Evaluation of the Becton Dickinson Directigen Test for Respiratory Syncytial Virus in Nasopharyngeal Aspirates

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A premarket trial of the Becton Dickinson Directigen respiratory syncytial virus membrane-based enzyme immunoassay compared the test with virus isolation for the detection of respiratory syncytial virus in 583 nasopharyngeal aspirates. After modification, the Directigen test showed a sensitivity of 83% and a specificity of 90%. It offers the potential for an efficient bedside test—without the need for any equipment—for the diagnosis of respiratory syncytial virus infection and requires only a 0.25-ml sample volume. However, for optimum reliability, freezing-thawing of samples and access to a confirmatory test were shown to be necessary.

Respiratory syncytial virus (RSV) is the most common respiratory virus pathogen in the pediatric community (6-9). In many countries with temperate or colder climates, a high rate of infection occurs each year during the winter months (1), leading to a significant increase in hospital admissions and use of diagnostic services. For many years after the pioneering work of Gardner and McQuillin (3), the immunofluorescence test has been the mainstay for the rapid diagnosis of respiratory viral infections. More recently, enzyme immunoassays (EIA) have offered an alternative rapid method for the laboratory diagnosis of these infections (2, 5, 10, 11). These assays require either experienced microscopists for the immunofluorescence test or a sufficient number of specimens for cost effectiveness in the case of EIA. Consequently, a simpler membrane EIA could offer an efficient and rapid method for the detection of RSV in nasopharyngeal aspirates (NPA), suitable for use within the hospital ward as a bedside test. This paper compares the use of such an assay with virus culture for the rapid diagnosis of RSV infection.

NPA were obtained from 583 patients (1 to 15 years of age) who attended the Adelaide Children’s Hospital for routine respiratory diagnosis. NPA were inoculated into HEp-2, HeLa-T, human diploid fibroblast, and primary monkey kidney cells. The mean time for a positive culture result for RSV in HEp-2 cells was 5 days; no RSV subtyping was performed. Cell cultures were examined for cytopathic changes at regular intervals, and a preliminary negative culture result was reported after day 7. The cultures were further incubated until day 28, with one passage on day 14, before a final negative result was confirmed. All samples were also examined with the Becton Dickinson Directigen RSV test. Each NPA (0.25 ml) was added to a Dispense Tube, followed by 3 drops of an extraction buffer. This was mixed thoroughly, and all of the extracted specimen was added to the ColorPac membrane test well. Then 4 drops of a wash fluid were added, followed by the addition of 4 drops of alkaline phosphatase-conjugated monoclonal antibodies to RSV fusion and nucleoproteins. This was incubated at room temperature for 2 min and the membrane well was washed with 8 drops of washing fluid. Four drops of each of two substrate reagents were then added sequentially, and the test result was read after 5 min. A positive result was indicated by a purple triangle with a white to light purple background within the membrane well. In each test batch, a positive control was included. All tests were read by the same individual without knowledge of culture results to avoid possible subjective bias in test interpretation.

Table 1 relates the initial results of the Directigen RSV test with virus culture as the reference. There were 52 NPA positive for RSV by virus culture, and 32 (62%) of these were positive in the Directigen test. Discrepant results were then examined in more detail. Among the 20 NPA which were negative in the Directigen RSV test but culture positive for the virus in the screen test, 11 were positive when repeated in the Directigen test (Table 2) with stored samples which were frozen and thawed once. In contrast, none of 20 Directigen-negative, culture-negative samples showed a posi-

| TABLE 1. Relation between the Directigen RSV test and virus culture for detection of RSV in NPA from 583 patients |
|------------------------|------------------------|------------------------|
| Culture result         | No. of Directigen RSV results* |
|                        | Positive | Negative | Total    |
| Positive               | 32       | 20        | 52       |
| Negative               | 52       | 479       | 531      |
| Total                  | 84       | 499       | 583      |

* Directigen sensitivity, 62% (32 of 52); Directigen specificity, 90% (479 of 531); predictive value of positive, 38% (32 of 84); predictive value of negative, 96% (479 of 499).

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TABLE 2. Relation between Directigen RSV test and virus culture for detection of RSV in NPA*

<table>
<thead>
<tr>
<th>Culture result</th>
<th>No. of confirmed Directigen results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>52</td>
<td>479</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>488</td>
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</tbody>
</table>

* After adjusting for confirmed positives in antibody blocking test and repeat testing of samples frozen and thawed once.

* Specimens were blocked with bovine anti-RSV serum in one ColorPac well and in another with bovine negative control serum after addition of wash reagent 1. A specimen was considered positive for RSV when the well treated with the bovine antiserum was lighter in intensity than that treated with negative control serum. Directigen sensitivity, 83% (43 of 52); Directigen specificity, 90% (479 of 531); predictive value of positive, 45% (43 of 95); predictive value of negative, 98% (479 of 488).
positive Directigen result after freezing-thawing. Conversely, in the sample of 52 NPA which were screen test positive by the Directigen RSV test but culture negative, 16 were subsequently confirmed positive in the former test after a blocking test with bovine anti-RSV serum (supplied by the manufacturer). The other 36 specimens showing reactions that were not blocked by (bovine) anti-RSV serum were considered false positive in the Directigen test. Table 2 relates these revised Directigen test results after blocking and repeat testing with virus culture. Thus, the sensitivity and specificity of the Directigen RSV test were 83% (43 of 52) and 90% (479 of 531), respectively, after adjusting for the confirmed positives and testing of specimens which were frozen and thawed once.

The diagnostic categories of these patients were further analyzed in relation to the patterns of RSV detection in both tests (Table 3). The highest rate of positives (45 of 104, 43%) was, as expected, seen in patients suffering from bronchiolitis or pneumonia. In patients with signs and symptoms of upper respiratory tract infection or asthma, 14% (34 of 242) were RSV positive by the Directigen test or culture. In the subgroup of patients with bronchiolitis or pneumonia, the sensitivity of the Directigen test (with reference to culture) was 91% (31 of 34), and in the subgroup with upper respiratory tract infection or asthma it was 77% (10 of 13). The specificity was the same in either group—91% (117 of 128 and 206 of 229).

The Becton Dickinson Directigen RSV test offers a specific and sensitive test for the rapid detection of RSV in NPA. Its high specificity of 90% and sensitivity of 83% compare well with those of other solid-phase EIA systems which require more stringent washing conditions and an automated spectrophotometer (2, 5, 10, 11). The detection of RSV (in the Directigen test) in NPA which were subsequently culture negative for the virus may have been influenced by transport of the specimens and the period from the onset of illness before the patients were sampled. In our study population, the mean duration of illness before the NPA was collected from the patient was 2.7 days (standard deviation, ±1.9) in the group which was Directigen and culture positive, compared with 3.4 days (standard deviation, ±2.4) in the group which was Directigen negative but culture positive. Various laboratories using the immunofluorescence test or EIA have also reported the detection of RSV (confirmed in blocking tests) in clinical specimens which were culture negative (4, 10, 12).

The short assay time of 15 min could make this an efficient bedside test, in particular for antiviral chemotherapeutic purposes or for infection control within the hospital. However, enhanced performance after one freeze-thaw cycle suggested that longer contact with extraction buffer or more vigorous sample disruption might be of value, and the current directions for use of the product have been modified accordingly; at present, this requirement will restrict bedside use of the test. The small volume of specimen needed (0.25 ml) would still allow for other rapid viral tests (and culture) with the remaining portion of the NPA. However, as with all such tests, regular access to virus culture is highly desirable to allow continuous monitoring of test performance and isolation of viruses other than RSV.

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LITERATURE CITED


