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Evaluation of the Abbott TESTPACK RSV Enzyme Immunoassay for Detection of Respiratory Syncytial Virus in Nasopharyngeal Swab Specimens

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The Abbott TESTPACK RSV assay (Abbott Laboratories, North Chicago, Ill.), a rapid (20-min) enzyme immunoassay, was compared with culture and direct immunofluorescence (DFA) of nasopharyngeal cells for the detection of respiratory syncytial virus (RSV) in nasopharyngeal swab specimens. Nasopharyngeal swab specimens, collected from 234 infants, were placed in viral transport medium. Portions of specimen in transport medium were used for each test. Of 234 specimens, 70 (30%) were culture positive, 103 (44%) were DFA positive, 107 (46%) were culture or DFA positive, and 112 (48%) were TESTPACK RSV positive. Of 19 specimens positive by TESTPACK RSV but negative by culture or DFA, 15 were positive by the blocking assay. A total of 122 specimens were culture, DFA, or blocking assay positive; TESTPACK RSV detected 108 specimens (sensitivity, 89%). The specificity, positive predictive value, and negative predictive value of TESTPACK RSV as compared with those of culture, DFA, and the blocking assay were 96, 96, and 89%, respectively. By comparison, the sensitivity, specificity, positive predictive value, and negative predictive value of combined culture and DFA were 88, 100, 100, and 88%, respectively. TESTPACK RSV is a rapid and reliable enzyme immunoassay for the direct detection of RSV antigen in nasopharyngeal swab specimens.

Epidemics of lower respiratory tract infections caused by respiratory syncytial virus (RSV) occur annually, accounting for a large proportion of pediatric hospital admissions during the winter (5). The availability of antiviral therapy with ribavirin coupled with the necessity of controlling the nosocomial spread of RSV have spurred efforts to develop rapid, same-day diagnostic methods for this virus. This premarket study compares the Abbott TESTPACK RSV assay (Abbott Laboratories, North Chicago, Ill.), a rapid (20-min) enzyme immunoassay, with culture and direct immunofluorescence (DFA) for the detection of RSV in nasopharyngeal swab specimens.

MATERIALS AND METHODS

Specimens. Nasopharyngeal swab specimens were obtained from 234 infants with respiratory symptoms by using swabs (Mini-Tip Culturette; American Scientific Products, St. Louis, Mo.). The ages of the patients ranged from 1 day to 2 years, with the exception of one patient, who was 3 years old. Specimens were collected during the 1987 and 1988 winter season. Duplicate swab specimens from each patient were placed in vials of viral transport medium (veal infusion broth plus 0.5% gelatin; gentamicin, 100 μg/ml; mycostatin, 100 U/ml; penicillin G, 200 U/ml; amphotericin B, 5 μg/ml; 0.00125% phenol red; 2.5 ml per vial) and were combined on arrival in the laboratory. Specimens from hospitalized patients usually arrived in the Virology Laboratory within 4 h after collection. Specimens received from outside hospitals were kept at 4°C until transport to the Virology Laboratory. Forty-five specimens were stored at −70°C prior to TESTPACK RSV analysis.

Viral culture. Commercially available cell culture tubes of MRC-5 cells (Ortho Diagnostics, Inc., Raritan, N.J.) and primary rhesus monkey kidney (RhMK) cells (Viromed Laboratories, Inc., Minneapolis, Minn.; Whittaker Bioproducts, Walkersville, Md.) were obtained weekly and maintained as described previously (3). HEp-2 cells were obtained in flasks at monthly intervals from Whittaker Bioproducts. Culture tubes were prepared twice weekly from cells that were trypsinized from the commercially purchased flasks or from subpassages of samples from these flasks and maintained as described previously (4). Prior to inoculation, all tubes were fed with maintenance medium.

Duplicate nasopharyngeal swab specimens were first vortexed, with the swab remaining in the transport medium, and were then combined. One tube of MRC-5 cells, two tubes of RhMK cells, and one tube of HEp-2 cells each were inoculated with 0.2 ml of vortexed specimen. The MRC-5 cell tube and one of the RhMK cell tubes were incubated at 36°C on a roller drum. The second RhMK cell tube and the HEp-2 cell tube were incubated at 33°C on a roller drum. Cell cultures were observed daily (except Sundays) for 7 days and every third or fourth day thereafter for a total of 14 days. HEp-2 cells were read daily (except Sundays). RhMK cell tubes, at 33°C, were hemadsorbed at day 7 postinoculation (or sooner if a suspicious cytopathic effect developed). RhMK cell tubes at 36°C were hemadsorbed at day 14 postinoculation. RSV was identified by the formation of large syncytial cells in MRC-5, RhMK, or HEp-2 cells that failed to hemadsorb guinea pig erythrocytes. Additionally, confirmation of RSV was performed by DFA of the cell culture whenever the DFA of the original specimen was negative. Foamy simian viruses were not evident in RhMK cell cultures that were considered positive for RSV. Other viruses were identified by their characteristic cytopathic effect (12). Hemadsorbing viruses (influenza or parainfluenza) were identified by indi-

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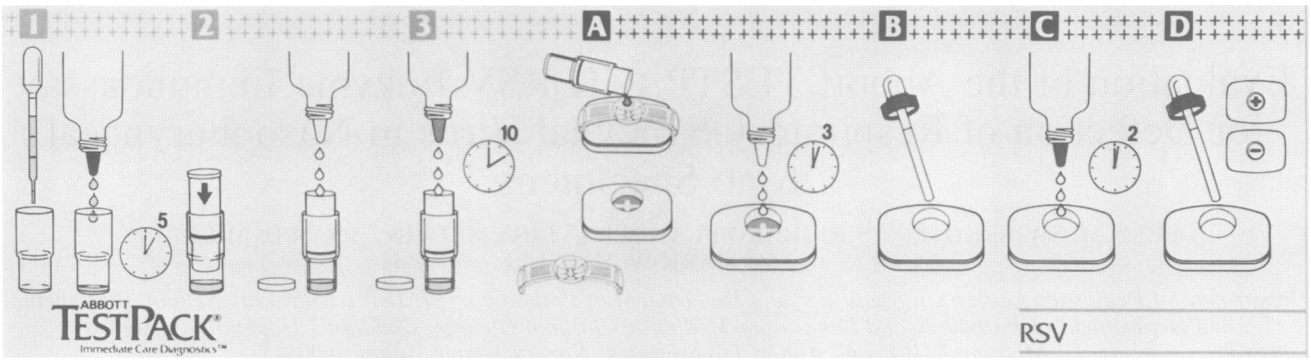

FIG. 1. TESTPACK RSV procedure. (Reprinted with permission from Abbott Laboratories.)

rect immunofluorescence (Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.).

DFA. A 1.5-ml sample of vortexed specimen was centrifuged at 1,660 × *g* for 5 min. The pellet was suspended in approximately 50 μl of phosphate-buffered saline (pH 7.3). This suspension was added to a well on a Teflon-coated (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) slide (Cell-Line Associates, Newfield, N.J.). Slides were dried under a warm stream of air and were then fixed in acetone at −20°C for 10 min. Control slides were made from RSV-infected RhMK cells, fixed as described above, and stored at −70°C.

Wells containing fixed cells were overlaid with 30 μl of fluorescein-conjugated monoclonal antibody to RSV (Imagen Respiratory Syncytial Virus test; Analytab Products, Plainview, N.Y.). Slides were incubated at 37°C in a humidified chamber for 15 min. Slides were rinsed with phosphate-buffered saline, submerged in phosphate-buffered saline for 5 min, and finally rinsed with distilled water. A control slide was stained as described above each day that the DFA was performed. Cover slips were mounted with mounting medium (Aqua-Mount; Lerner Laboratories, New Haven, Conn.), and slides were read with a microscope (Optiphot; Nikon), with incident UV light obtained from a 100-W high-pressure mercury lamp. Slides containing fewer than about 50 cells were rejected for insufficient cell number. The presence of a single cell exhibiting characteristic cytoplasmic fluorescence was sufficient to consider the specimen positive.

Enzyme immunoassay. The Abbott TESTPACK RSV was evaluated in a premarket study. The procedure, as illustrated in Fig. 1, was performed as follows. Specimen in viral transport medium (0.75 ml) was first treated with sample treatment buffer (reagent 1) and then filtered to clarify the specimen. Microparticles coated with bovine RSV antibody (reagent 2) were added to the specimen simultaneously with biotin-labeled bovine RSV antibody (reagent 3). After a 10-min incubation at room temperature, the solution was transferred to a reaction disk; and alkaline phosphatase-labeled anti-biotin antibody (reagent A), wash (reagents B and D), and substrate (reagent C) were added sequentially. Samples which contained RSV antigen formed a sandwich on the reaction disk consisting of antibody-coated microparticle–antigen–biotinylated antibody–anti-biotin enzyme conjugate. Specimens which produced a positive sign were considered positive for RSV antigen, while specimens which produced a negative sign were considered negative. The negative sign was produced by reaction of the biotinylated antibody and enzyme-labeled anti-biotin conjugate with a microparticle-RSV antigen complex already on the reaction disk. This procedural control controlled for the proper addition of reagents 3, A, B, and C. In addition, a white positive sign, formed by the microparticles against a blue field, was visible when the focuser was removed from the reaction disk, prior to the addition of reagent A. This controlled for the proper addition of reagents 1 and 2. If reagents were not added properly, one of the two procedural controls would not appear. Positive results were graded 1+, 2+, 3+, or >3+ based on the intensity of the vertical line. Any degree of color on the vertical line that was visibly darker than the background was considered positive (1+). A vertical line that was more intense in color than the horizontal line was interpreted as >3+. Positive (1+) and negative controls were tested weekly. Each new lot of uninoculated viral transport medium also was tested in duplicate. Specimens positive by TESTPACK RSV but negative by culture were tested in a blocking assay. The specimen was mixed with rabbit anti-RSV serum or diluent, and the TESTPACK RSV assay was performed as usual. Specimens that were ≥2+ by TESTPACK RSV were diluted before blocking was performed. A clearly significant reduction in the reaction of the treated specimen as compared with that of the untreated specimen indicated the presence of RSV antigen. For the majority of blocking assays (32 of 40), the treated (blocked) sample reverted to negative.

RESULTS

A total of 234 specimens were evaluated. A total of 70 specimens (30%) were culture positive for RSV; all 70 of these specimens (100%) were TESTPACK RSV positive. Forty-two specimens were culture negative but TESTPACK RSV positive (Table 1). A blocking assay was performed on 40 of the 42 discrepant samples; 36 were blocking assay positive. Of 42 discrepant samples, 38 were either blocking assay or DFA positive. Three of the four discrepant specimens that were blocking assay negative or DFA negative were low-level positives (1+) by TESTPACK RSV. Of the 112 specimens that were TESTPACK positive, 90% produced reactions that were 2+ or greater.

Of 234 specimens, 221 had sufficient cells for DFA; of these, 103 were DFA positive; of the 103 DFA-positive specimens, 89 (86%) were TESTPACK RSV positive (Table 1). Of 21 DFA-negative and TESTPACK RSV-positive samples, 17 were either culture positive or blocking assay positive.

A total of 107 specimens were either culture or DFA positive; 93 of these 107 specimens (87%) were TESTPACK

TABLE 1. Comparison of TESTPACK RSV with culture and DFA for detection of RSV antigen

TESTPACK RSV result	No. of specimens by:							
	Culture		DFA[a]		Culture or DFA		Culture, DFA, or blocking assay	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Postive	70	42	89	21	93	19	108	4
Negative	0	122	14	97	14	108	14	108

[a] Thirteen specimens contained insufficient cells for DFA.

RSV positive (Table 1). Nineteen specimens were TESTPACK RSV positive but were negative by both culture and DFA. Of 18 specimens with a sufficient volume for blocking, 15 were blocking assay positive.

Finally, 122 specimens were culture, DFA, or blocking assay positive; 108 (89%) were TESTPACK RSV positive (Table 1). All 14 specimens that were false negative by TESTPACK RSV were positive by DFA only. Of the 14 specimens positive by DFA only, 13 had less than 10 fluorescing cells per slide. Four specimens were TESTPACK RSV positive but negative by all other tests; three of four were 1+ by TESTPACK RSV. The fourth specimen was repeatedly 2+ by TESTPACK RSV but could not be tested by the blocking assay because of an insufficient specimen volume.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TESTPACK RSV compared with those of culture alone; DFA alone; culture or DFA combined; and culture, DFA, or blocking assay are given in Table 2. When compared with culture, the TESTPACK RSV showed 100% sensitivity but a positive predictive value of only 63%. However, the performance of TESTPACK RSV versus that of culture improved significantly when the results of the blocking assay were considered (sensitivity, 100%; specificity, 95%; PPV, 95%; NPV, 100%). When compared with culture, DFA, or blocking assay, the sensitivity, specificity, PPV, and NPV of TESTPACK RSV were 89, 96, 96, and 89%, respectively.

The relative performances of TESTPACK RSV, culture, and DFA when a total of 122 specimens were considered true positives, i.e., culture, DFA, or blocking assay positive, and compared in Table 3. TESTPACK RSV was more sensitive than culture and comparable to the combined use of culture and DFA for the detection of RSV.

Five RSV-positive (culture or blocking assay positive) specimens yielded additional viruses: cytomegalovirus (two), influenza A (one), parainfluenza type 1 (one), and rhinovirus (one). Ten viruses were isolated from RSV-negative (all tests negative) specimens: adenovirus (one), enterovirus (one), influenza A (six), and rhinovirus (two).

In an attempt to determine whether the 14 specimens that were positive only by DFA were true-positive or false-positive results, the medical records (excluding laboratory results) of these patients were reviewed by a single individual (M.W.K.) who was blinded to the culture results. An equal number of records of patients who were culture and DFA positive also were reviewed. The two groups did not differ with respect to age, admitting diagnosis, admission respiratory rate, reason for requesting DFA, duration of hospitalization, use of ribavirin therapy, prevalence of upper respiratory tract infection, or fever. All 14 (100%) patients whose specimens were positive both by culture and DFA had evidence of either respiratory distress or wheezing, while 8 of 14 (57%) patients whose specimens were DFA positive and culture negative had neither of these signs ($P < 0.001$; by the Fisher exact test).

DISCUSSION

The Abbott TESTPACK RSV assay is a rapid (20-min) enzyme immunoassay that has a performance comparable to that reported for currently available enzyme immunoassays which have longer turnaround times, ranging from 90 min to 5 h (2, 6–8, 10, 11, 13, 17). This test requires no instrumentation and can be read visually. Several unique features of the TESTPACK RSV, such as the large sample volume (750 μl) and the use of antibody-coated microparticles, biotinylated antibody, a focuser to concentrate the reaction, and enzyme-labeled anti-biotin antibody, contribute to the rapid assay format.

The TESTPACK RSV was more sensitive than culture for the detection of RSV. This finding was not surprising in light of the relative insensitivity of culture compared with that of DFA (9, 16). Ahluwalia and Hammond (2) found that culture detected only 75% of confirmed enzyme-linked immunosorbent assay-positive specimens. Moreover, McIntosh et al. (15) have demonstrated that specimens remained enzyme immunoassay positive even after storage at room temperature for 1 week, while the majority of specimens were culture negative.

The performance of TESTPACK RSV was comparable to that of combined culture and DFA and to that of DFA alone. However, specimens that contained <10 positive cells by DFA were not detected by TESTPACK RSV. Because the

TABLE 2. Performance characteristics of TESTPACK RSV compared with those of culture and DFA

TESTPACK RSV vs:	%			
	Sensitivity	Specificity	PPV	NPV
Culture	100	74	63	100
Culture or blocking assay	100	95	95	100
DFA	86	82	81	87
Culture or DFA	87	87	83	89
Culture, DFA, or blocking assay	89	96	96	89

TABLE 3. Relative performance characteristics of TESTPACK RSV, culture, and DFA[a]

Test method	%			
	Sensitivity	Specificity	PPV	NPV
TESTPACK RSV	89	96	96	89
Culture	57	100	100	76
DFA	86	100	100	86
Culture and DFA	88	100	100	88

[a] A total of 122 specimens were considered true positives, i.e., culture, DFA, or blocking assay positive.

interpretation of DFA results is very subjective, we cannot rule out the possibility that some of the DFA-positive and TESTPACK-negative specimens were false positives. Indeed, of the 14 specimens that were DFA positive but culture and TESTPACK RSV negative, 8 were from patients without evidence of lower respiratory tract disease, i.e., no wheezing or respiratory distress. If these specimens are considered false positives by DFA, the sensitivity, specificity, PPV, and NPV of TESTPACK RSV compared with those of culture, true-positive DFAs, and blocking assay were 95% (108 of 114), 97% (116 of 120), 96%, and 95%, respectively.

A minor disadvantage of this assay is the limitation on the number of tests that can be performed at one time. In our experience, no more than eight specimens could be tested in one run. During the winter, it is not uncommon for our laboratory to process up to 20 specimens for RSV per day. However, multiple runs of the TESTPACK RSV can be performed in a shorter time than one run of DFA.

The Abbott TESTPACK RSV is a reliable alternative to culture and DFA for the direct detection of RSV in nasopharyngeal swab specimens. We anticipate similar results with nasal wash and nasal aspirate specimens which have been reported to be superior to nasopharyngeal swab specimens for the diagnosis of RSV (1, 14). The rapid turnaround time (20 min) should facilitate the prompt institution of infection control procedures and therapy when necessary. Because up to 20% of RSV-negative specimens may contain other viruses (4), we recommend the culture of TESTPACK RSV-negative specimens. We also recommend the culture of RSV-positive specimens from patients who are immunocompromised or who have severe disease to detect other viruses, in addition to RSV.

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