Detection of Respiratory Syncytial Virus in Samples Frozen at −20°C

Respiratory syncytial virus (RSV) is well recognized as the single most important pathogen accounting for acute viral infections of the lower respiratory tract in infants and young children. Outbreaks of RSV infections occur each year in the winter and early spring (1, 4). Rapid detection of RSV is mandatory for early diagnosis, isolation measures, and, if judged necessary, antiviral therapy. Several rapid diagnostic methods, including enzyme immunoassay and immunofluorescence (IF), which rely on detection of RSV antigen in respiratory secretions, have been increasingly used for these purposes. The shell vial (SV) procedure and conventional cell culture allow detection of small amounts of virus that can be missed by the rapid antigen detection assays. RSV being a labile virus, appropriate guidelines for collection, transport, and inoculation of specimens must be followed (2). For respiratory infections, nasopharyngeal aspirates give the highest yield of positive test results. Samples should be applied to the slides and fixed in acetone immediately after collection (3).

Because of these concerns, the present study was undertaken in order to select the algorithm for the routine diagnosis of RSV infection and ascertain the effect of freezing the sample overnight at −20°C on detection of RSV antigen in the nasopharyngeal aspirate.

The study was undertaken in two periods. Over the first period (November 1994 to April 1995), RSV antigen was investigated in 100 children younger than 2 years old with clinically defined acute lower respiratory tract infection by IF (RSV-Mab test; Gull, Salt Lake City, Utah) performed with nasopharyngeal aspirates. In this period, samples for IF staining were prepared both promptly and after freezing them overnight at −20°C to see the effect of freezing on detection of RSV antigen. In the second period (November 1997 to February 1998), RSV was investigated in 54 children younger than 3 years with clinically defined acute lower respiratory tract infection by antigen detection using both IF (Argene; Biosoft, Varilhes, France) and SV assay on the nasopharyngeal aspirates to compare the sensitivities of these assays. All specimens were transferred to sterile containers and transported to the laboratory in viral transport medium immediately after collection. One portion was cultured for RSV, and a second portion was tested by IF for RSV.

For virus isolation by SV assay, Hep-2 epidermoid carcinoma cells were used. Briefly, the medium from the shell vials was aspirated and 0.2 ml of specimen was added directly to each of three shell vials. The vials were recapped and centrifuged at 700×g for 1 h. Excess inoculum was removed, fresh medium was added, and the vials were incubated at 37°C for 40 h.

For IF, a fluorescein isothiocyanate anti-RSV antibody was used for direct detection and culture-based identification. Specimens for antigen detection with fewer than three columnar epithelial cells per field were considered to give inconclusive results. Kits (RSV-Mab test [Gull] and Argene [Biosoft]) were used according to the manufacturers’ instructions. Slides were viewed at 400× magnification on an Olympus microscope.

Ten of the samples in the first-period preparations which were frozen at −20°C were stained with hematoxyline-eosin to study the effect of a −20°C incubation on the morphology of ciliated cells. In the first period, no difference in antigen detection was recorded when frozen samples were investigated in parallel with preparations prepared promptly. Of 100 samples, 55 were positive by both detection methods. No change in the morphology of ciliated cells was observed in preparations from samples frozen at −20°C.

In the second period, 18 of 54 (33%) specimens were positive by one or both methods. Eight samples were positive by both IF and SV assay. Three and seven of the specimens were positive only by IF and SV assay, respectively. For the three specimens that were positive only by IF, this result was interpreted to be due to the loss of viability of virus during transport.

On the basis of our results, all appropriate respiratory specimens from pediatric patients with a clinical diagnosis compatible with an RSV infection should be screened for RSV by IF as an initial procedure; only IF-negative specimens should be tested by the SV assay during the winter epidemic period. The specimens can be stored overnight at −20°C before the slide is prepared from the material.

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REFERENCES


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