

Comparison of the RSV Respi-Strip with Direct Fluorescent-Antigen Detection for Diagnosis of Respiratory Syncytial Virus Infection in Pediatric Patients

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The RSV Respi-Strip was compared to the SimulFluor respiratory screen for detecting respiratory syncytial virus in nasopharyngeal aspirates from pediatric patients. Of samples tested, 115/239 (49%) were positive by direct fluorescent-antigen detection. The sensitivity and specificity of the RSV Respi-Strip were 92% (95% confidence interval [CI], 86% to 96%) and 98% (95% CI, 94% to 100%), respectively, with a diagnostic efficiency of 95%.

Respiratory syncytial virus (RSV) is the most common virus causing respiratory infections in young children, occurring as a winter epidemic annually in temperate climates (11). Direct fluorescent-antigen detection (DFA) has become a common rapid method of detection, comparing favorably with tissue culturing (6, 7, 9, 13). Processing and reading of samples by DFA still requires specialized equipment and staff. As such, it is difficult to provide 24 h per day. Several immunoassay antigen detection kits are available, but most require multiple steps in processing (5, 6). Lateral-flow immunochromatographic assays have been developed with fewer steps and reliable results (8, 10, 12). Coris-BioConcept manufactures an RSV detection kit (RSV Respi-Strip [RSV-RS]) which requires only two reagents (extraction buffer and the immunostrips) and two incubations and makes results available within 25 min. We wished to evaluate whether this assay could be implemented in our regional diagnostic laboratory for the detection of RSV in samples from pediatric patients.

All specimens in this study were from pediatric patients (mean age, 16 months; age range, 1 week to 17 years) and were submitted from 26 January to 19 February 2004. Samples were collected by nasopharyngeal aspiration done in emergency departments or pediatric wards. Direct fluorescent-antigen testing for RSV (RSV-DFA) was performed using a SimulFluor respiratory screen DFA test (Chemicon International). The result of this test was selected as the a priori gold standard. All samples were processed according to the manufacturer's instructions with centrifugation and spotting of 30 μ l of resuspended cells on glass slides prior to inoculation into transport media. The cytospin method was not used. Stained slides were read with a fluorescent microscope and a fluorescein isothiocyanate filter. Epithelial cells with intracellular yellow fluorescence were confirmed as positive with pink fluorescence using a tetramethylrhodamine isothiocyanate filter. Two or more

distinctly positive cells/slide were required for a positive test result.

RSV-RS testing was performed on the same specimens submitted for DFA testing. The testing technologist was blinded to the DFA testing result. Testing was performed in accordance with the manufacturer's package insert. A 0.25-ml aliquot of nasopharyngeal aspirate was mixed with 0.25 ml of extraction buffer in a 3-ml tube and incubated at room temperature for 10 min. Lateral flow strips were then inserted into the tube and incubated for 15 min prior to reading. The presence of a positive control line with a positive test line was considered a positive result.

Results from RSV-DFA and RSV-RS testing were transferred to a Microsoft Excel spreadsheet and analyzed using Analyze-It clinical laboratory software (Analyze-It Software Ltd., Leeds, United Kingdom). Cost analysis was done by combining the labor cost/run and reagent cost/run including controls and dividing by the number of samples tested. All costs are in Canadian dollars.

A total of 236 samples, of which 115 (49%) were positive by DFA (Table 1), were included in this study. Of these, 106 were positive by the RSV-RS (sensitivity, 92%; 95% CI, 86% to 96%). The RSV-RS gave positive results for two samples that were negative by DFA (specificity, 98%; 95% CI, 94% to 100%). The positive and negative likelihood ratios for the RSV-RS were 46 and 0.08, respectively. The overall diagnostic efficiency was 95%. Cost analysis showed that small sample numbers favored the use of the RSV Respi-Strip and testing of larger numbers of samples favored DFA testing (Table 2).

In our study, we found that the RSV Respi-Strip assay was a rapid, easy-to-perform test for detecting RSV in samples from pediatric patients. The RSV-RS was less costly than DFA testing when fewer than five samples were being tested in the same run. This cost includes the use of controls with each run. Our current laboratory policy would be to run controls on similar low-complexity tests only once per day. Under these conditions, the RSV-RS would be more economical than DFA testing when testing is batched in any volume of less than seven samples per run. RSV-RS testing was both less sensitive and

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TABLE 1. Performance characteristics

DFA result	No. of samples tested with RSV Respi-Strips giving indicated result		Total
	Positive	Negative	
Positive	106	9	115
Negative	2	119	121
Total	108	128	236

less specific than RSV-DFA testing. As such, when the incidence of RSV infection was 20%, the positive predictive value would be less than 90%. Positive test results would need to be confirmed in this situation, but negative results would essentially exclude the diagnosis, as the negative predictive value would be 99%. Once the epidemic occurs and the positivity rate in clinical samples climbs to above 25%, the positive and negative predictive values of the RSV-RS are both >95% until the incidence is greater than 45%. Above this level, the negative predictive value drops, and negative results may need to be confirmed if clinically indicated.

Our study has several limitations. First, DFA testing was used as the gold standard, and discordant samples were not reconciled. Several studies have found that for RSV infection, tissue culture does not add significantly to the number of infections detected compared to DFA testing. One study using gene amplification as the gold standard showed antigen detection to have an 82% sensitivity and a 94% specificity for the detection of RSV (1). While gene amplification technology has become the new gold standard for the detection of many infectious agents, there is no commercial assay currently available with a turnaround time similar to those of antigen detection assays (4). Second, the population studied in this paper includes only pediatric patients. As no samples were tested from adults, we cannot comment on the performance of this assay in that population. Others have found that DFA testing (2, 3) and chromatogenic antigen detection assays (2) for re-

spiratory viruses have much lower sensitivities in this population than in pediatric patients. Until the RSV-RS assay is studied for adults, use should be limited to pediatric samples. Last, the RSV-RS assay provides only a single result. The SimulFluor respiratory screen assay tests for the presence of other respiratory viruses in the same sample. Our current algorithm is to do further testing on samples that screen positive for other respiratory viruses. Implementing the RSV-RS assay for STAT specimens would reduce turnaround times but also increase costs, as further workup by DFA testing would still be required for detecting other respiratory viruses.

In summary, the RSV Respi-Strip assay can be used as a rapid method for detecting RSV in samples from pediatric patients. It is technically easy to perform and has performance characteristics for implementation as a STAT or point-of-care test during the annual RSV epidemic.

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TABLE 2. Cost analysis

Method	No. of samples processed	Hands-on time (min)	Cost (Canadian dollars)		
			Reagent/run ^a	Total/run	Per result
RSV-DFA	1	20	34.13	44.47	44.47
	5	35	40.18	58.93	11.79
	10	60	49.16	80.21	8.02
RSV-RS	1	6	22.35	25.46	25.46
	5	15	56.59	64.35	12.87
	10	30	99.39	114.92	11.49

^a Includes cost of using a positive and negative control for each run.