JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1981, p. 258-265
0095-1137/81/020258-08$02.00/0

Vol. 13, No. 2

Detection of Respiratory Syncytial, Parainfluenza Type 2, and Adenovirus Antigens by Radioimmunoassay and Enzyme Immunoassay on Nasopharyngeal Specimens from Children with Acute Respiratory Disease

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Four-layer antispecies radioimmunoassay (RIA) and enzyme immunoassay (EIA) procedures were developed for the detection of respiratory syncytial virus (RSV), parainfluenza type 2 virus, and adenovirus antigens in nasopharyngeal specimens from children hospitalized for acute respiratory disease. Polystyrene beads (RIA) or flat-bottomed polystyrene microtiter plates (EIA) were used as the solid phases, guinea pig anti-virus immunoglobulins were used as the captive antibodies, rabbit anti-virus immunoglobulins were used as the secondary antibodies, and ^{125}I-labeled sheep anti-rabbit (RIA) or horseradish peroxidase-labeled swine anti-rabbit (EIA) immunoglobulins were used as the indicator antibodies. A comparison of the EIAs and RIAs with routinely used immunofluorescence (IF) techniques was made with 164 nasopharyngeal specimens collected from children with acute respiratory disease. Only 3 of 66 RSV IF-positive specimens were negative in RSV RIA, and of 83 RSV, parainfluenza type 2 virus, and adenovirus IF-negative specimens, 1 was positive in RSV RIA. Of 4 parainfluenza type 2 virus IF-positive and 11 adenovirus IF-positive specimens, each was positive in corresponding RIAs, and all 83 IF-negative specimens were negative in parainfluenza type 2 virus and adenovirus RIAs. The results of the RSV, parainfluenza type 2, and adenovirus EIAs confirmed the results of corresponding RIAs in each selected case tested. The RIAs and EIAs were found to be as specific and sensitive as IF techniques, and more practical in the rapid detection of respiratory viruses in nasopharyngeal secretions.

Viral respiratory diseases are common in all age groups, but especially in young children. The diagnosis of these diseases has been based traditionally on virus isolation from throat washings or the detection of a significant rise in virus-specific antibodies. Virus isolation, however, is expensive and time consuming, often taking several days to perform, and serological diagnosis usually requires two serum specimens taken 2 to 3 weeks apart to allow for detection of the immune response.

The development of sensitive and specific immunofluorescence (IF) techniques for the detection of viral antigens in the cells of the nasopharyngeal secretions, collected at the acute stage of the disease, has greatly improved the rapid laboratory diagnosis of viral respiratory diseases (4). The problem of specimen transportation, however, has caused some limitations to the use of the technique since cell preparations from the nasopharyngeal secretions are destroyed rapidly during transportation by the proteolytic enzymes present in the specimens. The method also requires a highly skilled microscopist to evaluate the test and is not easily automated.

Radioimmunoassay (RIA) and enzyme immunoassay (EIA) methods recently have been applied in the direct detection of viral antigens in clinical specimens (2, 6, 9, 10, 12, 13). These methods offer high sensitivity and specificity, do not require any laborious pretreatment of the specimens, and allow the testing of a large number of specimens per day. Recent advances in rapid viral diagnosis have suggested that similar RIA and EIA methods may also be used in the diagnosis of respiratory diseases (1, 3). In the present study, four-layer antispecies RIA and EIA methods were developed for the detection of respiratory syncytial virus (RSV), parainfluenza type 2 virus, and adenovirus antigens in nasopharyngeal specimens collected from children with acute respiratory disease. The results obtained with methods developed are reported and compared with those of corresponding IF techniques routinely used in this laboratory for the diagnosis of respiratory diseases.

MATERIALS AND METHODS

Propagation of parainfluenza type 2 virus and RSV. Parainfluenza type 2 virus and RSV (Randall strain) used in the preparation of viral nucleocapsids were grown in roller cultures of Vero cells at 35°C. Basic Medium Eagle with 0.2% bovine serum albumin (Armour Pharmaceutical Co., Ltd., Eastbourne, England), 5% (vol/vol) tryptose phosphate (Difco Laboratories, Detroit, Mich.), and antibiotics was used as the maintenance medium. When the cells showed an extensive cytopathic effect, usually in 5 or 6 days, they were detached by scraping with a rubber policeman, washed twice with phosphate-buffered saline (PBS), pH 7.35, and stored as a 20% (vol/vol) suspension in PBS at −20°C until used.

Purification of viral nucleocapsids. The virus-infected cells were homogenized in a Sorvall Omnimixer (Sorvall Inc, Newtown, Conn.) at full velocity for 2 min on ice. After the homogenization, 1% trypsin (Difco Laboratories) was added to give a final concentration of 0.25% (wt/vol), and the cell suspension was incubated at room temperature for 60 min with constant stirring, followed by centrifugation for 20 min at 2,000 rpm. The supernatant fraction was then centrifuged on CsCl step gradients (3 ml of 25%, 2 ml of 30%, and 1 ml of 40% CsCl) in a Spinco SW 27 rotor at 25,000 rpm for 3 h at 4°C, and the nucleocapsid band at the 30% CsCl layer was collected and dialyzed overnight against PBS, pH 7.35. A final concentration of 0.05% (wt/vol) of trypsin was then added to the nucleocapsid solution, and the mixture was incubated at room temperature for 30 min with constant stirring. The trypsin-treated nucleocapsid material was then recentrifuged into 30% CsCl as described above and layered on a third CsCl step gradient (20, 25, 30, 35, and 40% CsCl, 1.2 ml each), which was centrifuged for 15 h at 25,000 rpm at 4°C in a SW 27 rotor. The purified nucleocapsid preparation was concentrated by centrifugation at 25,000 rpm for 45 min at 4°C in a Spinco SW 27 rotor, and the pellet was collected. The protein content was determined by the method of Lowry et al. (8). The purification of group-reacting adenovirus type 2 hexon antigen used as the immunizing antigen for the preparation of hyperimmune sera against adenovirus has been reported earlier (6).

Immunizations. Rabbit antisera to parainfluenza type 2 virus and RSV were prepared by intradermally immunizing rabbits with 300 μg of purified virus protein per animal in PBS diluted 1:1 in incomplete Freund adjuvant. Intradermal booster inoculations (200 μg per animal) were given 3 and 6 weeks after the first injection. One week after the final injection, the animals were exsanguinated, and the sera were preserved at −70°C. Guinea pigs were immunized in a similar way except that 100 μg of antigens per animal were used. The guinea pig sera were pooled and preserved as above. The titers of both rabbit and guinea pig hyperimmune sera for RSV were between 1:500,000 and 1:1,000,000, and those for the parainfluenza type 2 virus were between 1:1,000,000 and 1:10,000,000 as measured by EIA with the parainfluenza type 2 and RSV cell lysates as antigens (5). The immunization procedure for preparation of group-reacting adenovirus type 2 hexon hyperimmune sera in rabbits and guinea pigs has been reported in detail elsewhere (6).

RIA reagents. Guinea pig and rabbit anti-parainfluenza type 2 virus, anti-RSV, and anti-adenovirus immunoglobulin fractions were prepared by precipitation of sera with an 18% (wt/vol) final concentration of sodium sulfate, followed by chromatography on a Sephadex G-25 column. Immunosorbent-purified sheep anti-rabbit immunoglobulin G antibody was labeled with ^{125}I by a modification of the chloramine T method of Hunter and Greenwood (7). Chromatography on Sephadex G-25 was used to separate the unreacted iodine from the radiolabeled protein. Fractions containing the radiolabeled protein were pooled, diluted with an equal volume of PBS, pH 7.35, containing 42% glycerol, 2% bovine serum albumin, and 0.2% NaN_3, and stored at 4°C until used. The specific activity of the labeled antiserum was 11 μCi/μg.

RIA procedures for parainfluenza type 2 virus and RSV antigens. Polystyrene beads (6.4 mm in diameter, Precision Plastic Ball Co., Chicago, Ill.) were coated with anti-parainfluenza type 2 or anti-RSV guinea pig immunoglobulin by incubating the untreated beads overnight in an antibody solution, containing either 1.25 μg of anti-parainfluenza type 2 immunoglobulin per ml (0.25 μg per bead) or 2.5 μg of anti-RSV immunoglobulin per ml (0.5 μg per bead). Carbonate buffer, pH 9.6, was used as diluent (14). Beads were stored in these antibody solutions at 4°C until used, usually for not more than 2 weeks. Nasopharyngeal specimens in 200-μl volumes in a single (vol/vol) dilution or serially diluted were pipetted into disposable polystyrene tubes and a polystyrene bead with adsorbed anti-virus immunoglobulin was then added. After overnight incubation at 37°C, the specimens were aspirated, and the beads were washed twice with 5 ml of tap water. A 200-μl volume of rabbit anti-parainfluenza type 2 immunoglobulin (4 μg/ml) or anti-RSV immunoglobulin (16 μg/ml) was then added to each tube, and the beads were incubated at 37°C for 1 h. The beads were washed as described above and a 200-μl volume of ^{125}I-labeled sheep anti-rabbit immunoglobulin was added to each tube, and the beads were incubated for 1 h more at 37°C. After the washing procedure, the beads were placed in clean tubes, and radioactivity was counted in an LKB 1280 gamma counter. Buffer blanks (dilution buffer only) and titrations of virus-infected cell lysate antigens used as positive controls (5) were included in each assay. As a dilution buffer, PBS (pH 7.35) containing 20% inactivated fetal calf serum (Gibco Europe, Glasgow, Scotland), 2% Tween 20, and 0.1% NaN_3 was used in all steps. The assay was standardized by diluting the iodinated anti-rabbit immunoglobulins to a concentration that gave 5,000 cpm bound (5,000 active cpm), when a 200-μl volume of the label was incubated with a bead adsorbed with 2 μg of purified rabbit immunoglobulin G. The value of 300 cpm (approximately two times the mean of the counts-per-minute values for the negative specimens) was taken as the cutoff value, and the specimens with a value of 300 cpm or more were considered as positive with the proviso that confirmatory tests indicated specific binding.

RIA confirmatory tests for parainfluenza type 2 and RSV antigens. To test the specificity of the binding, blocking tests were performed on selected specimens. The tests were done in the same way as described above except that the anti-parainfluenza type 2 or anti-RSV guinea pig serum was added before the secondary rabbit antibody. The tests were done as follows: 200-μl volumes of the specimens were pipetted into three tubes, a bead with adsorbed anti-virus immunoglobulin was added to each of the three tubes, and the beads were incubated overnight at 37°C. After the washing procedure, a 200-μl volume of the anti-parainfluenza type 2 guinea pig serum (1:4,000 dilution) or anti-RSV guinea pig serum (1:500 dilution) was added to the first tube, and the same volume of corresponding dilutions of normal guinea pig sera (preinfection sera) was added to the second tube; to the third tube only dilution buffer was added. After incubation at 37°C for 1 h, a 200-μl volume of the rabbit anti-parainfluenza type 2 immunoglobulin (8 μg/ml) or rabbit anti-RSV immunoglobulin (32 μg/ml) was added to each of the three tubes (without the removal of the previous 200-μl sample), giving a total volume of 400 μl. The beads were then incubated for 1 h at 37°C, and the rest of the tests were performed as the assay proper. The tests were considered positive if a 50% or greater decrease in counts-per-minute values was seen with the beads incubated with guinea pig hyperimmune sera, as compared with beads incubated with normal guinea pig sera or dilution buffer.

Adenovirus RIA and adenovirus confirmatory test. The radioimmunoassay procedure for the detection of adenovirus antigens and the corresponding confirmatory test were identical to the parainfluenza type 2 and RSV RIA tests and have been reported earlier in detail (6).

EIA for parainfluenza type 2 virus, RSV, and adenovirus antigens. The EIAs for parainfluenza type 2, RSV, and adenovirus antigens and the confirmatory tests were identical to the corresponding RIA methods. The same incubation times and the same concentrations of reagents were used. The tests were done as follows: polystyrene flat-bottomed microtiter plates (catalogue no. 76-201-05, Flow Laboratories Ltd., Irving, Scotland) were used as the solid phase instead of polystyrene beads because the color reactions could be measured directly through the bottoms of the microtiter plate wells. The plates were coated in 100-μl volumes with either 0.25 μg of the anti-parainfluenza type 2 virus, 0.25 μg of anti-adenovirus, or 0.5 μg of anti-RSV immunoglobulins per well. Volumes (100 μl) of the specimen dilutions were pipetted into each of the wells, incubated overnight at 37°C, followed by washing twice with PBS (pH 7.35) containing 0.1% Tween 20 and the subsequent addition of a 100-μl volume per well of the appropriate rabbit anti-virus immunoglobulins. After a 1-h incubation at 37°C and the washing procedure, a 100-μl volume per well of horseradish peroxidase-conjugated swine antibodies against rabbit immunoglobulins (Orion Diagnostica, Helsinki, Finland) in a dilution of 1:2,000 was added. After an additional 1-h incubation at 37°C and the washing procedure, a 100-μl volume of the substrate solution, consisting of 3 mg of orthophenylene diamine (Koch-Light Laboratories, Colnbrook, Bucks, England) per ml in 0.1 M citrate-Na_2HPO_4 buffer (pH 5.5) and 10 μl of 30% H_2O_2 per 15 ml in the same buffer, was added to each of the wells. The reaction was stopped by adding a 100-μl volume per well of 1 N HCl after the incubation for 1 h at room temperature in the dark. The intensity of the color was determined with a Titertek Multiscan spectrophotometer (Flow Laboratories, Ltd., Irving, Scotland). As a diluent, PBS (pH 7.35) containing 20% inactivated fetal calf serum, 2% Tween 20, and 10^{-4} M merthiolate was used in all steps, except for the substrate solution. The EIA confirmatory tests were identical to the RIA confirmatory tests, except that 100-μl volumes of the reagents, instead of 200-μl volumes, were used.

IF. A standard indirect immunofluorescence method was used for the detection of adenovirus, parainfluenza type 2 virus, and RSV antigens in the cells of nasopharyngeal secretions of children with acute respiratory disease (4). Bovine anti-RSV (Wellcome Research Laboratories, Beckenham, England), a guinea pig antiserum to parainfluenza type 2 virus (kindly supplied by G. Åstad, Statens Institut for Folkenhelse, National Institute of Public Health, Oslo, Norway), and a rabbit antiserum to adenovirus type 2 (kindly supplied by Monica Grandien, State Bacteriological Laboratory, Stockholm, Sweden) were used as the detector antibodies. As indicator antibodies, fluorescein-conjugated rabbit anti-bovine, sheep anti-guinea pig, and sheep anti-rabbit immunoglobulins (Wellcome Research Laboratories) were used.

Specimens. The nasopharyngeal specimens were obtained from 164 children, between 1 month and 9 years old, hospitalized with acute respiratory disease in Turku University Hospital and Turku City Hospital during epidemics in 1978, 1979, and 1980. The specimens were obtained within 2 days after admission to the hospital by aspirating the secretions with a mucus extractor through the nostrils from the nasopharynx. The specimens were then stored at 0°C on ice during transportation and processed further within 2 to 4 h.

Specimen handling. The nasopharyngeal specimens were processed by the technique of Gardner and McQuillin (4). Briefly, the specimens were diluted with 1 to 2 ml of sterile PBS, pH 7.35, and the mucus was broken up with a wide-bore Pasteur pipette. The specimens were then centrifuged at 1,000 rpm for 10 min, and the supernatant fluid containing the soluble viral antigens (mucus fraction) and the pellet containing the cells were collected. The cells were further suspended in PBS (pH 7.35), the remaining unbroken mucus was removed with a Pasteur pipette, and the cells were recentrifuged at 1,000 rpm for 10 min at 4°C. The washed cells were then diluted with PBS (pH 7.35) to obtain optimal cell concentration, and slide preparations were made for IF studies. The rest of the cell material (cell fractions) and the mucus fractions were stored at −70°C for RIA and EIA studies. From those specimens with sufficient volume, part of the original nasopharyngeal specimens without further processing were stored at −70°C for RIA and EIA studies. Before testing in RIA and EIA, the specimens (original nasopharyngeal specimens, mucus, and cell fractions) were diluted ½ in PBS containing 20%

inactivated fetal calf serum, 2% Tween 20, and 10^{-4} M merthiolate and sonicated for 1 to 3 minutes with a Branson Sonifier cell disrupter B15 (Branson Instruments Co., Stamford, Conn.) to solubilize the mucus and to homogenize the specimens. The specimens were then further diluted in the same buffer and tested at a 1/20 dilution of the original nasopharyngeal specimens, and a 1/5 dilution of the mucus and the cell fractions. However, the actual dilution of the mucus fractions was much higher than 1/5 and varied considerably because of the original dilution in PBS as indicated above.

RESULTS

The sensitivities of the RIAs for adenovirus, parainfluenza type 2 virus, and RSV antigens were determined by testing dilutions of the viral antigens based upon the protein content. The sensitivity of the adenovirus RIA and EIA for the highly purified adenovirus type 2 hexon antigen was approximately 1 ng/ml. The sensitivities of the RSV and parainfluenza type 2 RIAs and EIAs for the semipurified RSV and parainfluenza type 2 cell culture antigens varied from 10 to 30 ng/ml (Table 1).

The cross-reactivities of the RIAs for adenovirus, parainfluenza type 2 virus, and RSV antigens were shown by cross-testing all the RIA-positive nasopharyngeal specimens in all three RIA tests. No cross-reactions between the tests were found except for two dual infections with RSV and adenovirus, confirmed by RSV and adenovirus IF. The specificity of parainfluenza type 2 RIA for the parainfluenza type 2 antigens was further shown by negative results in cross-tests with RIA for parainfluenza type 1 and type 3 viral antigens.

Representative RIA titration results of RSV IF-positive and of RSV IF-negative nasopharyngeal secretions, and the corresponding mucus and cell fractions, are presented in Table 2. The results indicate that some of the RSV IF-positive nasopharyngeal secretions were positive in RIA up to a dilution of 1:1,280 or even higher. RSV antigens were usually also detectable simultaneously in the mucus and the cell fractions. The counts-per-minute values of the negative specimens were low, varying from 50 to 150 cpm.

The original nasopharyngeal secretions were available for 29 RSV IF-positive and 83 RSV IF-negative specimens (Fig. 1a and b). Each of 83 secretions representing IF-negative specimens was negative in RIA except 1, which had a counts-per-minute value slightly above the cut-off line. Of 29 secretions representing IF-positive specimens, 28 were positive in RIA, and 1 specimen was negative.

The mucus fractions were available for 24 of 29 RSV IF-positive and 78 of 83 RSV IF-negative specimens shown in Fig. 1a and b. In addition, 37 additional mucus fractions were available from RSV IF-positive specimens. Figures 1c and d indicate the RIA results for all 139 mucus fractions. Each mucus fraction from the RSV IF-negative specimens was negative in RIA, but only 50 of 61 mucus fractions from the RSV IF-positive specimens were positive in RIA. For 3 of these 11 mucus specimens with a negative RIA result, the original secretions were available, and 2 were positive in RIA. When the cell fractions from these 11 specimens were tested, 6 additional specimens were positive in RIA. Thus, three specimens were negative in RIA, but positive in IF. However, for only one of these three specimens was the original specimen available, and this was negative (Fig. 1a).

A confirmatory RIA was done on selected RSV IF-positive nasopharyngeal secretions and mucus fractions showing low (between 300 and 1,000 cpm) binding in RSV RIA. The results indicated a specific binding in each case. The confirmatory RIA also proved the specificity of the binding of the specimen with a weakly positive result in RSV RIA but a negative result in IF (see Fig. 1b).

Four parainfluenza type 2 IF-positive and 83 IF-negative specimens were available for testing in RIA. Titrations in the parainfluenza type 2 RIA of the four IF-positive and of one IF-nega-

TABLE 1. *Sensitivity comparison of RIA and EIA for RSV, parainfluenza type 2 virus (para 2), and adenovirus (adeno) antigens*[a]

Virus	cpm at protein content (ng/ml) of virus preparation								Sensitivity (ng/ml)
	1,000	300	100	30	10	3	1	0	
RSV	1743 (≥2.000)[b]	1,065 (1.129)	359 (0.564)	175 (0.314)	130 (0.186)	90 (0.122)	103 (0.111)	85 (0.124)	10–30
Para-2	2219 (≥2.000)	1,454 (1.315)	602 (0.361)	267 (0.190)	136 (0.099)	81 (0.074)	79 (0.079)	89 (0.072)	10–30
Adeno	NT NT	NT NT	2,847 (≥2.000)	2,028 (≥2.000)	1,312 (1.464)	638 (0.562)	365 (0.305)	134 (0.139)	1

[a] Twice the mean value of the three buffer blanks (0 ng/ml) was taken as the cutoff value in each test.
[b] Figures in parentheses indicate absorbance at 492 nm. NT, not tested.

TABLE 2. *Representative results of RSV antigen detection by the RIA on three IF-positive (patients 1, 2, and 3) and three IF-negative (patients 4, 5, and 6) nasopharyngeal specimens collected from children hospitalized with acute respiratory disease*

Patient	Age	IF results	cpm at specimen dilution of:							
			Nasopharyngeal secretion						Mucus (1:5)	Cells (1:5)
			1:20	1:80	1:320	1:1,280	1:5,120	1:20,480		
1	1 yr, 1 mo	+	2,402	2,352	2,103	1,349	618	273	2,568	2,498
2	6 mo	+	1,749	1,492	1,106	567	244	183	1,146	176
3	6 yr, 10 mo	+	546	457	348	186	171	141	296	244
4	3 yr, 4 mo	−	157	NT[a]	NT	NT	NT	NT	151	154
5	4 mo	−	102	NT	NT	NT	NT	NT	98	115
6	11 mo	−	122	NT	NT	NT	NT	NT	112	93

[a] NT, Not tested.

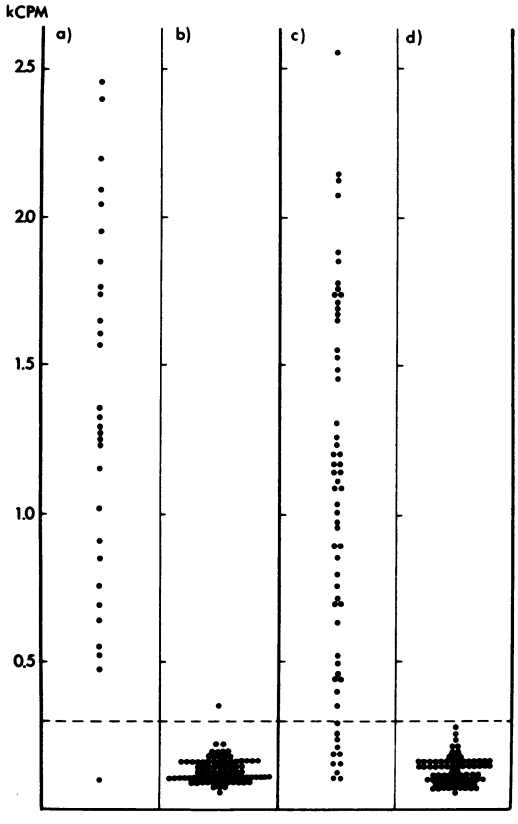

FIG. 1. *RIA results expressed as counts-per-minute values for 29 RSV IF-positive (a) and 83 RSV IF-negative (b) original nasopharyngeal specimens, and for corresponding 61 RSV IF-positive (c) and 78 RSV IF-negative mucus fractions (d) of the original nasopharyngeal specimens, collected from children hospitalized with acute respiratory disease. The dotted line represents the cutoff line at 300 cpm.*

tive specimens are shown in Table 3. All IF-positive specimens were clearly positive in RIA whether the tested material was the original nasopharyngeal secretion, the mucus, or the cell fractions. The counts-per-minute values of the negative specimens were very low, usually 50 to 80 cpm.

Eleven adenovirus IF-positive and 83 IF-negative specimens were available for the RIA studies. The mucus fractions were obtained from 8, the cell fractions were obtained from 6, and the original nasopharyngeal secretion was available from 3 of the 11 IF-positive specimens. All specimen fractions for the 11 IF-positive specimens were positive, and all IF-negative specimens were negative in adenovirus RIA, and the counts-per-minute values were comparable to those obtained in RSV and parainfluenza type 2 RIAs (Table 4).

All the adenovirus and the parainfluenza type 2 positive specimens, selected RSV positive specimens, and selected negative specimens were tested also in EIA (Table 5). The results of the EIAs confirmed those of the corresponding RIAs in each case, and the confirmatory EIAs indicated specific reactions. The absorbance values for the negative specimens were very low, usually between 0.03 and 0.1.

DISCUSSION

Despite the fact that the number of the positive specimens tested, particularly for adenovirus and parainfluenza type 2 virus, were rather limited, the results indicated that respiratory virus antigens can be detected by four-layer antispecies RIA and EIA on nasopharyngeal secretions collected from children with acute respiratory diseases. This finding provides rapid and practical ways for the specific diagnosis of viral respiratory diseases. RSV, parainfluenza type 2 virus, and adenoviruses were selected as representative respiratory viruses, but identical assays are now under development in our laboratory for additional respiratory viruses.

The developed assays were compared with the

TABLE 3. *Representative results of parainfluenza type 2 virus antigen detection by the RIA on four IF-positive (patients 7, 8, 9, and 10) and one IF-negative (patient 2) nasopharyngeal specimens and mucus and cell fractions of the nasopharyngeal specimens, collected from children hospitalized with acute respiratory disease*

Patient	Age	IF results	Specimen[a]	cpm at specimen dilution of:						
				1:5	1:20	1:80	1:320	1:1,280	1:5,120	1:20,480
7	9 yr	+	A	NT[b]	1927	1,079	518	212	113	81
8	3 yr, 8 mo	+	A	NT	2,633	2,576	2,065	991	268	116
			B	2,751	2,391	1,501	617	181	79	NT
			C	2,812	2,269	1,216	522	148	72	NT
9	1 yr, 7 mo	+	B	821	225	105	103	77	59	NT
			C	1,812	781	245	88	45	52	NT
10	5 yr, 11 mo	+	B	1,184	705	308	139	85	50	NT
			C	2,847	2,145	937	294	117	56	NT
2	6 mo	−	A	NT	50	NT	NT	NT	NT	NT
			C	48	NT	NT	NT	NT	NT	NT

[a] A, Nasopharyngeal specimen; B, mucus fraction; C, cell fraction.
[b] NT, Not tested.

TABLE 4. *Representative results of adenovirus antigen detection by the RIA on three IF-positive (patients 11, 12, and 13) and one IF-negative (patient 14) nasopharyngeal specimens and cell and mucus fractions of the nasopharyngeal specimens, collected from children hospitalized with acute respiratory disease*

Patient	Age	IF results	Specimen[a]	cpm at specimen dilution of:						
				1:5	1:20	1:80	1:320	1:1,280	1:5,120	1:20,480
11	10 mo	+	A	NT[b]	2,113	1,708	1,082	415	179	76
			B	1,565	1,209	986	506	139	100	NT
12	5 mo	+	C	2,165	1,772	1522	1,002	444	133	NT
			B	1,911	1,467	788	345	133	68	NT
13	2 yr, 4 mo	+	C	532	320	170	104	77	70	NT
14	1 yr, 3 mo	−	A	NT	85	NT	NT	NT	NT	NT
			B	94	NT	NT	NT	NT	NT	NT

[a] Letter designations as in Table 3.
[b] NT, Not tested.

standard indirect IF test (4), which has been the routine test for respiratory virus antigens in our diagnostic unit for several years. The highly specific immunofluorescence technique has proved to be at least as sensitive a test as virus isolation in our laboratory, and the work of Gardner and McQuillin (4) and more recently Minnich and Ray (11) has shown that the agreement between virus isolation and IF is more than 90% for RSV, between 76 and 99% for parainfluenza type 2 virus, and between 35 and 65% for adenovirus.

When the results of IF were compared with those of RIA and EIA, only one specimen was negative in RSV RIA (and also RSV EIA) on the original nasopharyngeal secretion but positive in RSV IF. Two other specimens were positive in RSV IF and negative in RSV RIA and EIA, but only the mucus and cell fractions from these specimens were available for RIA and EIA studies. Another specimen, positive in RSV RIA and EIA and proved specific by confirmatory tests, was negative in RSV IF. Complete agreement was found between the adenovirus and the parainfluenza type 2 virus RIA and EIA, and the corresponding IF even though only the mucus or cell fractions were available from many specimens.

TABLE 5. *Representative results of RSV, parainfluenza type 2 virus (para 2), and adenovirus (adeno) antigen detection by EIA on three IF-positive (patients 1, 10, and 11) nasopharyngeal specimens and one negative (patient 15) nasopharyngeal specimen collected from children hospitalized with acute respiratory disease*

Patient	IF results	Virus	Absorbance at 492 nm at specimen dilution of:						
			1:5	1:20	1:80	1:320	1:1,280	1:5,120	1:20,480
1	+	RSV	NT[a]	1.919[b]	1.757	1.276	0.571	0.172	0.072
10	+	Para 2	1.434[c]	0.666	0.278	0.116	0.079	0.059	NT
11	+	Adeno	NT	≥2.0[b]	1.544	0.838	0.261	0.104	0.042
15	−	RSV	NT	0.043[b]	NT	NT	NT	NT	NT
15	−	Para 2	NT	0.064[b]	NT	NT	NT	NT	NT
15	−	Adeno	NT	0.066[b]	NT	NT	NT	NT	NT

[a] NT, Not tested.
[b] Nasopharyngeal specimen.
[c] Mucus fraction of the nasopharyngeal specimen.

With the mucus fractions of the specimens, a lower positive rate was found as compared with the original nasopharyngeal secretions. This indicates that the dilution of the specimen and the cells present in the original nasopharyngeal secretions may influence the outcome of RIA and EIA. The sonication used in the preparation of specimens might release additional antigens from the cells, increasing the sensitivity of the RIA and EIA when original nasopharyngeal specimens are used as specimens instead of mucus fractions. Thus, throat washings, often collected instead of nasopharyngeal secretions as routine specimens in viral respiratory diseases, may not contain sufficient concentrations of viral antigen for RIA and EIA methods. Indeed, our preliminary results with the detection of adenovirus antigens from throat washings of army trainees with adenovirus infections have confirmed these findings; viral antigens were detectable in fewer than half of the cases positive by virus isolation (Halonen and Sarkkinen, unpublished data).

The use of nasopharyngeal secretions as specimens for RIA and EIA instead of throat washings, however, requires the disruption of the mucus present in the secretions. The mechanical disruption of the mucus with a Pasteur pipette often used in the preparation of cells for IF studies is too tedious and results in too much dilution of the specimens. In the early stage of the study, several chemical treatments were tried, including trypsinization and treatments with 20% *N*-acetyl cysteine (3) and with 0.5% deoxycholate. However, all these treatments produced variable results, and at least *N*-acetyl cysteine decreased the specific counts-per-minute or absorbance values of the specimens as compared with the untreated controls. Sonication of the specimens for a maximum of 3 min with a sonicator equipped with a microtip was found to be the optimal treatment for solubilizing the secretions. This treatment resulted in the total disruption of the mucus without any need to dilute the specimens with large amounts of dilution buffer. It was also found that as much as a twofold increase in the specific counts-per-minute and absorbance values was detected with some secretions when the specimens were treated by sonication only.

The confirmatory tests were not necessary to prove the specificity of the binding in RIA and EIA since no false-positive reactions were found. This is in contrast with our earlier findings with the use of the RIA and EIA techniques in the detection of rotavirus and adenovirus antigens in stool specimens (6, 13, 13a). However, in spite of this finding we feel that low positive reactions (between 300 and 1,000 cpm in RIA, and between the absorbance values of 0.3 and 1.0 in EIA) should be confirmed in the blocking tests to make certain that the reactions are specific. This is particularly important since the experience gained so far in the use of the RIA and EIA for the detection of respiratory virus antigens is limited.

Only a few reported studies dealt with direct antigen detection by RIA or EIA, from the clinical specimens of patients with respiratory disease. With EIA for RSV, Chao et al. (3) found positive results for 23 of 29 nasopharyngeal secretions positive for RSV by tissue culture isolation, but only 19 if the positive/negative ratio of 2.1 was taken as the cutoff line. However, they treated their specimens with *N*-acetyl cysteine, which according to our results is less efficient than sonication in disrupting mucus and also decreases the specific counts-per-minute and absorbance values. Berg et al. (1) reported the detection of influenza A antigens by inhibition EIA in all 12 volunteers infected with influenza A/Victoria/3/75 wild type. They concluded that

the sensitivity of a conventional EIA was not enough to detect the influenza A antigens in the throat washings, but required the use of an enzyme-linked fluorescence assay or an ultrasensitive enzymatic radioimmunoassay, reported by them to be 1,000 times more sensitive than the conventional EIA. Our unpublished results for the detection of adenovirus antigens from throat washings indicate that the sensitivity of a conventional four-layer RIA and EIA is on the borderline for the throat washings but is high enough for nasopharyngeal specimens collected from hospitalized children.

The RIA procedures for the detection of rotavirus and adenovirus antigens from stool specimens (6, 13) have been used with excellent results for more than 1.5 years in our routine diagnostic unit. In the present study, both RIA and EIA methods were developed for the detection of viral antigens from nasopharyngeal specimens. The results of the present and an earlier study (13a) have confirmed that both methods are equally sensitive, specific, and also practical in the detection of viral antigens from clinical specimens. The choice between these two methods is thus mainly based on the special requirements of the laboratory and the equipment available. However, whichever test is chosen, both a four-layer immunoassay, with only the antispecies antibody labeled with a radioactive tracer or conjugated with enzyme, or a direct immunoassay with a labeled viral antibody, can be used. The four-layer immunoassay is possibly the best choice when several viral antigens must be tested for each specimen, but for certain viruses the direct immunoassay might be the method of choice.

ACKNOWLEDGMENTS

The excellent technical assistance of Kaija Johansson and Seija Hurten is gratefully acknowledged.

This study was supported by grants from the Sigrid Jusélius Foundation and the Daisy and Yrjö Eskola Foundation.

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