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Comparison of Enzyme-Linked Immunosorbent Assay, Indirect Immunofluorescence Assay, and Virus Isolation for Detection of Respiratory Viruses in Nasopharyngeal Secretions

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Nasopharyngeal secretions obtained from 94 children with acute respiratory illness were examined for the presence of respiratory syncytial virus (RSV), adenovirus, and influenza virus type A by virus culturing (virus isolation technique [VIT]), immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA). Similar results were obtained in at least two tests for RSV, influenza virus type A, and adenovirus in 92 (97.9%), 88 (93.6%), and 88 (93.6%) cases, respectively. Both rapid virus detection methods showed good specificity for the diagnosis of these virus infections (≥90.7%) and were more sensitive than was VIT for RSV detection. In a more accurate statistical analysis, the indexes of agreement between VIT and ELISA were substantial for RSV ($\kappa = 0.69$; $z = 5.5$; $P < 0.0001$), influenza virus type A ($\kappa = 0.67$; $z = 5.3$; $P < 0.0001$), and adenovirus ($\kappa = 0.71$; $z = 6.0$; $P < 0.0001$), while it was almost perfect for RSV when ELISA was compared with IFA ($\kappa = 0.88$; $z = 5.7$; $P < 0.0001$). Although the observed agreement was good in the comparison of these two tests for these three viruses (89%), the indexes of agreement were moderate in the comparison of IFA and VIT for RSV ($\kappa = 0.55$; $z = 2.0$; $P < 0.05$), influenza virus type A ($\kappa = 0.42$; $z = 9.7$; $P < 0.0001$), and adenovirus ($\kappa = 0.41$; $z = 6.5$; $P < 0.0001$) and of ELISA and IFA for influenza virus type A ($\kappa = 0.55$; $z = 7.0$; $P < 0.0001$) and adenovirus ($\kappa = 0.59$; $z = 6.8$; $P < 0.0001$). All of the statistical evaluations demonstrated better agreement between ELISA and VIT for influenza virus type A and adenovirus.

Respiratory viruses are currently identified in many clinical laboratories only by inoculating a variety of cell cultures with respiratory secretions collected from infected patients. Although offering a sensitive and specific approach to the diagnosis of most common respiratory viruses, virus culturing takes several days to complete. To find a rapid, specific, and sensitive method of diagnosis, investigators have used different techniques for the detection of viral antigens in clinical specimens, and the value of these techniques has been discussed (1–9, 11–14, 16, 17, 19–29). In recent years, more intensive studies have been done, mainly of the diagnosis of respiratory syncytial virus (RSV); trials of antiviral therapy for RSV infection are now being conducted (15).

The purpose of this study was to simultaneously compare enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and the standard virus isolation technique (VIT) for the detection of virus or viral antigens in the nasopharyngeal secretions (NPS) of children with acute respiratory illness and to evaluate their use in the diagnosis of RSV, influenza virus type A, and adenovirus, some of the most common viruses responsible for this kind of disease.

MATERIALS AND METHODS

Patients. During the period from January 1985 through December 1986, nasopharyngeal specimens from 94 infants and children were studied. The patients were either seen in the emergency room or hospitalized with acute respiratory disease. The time of sampling ranged from 2 to 8 days after the onset of symptoms.

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Collection of specimens and processing. NPS were obtained by gentle suction with a plastic catheter, a mucus trap, and irrigation with a small volume (about 2.0 ml) of sterile phosphate-buffered saline (PBS). The nasal aspirate obtained was transported in ice to the laboratory within 2 h.

VIT. Specimens treated with antibiotics were inoculated into tubes containing monolayers of HEp-2, BHK-21, Vero, and MDCK cell cultures. The HEp-2 and BHK-21 cell cultures were maintained in Eagle's minimal essential medium with 2% fetal calf serum, while Vero and MDCK cell cultures were maintained in Eagle's minimal essential medium with 2 μg of trypsin per ml. The cells were fed with fresh medium every 3 days. Two tubes of each cell culture were inoculated with 0.1 ml of specimen per tube and incubated at 35°C in stationary racks. The cultures were examined every 2 days for cytopathic effects or for the presence of hemadsorption for 14 days. The hemadsorption test was done 7 days after inoculation of clinical specimens into tubes of Vero (guinea pig erythrocyte) and MDCK

TABLE 1. Positivity of VIT, IFA, and ELISA for RSV, influenza virus type A, and adenovirus in the NPS of 94 children

Virus	No. (%) of specimens found positive by:			
	VIT	ELISA	IFA	VIT+ELISA+IFA
RSV	8 (8.5)	15 (16.0)	14 (14.9)	16 (17.0)
Influenza virus type A	8 (8.5)	8 (8.5)	12 (12.8)	15 (16.0)
Adenovirus	11 (11.7)	9 (9.6)	13 (13.8)	17 (18.1)
Total	27 (28.7)	32 (34.0)	39 (41.5)	48 (51.1)

TABLE 2. Comparison of IFA, ELISA, and VIT for diagnosis of RSV, influenza virus type A, and adenovirus infections in the NPS of 94 children

Possible result	No. of specimens with the indicated result[a]								
	RSV			Influenza virus type A			Adenovirus		
	I/V	E/V	E/I	I/V	E/V	E/I	I/V	E/V	E/I
−/−	78	80	78	82	81	80	78	79	79
−/+	1	0	2	4	1	2	7	2	2
+/−	8	6	1	4	5	6	4	4	6
+/+	7	8	13	4	7	6	5	9	7

[a] I, IFA; V, VIT; E, ELISA.

(chicken erythrocyte) cell cultures. The isolated virus was identified by IFA with a specific antibody (Wellcome Laboratory, United Kingdom). One blind passage was done for the negative specimens before the specimens were discarded. Influenza virus isolation was also attempted by inoculating NPS into the amniotic sacs of 10-day-embryonated hen eggs.

IFA. Cells from nasopharyngeal aspirates were prepared for IFA for RSV, adenovirus, and influenza virus type A as described previously (30). The reagents for IFA (Wellcome Laboratory) were provided by the Central Public Health Laboratory, London, United Kingdom. NPS were diluted in PBS, centrifuged, suspended in PBS, and centrifuged. This procedure was repeated twice. The washed cells were resuspended in a few drops of PBS, and two cell smears on each microscope slide were prepared and then fixed in nonhydrous acetone for 10 min at 4°C. Indirect IFA was used. The specimens were examined under a fluorescence microscope with a halogen lamp (Nikon; 50 W). All of the clinical specimens were read by two observers. The doubtful or discrepant samples were restained and read at least twice to check for reproducibility.

ELISA. The ELISA has been described elsewhere (14). NPS were diluted 1/10 with PBS, sonicated in a cell disruptor for 2 min, congealed and discongealed three times, and centrifuged. The supernatant was processed for ELISA. The wells of microtiter plates (Dynatec) were coated with 100 μl of adequate dilutions of a capture virus antiserum specific for each virus (produced either in guinea pigs for influenza virus type A and adenovirus or in bovines for RSV). After incubation for 3 h at 37°C, the wells were washed four times in phosphate buffer containing 0.05% Tween 20. Specimens (100 μl) were added to duplicate wells for each virus investigation and incubated overnight at 37°C. The wells were again washed, and 100 μl of the appropriate rabbit serum was added. After incubation for 1 h at 37°C and a wash, 100 μl of an anti-rabbit immunoglobulin–alkaline phosphatase conjugate was added. After incubation for 1 h at 37°C and another wash, the substrate *p*-nitrophenylphosphate was added. Both antisera and control antigens were provided by the National Bacteriological Laboratory, Stockholm, Sweden. The alkaline phosphatase conjugate and the substrate *p*-nitrophenylphosphate were acquired from Sigma Chemical Co.

Statistical analysis. The percentages of sensitivity, specificity, agreement, and predictive values were calculated with standard formulas. The kappa (κ) statistical method was used for a more accurate evaluation of the index of agreement (18), and the standard error was calculated with the *z* test with the corresponding *P* value (10).

RESULTS

Nasopharyngeal aspirates collected from 94 children with acute respiratory illness between January 1985 and December 1986 were tested simultaneously by IFA. ELISA, and VIT for the presence of RSV, influenza virus type A, and adenovirus.

Results of the comparison of the two antigen detection assays with VIT are summarized in Tables 1 and 2. ELISA and IFA detected viral infections in 32 and 39 cases, respectively, while only 27 were found positive by VIT. Of the 32 IFA-positive specimens, 16 yielded viruses in cell cultures. However, 12 culture-positive specimens were missed by IFA. One sample IFA positive for influenza virus A yielded enterovirus in cell cultures. ELISA detected viral antigens in 39 specimens, 25 of which were also found positive by VIT. Only three VIT-positive specimens were missed by ELISA. In the ELISA-IFA comparison, 26 specimens were found positive by both assays, 6 were found positive only by IFA, and 13 were found positive only by ELISA.

Overall, similar results were obtained in at least two tests for RSV, influenza virus type A, and adenovirus in 92 (97.9%), 88 (93.6%), and 88 (93.6%) cases, respectively. Six positive cases were detected only by IFA (two positive cases for each virus). Five positive cases for influenza virus type A (three cases) and adenovirus (two cases) were detected only by ELISA, while only VIT detected influenza virus type A in one case and adenovirus in two cases. Of a total of 94 NPS examined, 48 (51.1%) yielded a specific virus in one, two, or all three tests.

In comparison with the VIT (considered the gold standard), both ELISA and IFA were reliable for RSV diagnosis, as the sensitivity, specificity, and agreement were 100, 93.0, and 93.6%, respectively, in ELISA and 87.5, 90.7, and 90.4%, respectively, in IFA. Although the positive predictive value was low (46.7%) and the index of agreement was moderate ($\kappa = 0.55$; $z = 2.0$; $P < 0.0001$) in the comparison

TABLE 3. Percentages of sensitivity, specificity, predictive values, and agreement and kappa indexes of ELISA, IFA, and VIT for RSV in the NPS of 94 children

Assays compared	%					Kappa index (z)
	Sensitivity	Specificity	Predictive value		Agreement	
			+	−		
ELISA and VIT	100 (8/8)	93.0 (80/86)	57.1 (8/14)	100 (80/80)	93.6 (88/94)	0.69 (5.51)[a]
ELISA and IFA	86.7 (13/15)	98.7 (78/79)	92.9 (13/14)	97.5 (78/80)	96.8 (91/94)	0.88 (5.75)[a]
IFA and VIT	87.5 (7/8)	90.7 (78/86)	46.7 (7/15)	98.7 (78/79)	90.4 (85/94)	0.55 (2.0)[b]

[a] $P < 0.0001$.
[b] $P < 0.05$.

TABLE 4. Percentages of sensitivity, specificity, predictive values, and agreement and kappa indexes of ELISA, IFA, and VIT for influenza virus type A in the NPS of 94 children

Assays compared	%					Kappa index (z)[a]
	Sensitivity	Specificity	Predictive value		Agreement	
			+	−		
ELISA and VIT	87.5 (7/8)	94.2 (81/86)	58.3 (7/12)	98.8 (81/82	93.6 (88/94)	0.66 (5.26)
ELISA and IFA	75.0 (6/8)	93.0 (80/86)	50.0 (6/12)	97.6 (80/82)	91.5 (86/94)	0.55 (7.0)
IFA and VIT	50.0 (4/8)	95.3 (82/86)	50.0 (4/8)	95.3 (82/86)	91.5 (86/94)	0.47 (9.75)

[a] $P < 0.0001$.

of IFA and VIT, the index of agreement between ELISA and IFA was almost perfect ($\kappa = 0.88$; $z = 5.7$; $P < 0.0001$) and the positive predictive value was good (92.8%), demonstrating that the positive ELISA and IFA results which were missed by VIT were true (Table 3).

Test results for influenza virus type A are summarized in Table 4. The specificity of IFA was higher than 95%, but the sensitivity of IFA was low (50%); the index of agreement was moderate ($\kappa = 0.47$; $z = 9.7$; $P < 0.0001$) in the comparison of IFA and VIT, demonstrating that an observed agreement higher than 91% is not reliable. Furthermore, the positive predictive value was low, indicating that both ELISA and IFA probably detected false-positive results. All of the statistical evaluations demonstrated better agreement between ELISA and VIT for this virus.

For adenovirus, the specificities of all of the assays were higher than 92.9%, although the sensitivity was good (81.8%) only for ELISA. Also, the index of agreement between ELISA and VIT was substantial ($\kappa = 0.71$; $z = 6.0$; $P < 0.0001$), while in the comparisons of ELISA and IFA ($\kappa = 0.59$; $z = 6.8$; $P < 0.0001$) and IFA and VIT ($\kappa = 0.44$; $z = 6.5$; $P < 0.0001$), the indexes of agreement were only moderate (Table 5).

Other viruses, such as influenza virus type B, parainfluenza virus types 1, 2 and 3, enterovirus, and herpes simplex virus, were detected by culturing in 12 NPS. IFA also detected influenza virus type B and parainfluenza virus types 1 and 3, which were considered negative in the present evaluation.

DISCUSSION

Our data correspond to the results obtained by other investigators who have applied IFA and ELISA to the diagnosis of respiratory viruses in NPS. For RSV, the sensitivity of IFA (compared with culturing) has been reported to be 45 to 98% (7, 11, 13, 24, 28). The sensitivity of ELISA (compared with culturing) has ranged from 41 to 95% (4–6, 8, 21). In studies including IFA and ELISA, it has been found that ELISA is less sensitive than IFA is for the diagnosis of RSV and influenza virus type A (6, 24). It has also been reported that radioimmunoassay, ELISA, and IFA are equally sensitive for the detection of RSV (29). ELISA proved to be highly specific and showed good agreement with both IFA and VIT. The specificity, agreement, and positive predictive value for RSV were higher for ELISA than for IFA when the two assays were compared. Freezing of specimens may have led to discrepancies between the results of VIT and ELISA, since RSV may not survive freezing, while the viral antigen is quite stable (24). In the present investigation, some specimens were stored at −70°C for several days before virus isolation was attempted. Also, the overgrowth of HEp-2 cells may have made the cytopathic effect undetectable, resulting in a seemingly increased sensitivity of ELISA (4). All of the factors mentioned probably contributed to the low sensitivity of VIT in the detection of RSV infection.

The sensitivity of rapid diagnostic tests for influenza virus type A obtained by other investigators varies from 43 to 93%, with a specificity of greater than 90% (9, 11, 14, 16, 23, 25, 27, 28). The sensitivity observed in the present study in the comparison of IFA and VIT for influenza virus type A was low (50.0%). Also, the positive predictive values of three comparisons were low, suggesting the probability that both ELISA and IFA detected false-positive reactions.

For adenovirus, the sensitivity observed was also low (45.5%). Similar results were obtained by other investigators, who also observed that IFA was less sensitive than VIT was for the detection of adenovirus (12, 13, 15, 21, 27). Since adenovirus can occasionally be isolated from NPS long after the infection, the detection of this virus by VIT does not necessarily indicate an acute adenovirus infection, while IFA seems to predominantly detect a recent infection (12).

The kappa index was used in the present investigation to determine the concordance of the assays with each other and to assess the reliability of the observed agreement, which does not correct the agreement expected by chance. If one considers the absence of a standard, there is no way of

TABLE 5. Percentages of sensitivity, specificity, predictive values, and agreement and kappa indexes of ELISA, IFA, and VIT for detection of adenovirus in the NPS of 94 children

Assays compared	%					Kappa index (z)[a]
	Sensitivity	Specificity	Predictive value		Agreement	
			+	−		
ELISA and VIT	81.8 (9/11)	95.2 (79/83)	69.2 (9/13)	97.5 (79/81)	93.6 (88/94)	0.71 (6.0)
ELISA and IFA	77.8 (7/9)	92.9 (79/85)	53.8 (7/13)	97.5 (79/81)	91.5 (86/94)	0.59 (6.8)
IFA and VIT	41.7 (5/12)	95.1 (78/82)	55.5 (5/9)	91.8 (78/85)	88.3 (83/94)	0.44 (6.5)

[a] $P < 0.0001$.

determining the assay of choice. The kappa index should correct for the extent of agreement expected by chance (18). In the present study, it was shown that, although the agreement expected was good in the comparison of all of the assays (≥89%), the kappa index demonstrated only a moderate agreement for the diagnosis of influenza virus type A and adenovirus infections in the comparisons of ELISA and IFA and of IFA and VIT. The index of agreement was substantial between ELISA and VIT for RSV ($\kappa = 0.69$; $z = 5.5$; $P < 0.001$), influenza virus type A ($\kappa = 0.67$; $z = 5.3$; $P < 0.0001$), and adenovirus ($\kappa = 0.71$; $z = 6.0$; $P < 0.0001$) and moderate between IFA and VIT for the three viruses studied. The index of agreement between ELISA and IFA was almost perfect for RSV ($\kappa = 0.88$; $z = 5.7$; $P < 0.0001$), demonstrating that the positive IFA and ELISA results were true and that VIT was less sensitive.

Other studies have already demonstrated that none of the methods shows complete agreement when compared with each other, because of the variability of the reagents and the variable quality of the clinical specimens used by different investigators. Furthermore, apparent false-positive results may occur when specimens are collected at a later stage of the disease, when VIT becomes negative but when antigen is detectable by IFA and by ELISA (21, 26). Meanwhile, VIT detects viruses for which no reagents are available for rapid diagnosis. It also must be considered that only VIT is able to detect infection with a variant or even a new virus.

The results obtained in the present study indicate that ELISA is a good method for the detection of RSV, influenza virus type A, and adenovirus and is in better agreement with VIT than IFA is.

Rapid tests for the diagnosis of respiratory viruses cannot replace VIT but, if used in conjunction with VIT, can provide a significant increase in positivity (3, 11). The use of two techniques, either IFA and VIT or ELISA and VIT, provides a significant increase in the proportion of positive results, consequently diminishing the false-negative results.

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