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Comparison of Two Rapid Methods for Detection of Respiratory Syncytial Virus (RSV) (TestPack RSV and Ortho RSV ELISA) with Direct Immunofluorescence and Virus Isolation for the Diagnosis of Pediatric RSV Infection

EVA E. THOMAS* AND LAURA E. BOOK

Department of Pathology, Faculty of Medicine, University of British Columbia, British Columbia's Children's Hospital, Vancouver, British Columbia V6H 3V4, Canada

Received 25 May 1990/Accepted 12 December 1990

The ability of two commercial immunoassays to detect respiratory syncytial virus (RSV) in respiratory specimens was evaluated as follows: 152 specimens were tested by TestPack RSV (Abbott), and 72 were tested by Ortho RSV ELISA (Ortho). Test outcomes were compared with those of virus isolation alone, direct immunofluorescence assay (DFA) alone, or virus isolation and/or DFA. TestPack RSV versus virus isolation showed 91% sensitivity, 96% specificity, 93% positive predictive value (PPV), and 95% negative predictive value (NPV). TestPack RSV versus DFA showed 89% sensitivity, 97% specificity, 96% PPV, and 93% NPV. When TestPack RSV performance was compared with that of virus isolation and DFA, the sensitivity was 87% and the specificity was 100%. Ortho RSV ELISA versus virus isolation showed 88% sensitivity, 87% specificity, 79% PPV, and 93% NPV. Ortho RSV ELISA versus DFA showed 91% sensitivity, 88% specificity, 81% PPV and 95% NPV. When Ortho RSV ELISA performance was compared with that of virus isolation and DFA, the sensitivity was 86%, the specificity was 89%, the PPV was 86%, and the NPV was 89%. The accuracy of the TestPack RSV in combination with ease of performance and no need for specialized equipment or special skills make it an attractive alternative to DFA for rapid direct detection of RSV.

Respiratory syncytial virus (RSV) is a major cause of upper and lower respiratory tract infections in children (15) and is frequently reported as causing nosocomial outbreaks (4). Access to rapid and accurate diagnostic services for the detection of RSV is of value, not only for the early initiation of antiviral treatment of children who are seriously ill with the virus but also for the prevention of nosocomial spread. The usefulness of the classical direct immunofluorescence assay (DFA) for RSV detection is dependent upon a skilled microscopist and prompt specimen processing (9, 13), and the method is not applicable to automation or large-scale testing. Enzyme-linked immunosorbent assays (ELISAs) for direct antigen detection are not usually as rapid as DFA (3, 4) and display various degrees of sensitivity and specificity (10, 15) as compared with virus isolation and DFA. These tests are not, however, dependent on an experienced microscopist or prompt specimen processing (9). ELISAs are suitable for large-scale testing (15), and the results can be objectively obtained by spectrophotometry (5, 15). Because of the limitations of both DFA and ELISAs, their use has been mainly restricted to specialized, well-equipped virus laboratories. TestPack RSV (Abbott Laboratories, Abbott Park, Ill.) is a rapid RSV detection immunoassay which is self-contained and requires neither specialized equipment nor extensive training. We evaluated the overall performance of TestPack RSV and Ortho RSV ELISA (Ortho Diagnostic Systems Inc., Raritan, N.J.), which uses the classical microtiter plate format for the detection of RSV in pediatric respiratory specimens.

Clinical specimens. A total of 152 respiratory specimens were collected from children who were admitted to British Columbia's Children's Hospital (ages, 2 weeks to 18 years) and who had acute respiratory disease from 1 October through 31 March 1987 to 1988 and 1988 to 1989. One hundred seventeen specimens were nasopharyngeal washings (obtained by the instillation of 1.5 ml of saline through a baby feeding catheter inserted in the child's nostril, with the recovery of 1 ml of fluid), 16 were tracheal aspirates, 10 were Auger suctions, 3 were sputum specimens, 1 was a throat swab specimen, and 5 were from unidentified sites. All specimens were transferred to sterile containers and transported to the laboratory immediately after collection, at which time one portion was cultured for viral agents, a second portion was tested by DFA for RSV, and a third portion was frozen at −70°C for future studies. The numbers of specimens tested with each test were as follows: virus isolation, 152; DFA, 152 (18 of which contained insufficient material); TestPack RSV, 152; and Ortho RSV ELISA, 72 (72 of which were cultured and 63 of which were tested by DFA). All specimens were coded, and one technologist conducted the tests without knowledge of DFA or culture results.

Virus isolation. Three commercially available cell types (Connaught Diagnostics, Willowdale, Ontario, Canada) were used: primary rhesus monkey kidney (PMK) cells, MRC-5 human lung diploid fibroblasts, and HEp-2 epidermoid carcinoma cells. A fresh specimen (1 ml) was added to two tubes each of HEp-2, PMK, and MRC-5 cells and incubated at 35°C overnight on a stationary rack. After 20 h of incubation, the medium was changed and cultures were transferred to a roller drum and maintained for 14 days. RSV, cytomegalovirus (CMV), enterovirus, and adenovirus were detected by their cytopathic effects. Influenza virus and parainfluenza virus were detected by hemadsorption.

* Corresponding author.

TABLE 1. TestPack RSV versus virus isolation and DFA

TestPack RSV result	No. of results					
	Culture (n = 152)		DFA (n = 134)		Culture and/or DFA[a] (n = 134)	
	+	−	+	−	+	−
+	50	4	49	2	54	0
−	5	93	6	77	8	72

[a] −, Negative by both culture and DFA; +, positive by culture or DFA or both.

TABLE 3. Accuracy of TestPack RSV

Test(s) that TestPack RSV was compared with	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Culture (n = 152)	91	96	93	95
DFA (n = 134)	89	97	96	93
Culture and/or DFA[a] (n = 134)	87	100	100	90

[a] Negative specimens were negative by both culture and DFA; positive specimens were positive by culture or DFA or both.

Virus identification was performed as follows: RSV by DFA (see below); influenza virus, parainfluenza virus, and adenovirus by the indirect immunofluorescence assay (Microscan Division, Bartels, Baxter Healthcare Co., West Sacramento, Calif.); enterovirus by electron microscopy; and CMV by DFA (Bartels Immunodiagnostic Supplies Inc., Bellevue, Wash.).

DFA. A fluorescein isothiocyanate-conjugated anti-RSV antibody (Ortho respiratory syncytial virus identification reagent; Ortho) was used for direct detection and culture confirmation. The manufacturer's instructions were followed with one modification: for rapid detection of RSV in fresh specimens, 1 ml of sample and 1 ml of dimercaptoethanol (Sigma Chemical Co., St. Louis, Mo.) were mixed and vortexed for 1 to 2 min prior to the addition of 4 ml of phosphate-buffered saline. After being processed, the specimens were assessed by the same technologist at a magnification of ×400 with a Leitz Laborlux 12 microscope with a mercury short-arc lamp and considered positive if they demonstrated typical cytoplasmic inclusions in one or more epithelial cells. The specimens were considered negative if there were at least three columnar epithelial cells present per field with no typical staining. Specimens with fewer than three columnar epithelial cells present per field and not exhibiting positive fluorescence were considered inconclusive.

TestPack RSV. The manufacturer's instructions for the TestPack RSV were followed. The principles of the test were described in detail previously (12). Specimens tested by the TestPack RSV had all been frozen at −70°C and were coded and tested at the same time.

Ortho RSV ELISA. The manufacturer's instructions for the Ortho RSV ELISA were followed. Because of a lack of specimens, only 72 could be tested. These specimens had all been frozen at −70°C.

Statistical methods. Sensitivity, specificity, and predictive values for positive and negative tests were calculated by standard procedures (6).

RSV was isolated with HEp-2, PMK, and MRC-5 cells from 55 of 152 specimens. The mean time for the appearance of cytopathic effects was 6.7 days (range, 2 to 19 days). One RSV culture-positive specimen also grew adenovirus. From 23 RSV culture-negative specimens other viruses were isolated as follows: 6 parainfluenza virus type 3, 2 influenza virus type B, 2 enterovirus, 6 CMV, 3 adenovirus, and 1 hemadsorbing agent (not identified). Three specimens each contained two viruses (CMV and adenovirus; influenza virus type A and adenovirus; and influenza virus type A and CMV). DFA, virus isolation, Ortho RSV ELISA, and TestPack RSV were not positive in any of these specimens, with one exception: one specimen growing adenovirus was found RSV positive by Ortho RSV ELISA but negative by virus isolation, DFA, and TestPack RSV.

DFA testing of smears was positive in 55 of 134 specimens and negative in 79 of 134 specimens. One hundred thirty-three specimens were tested by both DFA and virus isolation. One hundred twenty (90%) test results were in concordance. Of the 13 (10%) discordant specimens, 5 were found positive by virus isolation and negative by DFA and 8 were found positive by DFA and negative by virus isolation. We compared TestPack RSV and Ortho RSV ELISA versus virus isolation alone and versus both virus isolation and DFA (Tables 1 and 2). The diagnostic accuracies of the evaluated tests were calculated for the same combinations (Tables 3 and 4). Only 134 specimens yielded DFA results, since 18 of 152 (12%) had an insufficient number of cells present and would have required repeat sampling. Of these 18, 2 were found positive by virus isolation, TestPack RSV, and Ortho RSV ELISA; 1 was found positive by virus isolation and TestPack RSV but negative by Ortho RSV ELISA; and the remaining 15 were found negative by the other assays. Two of 152 specimens tested by TestPack RSV required retesting, and 3 of 152 showed weak reactions. No specimens tested by Ortho RSV ELISA required retesting.

Seventy-two specimens were tested by both Ortho RSV ELISA and TestPack RSV. Twenty-three were found posi-

TABLE 2. Ortho RSV ELISA versus virus isolation and DFA

Ortho RSV ELISA result	No. of results					
	Culture (n = 72)		DFA (n = 63)		Culture and/or DFA[a] (n = 65)	
	+	−	+	−	+	−
+	23	6	21	5	24	4
−	3	40	2	35	4	33

[a] See Table 1 footnote.

TABLE 4. Accuracy of Ortho RSV ELISA

Test(s) that Ortho RSV ELISA was compared with	%			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Culture (n = 72)	88	87	79	93
DFA (n = 63)	91	88	81	95
Culture and/or DFA[a] (n = 65)	86	89	86	89

[a] See Table 3 footnote.

tive by both (22 of these were culture positive and 1 was culture negative), and 42 were found negative by both (40 of these were culture negative and 2 were culture positive). One was found positive by TestPack RSV and negative by Ortho RSV ELISA (this specimen later grew RSV). Six were found positive by Ortho RSV ELISA and negative by TestPack RSV (five were found negative by DFA and virus isolation and one was found positive by DFA and virus isolation).

The question of an appropriate "gold standard" in comparative studies of rapid RSV diagnosis has been debated in the literature, with some authors suggesting that virus isolation should be the mainstay (11, 14) and others arguing that DFA and ELISAs are more sensitive and could in most circumstances replace virus isolation (7, 14). In this study, in which all specimens were received within 2 h of collection, 8 of 133 (6%) were found positive by DFA and negative by virus isolation and 5 of 133 (4%) were found negative by DFA and positive by virus isolation. In addition, 24% of all virus-positive respiratory specimens did not contain RSV. A low virus load or a poor quality of the DFA specimens could explain a positive RSV culture with a negative DFA result. A negative RSV culture with a positive DFA result could be explained by specimen collection taking place several days after the onset of symptoms or inactivation of the virus because of ongoing ribavirin treatment or prolonged specimen transportation time. The use of a mucolytic agent prior to specimen processing and an experienced interpreter decrease the risk of nonspecific fluorescence. Because of these concerns, we chose to compare our test results not only with those of virus isolation and DFA separately but also with those of virus isolation and DFA together.

TestPack RSV performed well in our study, in which the majority of specimens were nasopharyngeal washings (77%) or tracheal aspirates (17%), with a sensitivity of 87% and a specificity of 100% when compared with DFA and virus isolation (Table 3). These results are in agreement with those of Swierkosz et al. who evaluated TestPack RSV with nasopharyngeal swab specimens (12). Nasopharyngeal washings have been reported to be superior to nasopharyngeal swabs for RSV recovery (1, 8), but our results suggest that either of the two specimen collection procedures is appropriate for analysis with TestPack RSV (12). In addition to its high accuracy, it was also a rapid test (20 min per specimen) and required no specialized technical skills or equipment. It was estimated that one technologist could handle 10 to 15 specimens in a single batch. The major drawback of TestPack RSV is the cost. It would be expensive to use this test on large scale, but it is well designed for use in "after-hours" testing or for a laboratory with a low specimen load and limited experience with RSV diagnosis.

Ortho RSV ELISA, which did not include a blocking antibody, had a sensitivity of 86% and a specificity of 89% when compared with DFA and virus isolation (Table 4). This assay has been evaluated by others (2, 7, 10), and although our results were more favorable, the specificity should ideally have been higher in our study. It has been our experience that it is easier to defend a low sensitivity than a low specificity to clinicians. The specificity in our study might have improved with the use of a blocking antibody, but the necessity of a confirmatory step for all positive results would increase the testing time. This test required special equipment, such as an ELISA washer and a reader, to make use of its potential for automation and large-scale testing. Other commercial ELISAs for RSV detection with better accuracy have been reported (10, 15), and although it is difficult to compare test results between laboratories with different specimens and test conditions, we cannot recommend Ortho RSV ELISA without the use of a blocking antibody.

Rapid detection of respiratory viral agents is becoming of increasing importance with the introduction antiviral agents such as ribavirin (15). Although interest in providing such assays commercially has been focused on RSV, other viral pathogens (e.g., influenza virus and parainfluenza virus) are also important in pediatric respiratory disease. It is our opinion that virus isolation, with its limitations (it is labor intensive and cannot detect inactivated virus), remains the best alternative for the definitive diagnosis of respiratory viral pathogens. However, with the availability of a specific, rapid, direct RSV assay, a positive result could exclude further culturing of the specimen, with some cost reduction for clinical virus laboratories. In this study, only 1 of 55 RSV culture-positive specimens grew another virus (adenovirus), indicating that the risk of missing a dual virus infection with such a policy is low. We still prefer DFA as a direct test because it allows the assessment of specimen quality and is less expensive than TestPack RSV. We are, however, using TestPack RSV for after-hours testing. In conclusion, TestPack RSV could safely be used for rapid RSV diagnosis in a small laboratory with no cell culturing facilities and limited DFA experience, with the caveat that a negative specimen should be sent off to a specialized laboratory for further analysis. The more widespread use of direct RSV testing should be encouraged, since it not only will help sick patients but also will provide good epidemiological data and improve our knowledge of this important pathogen.

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