reagents are easily prepared and the reading does not appear to produce difficulties.

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