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### Evaluation of Direct Immunofluorescence, Dot-Blot Enzyme Immunoassay, and Shell-Vial Culture for Detection of Respiratory Syncytial Virus in Patients with Bronchiolitis

Respiratory syncytial virus (RSV) is recognized as the principal causative agent of acute lower respiratory tract infections, affecting mainly infants and young children. The majority of these infections occur in annual epidemic outbreaks in winter and early spring. It is, therefore, important to obtain a rapid, early diagnosis that permits identification of infected children (1, 2).

There are two rapid techniques for the diagnosis of infection by RSV, both based on viral antigen detection in respiratory secretions: enzyme immunoassay (EIA) and direct immunofluorescence. Isolation of the virus in cell culture has been considered the gold standard with which all new techniques of detection and isolation of RSV should be compared (3, 4). However, shell-vial culture has been shown to be more rapid and more sensitive than classic cell culture, suggesting

that it may be used as an alternative comparative method for the study of epidemic outbreaks of bronchiolitis by RSV (5, 6).

We performed a prospective study of the efficacy of two rapid methods of antigen detection, a dot-blot (DB) EIA (Directigen RSV, Becton Dickinson, USA) and direct immunofluorescence (Monofluokit RSV, Pasteur Diagnostics, France), compared with the shell-vial method of isolation in culture for the detection of RSV in 229 nasopharyngeal aspirates obtained from children soon after the onset of bronchiolitis. The DB-EIA technique was performed using 250 µl of diluted sample. The sample was prepared and the results read in accordance with the manufacturer's instructions. For the direct immunofluorescence technique, 200 µl of the sample was centrifuged in a cytospin (Cytospin 3, Shandon, UK) at 700 x g for 10 min. After drying, the smears were fixed with acetone for 10 min at -20°C and then stained with a direct RSV fluorescent antibody stain (Monofluokit RSV). The slides were incubated at 36°C in a humidity chamber for 30 min, after which they were rinsed in phosphate-buffered saline. The slides were viewed at x 400 on a fluorescence microscope.

For the shell-vial technique 200 µl of the sample was inoculated into two Hep-2 vials (Viracell, Ingelheim Diagnostica, Spain). The vials were then centrifuged at 700 x g for 45 min. They were allowed to rest at 36°C for 60 min and the supernatant was discarded. One ml of maintenance medium, MEM with 1 % fetal bovine serum, was added to each sample. The vials were incubated at 36°C for two days if both rapid tests were positive and for three days if only one test was positive or if both were negative. After incubation the monolayers were stained with anti-RSV (Monofluokit RSV). The monolayers were viewed at x 200 and x 400 on a fluorescence microscope.

Sensitivity, specificity, and positive and negative predictive values for the DB-EIA and the direct immunofluorescence technique were calculated by comparison with isolation of RSV in the shell-vial culture.

Of the 229 samples studied, 130 (56.8 %) were considered positive for RSV. In this group of 130 samples, we detected 116 cases of RSV infection (89.2 %) and 14 cases of infection by other viruses. Of the total samples studied, RSV accounted for 50.6 % of the viral infections. The results obtained with the different techniques are shown in Table 1. The DB-EIA detected 74 cases (63.7 %) of RSV infection, direct immuno-

**Table 1:** Comparison of dot-blot EIA, direct immunofluorescence, and shell-vial culture for detection of respiratory syncytial virus in 229 nasopharyngeal aspirate specimens.

Dot-blot EIA	Direct IF	Shell-vial culture	No. (%) with given result
+	+	+	64 (55.2)
+	+	-	7 (6.0)
-	+	+	26 (22.4)
+	-	-	2 (1.7)
-	+	-	3 (2.5)
-	-	+	13 (11.2)
+	-	+	1 (0.8)
-	-	-	99 (43.2)

IF = immunofluorescence.

fluorescence 100 (86.2 %) cases, and shell-vial culture 104 (89.6 %). Of the 104 cases detected by positive shell-vial culture, the DB-EIA detected 65 (62.5 %) and direct immunofluorescence 90 (86.5 %). Compared with the shell-vial method, the DB-EIA technique had a sensitivity of 62.5 %, a specificity of 91.8 %, a positive predictive value of 87.8 %, and a negative predictive value of 72.3 %. The results obtained with the direct immunofluorescence technique were 86.5 %, 90.9 %, 90 %, and 87.8 %, respectively. Statistically significant differences between direct immunofluorescence and the DB-EIA were found for sensitivity ( $p = 0.0002$ ) and the negative predictive value ( $p = 0.01$ ).

Using the results reported by Johnston and Siegel (7) as a starting point, we used the shell-vial method as the sole reference for the comparison of results obtained with the rapid antigen techniques. These authors have shown that the shell-vial method is superior to conventional cell culture for detecting viable RSV. Of all techniques employed, the shell-vial obtained the highest percentage of positivity (89.6 %), higher than the 75 % obtained by Smith et al. (8) or the 73 % reported by Johnston and Siegel (7). In addition, in 11.2 % of the cases the diagnosis was obtained solely by isolation in the shell-vial culture. This percentage is also higher than the 4.6 % reported by Johnston and Siegel (7).

When the two rapid antigen detection techniques were compared with the shell-vial culture, sensitivity was 62.5 % for DB-EIA and 86.5 % for direct immunofluorescence. Our results confirm those reported by Halstead et al. (9), in which the DB-EIA (Directigen RSV) had a sensitivity of 75.8 % compared with conventional culture. Nevertheless, this technique has the advantage of speed and simplicity, with results available in

15 min. In two cases (1.7 %) positivity was obtained solely by the DB-EIA. One of these was considered a false-positive result, in view of the isolation of adenovirus in the shell-vial culture. In three cases (2.5 %) the diagnosis was provided solely by direct immunofluorescence. In all these the number of infected epithelial cells was very low. For this technique the quality of the specimen is critical and cannot always be relied upon. In those cases (6 %) in which both rapid techniques gave positive results and the shell-vial culture negative results, we believe that the lability or instability of RSV hindered its inability to grow in Hep-2 cells.

None of the techniques used in our study was able to detect all cases of infection by RSV. It is necessary, in the interest of diagnostic efficacy, to use at least two techniques, one of the rapid antigenic group and one type of cell culture, preferably the shell-vial method. In 14 cases (10.8 %) in the present study, a virus other than RSV was isolated during the epidemic of bronchiolitis. We recommend that shell-vial culture be used routinely for detection of non-RSV virus in order to ensure complete information regarding the etiology of viral respiratory infections.

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