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Enzyme Immunoassay for Respiratory Syncytial Virus: Rapid Detection in Nasopharyngeal Secretions and Evaluation of Isolates Representing Different RSV Subgroups

Marilda M. Siqueira,[1] Jussara P. Nascimento,[1] Silvana A.R. Portes,[1] and Wilhelm Schuy[2]

[1]Departamento de Virologia, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [2]Behringwerke AG, Marburg, Germany

The presence of respiratory syncytial virus (RSV) was investigated by immunofluorescent antibody (IFA) technique and by an enzyme immunoassay (EIA) in 169 samples of nasopharyngeal secretions of infants and children with acute respiratory infections. Of 31 samples positive by EIA, 25 were positive by IFA. In 24 samples from a retrospective study, RSV positive by IFA and/or tissue culture isolation (TCI), 22 were also positive by EIA. The EIA was also evaluated with 111 RSV isolates in Hep2 cell cultures representing different RSV subgroups. All were positive by EIA.

Key words: RSV groups A and B, enzyme immunoassay, rapid diagnosis, immunofluorescence, respiratory viruses

INTRODUCTION

Respiratory syncytial virus (RSV) is the major cause of acute respiratory viral infections in infants and young children worldwide (1). RSV produces a sharply defined epidemic every year with a large proportion of hospital admissions (2). Until some years ago RSV was thought to be a serologically monotypic virus. Recently, different investigators using monoclonal antibodies (MAbs) against virus structural proteins have recognized two major antigenic groups: A and B or 1 and 2 (3,4). This finding is important for better understanding of RSV immunology, for vaccine development, and the critical use of MAbs for rapid diagnosis.

Rapid detection of RSV is important for clinical management and the appropriate use of antiviral drugs. An established diagnostic method is based on the demonstration of RSV antigens in specimens of nasopharyngeal secretion (NPS) by indirect fluorescent antibody (IFA) test (5). Alternative methods such as enzyme immunoassay (EIA), radioimmunoassay (RIA), and time-resolved fluoroimmunoassay (TR-FIA) using polyclonal or monoclonal antibodies have been described (6,7,8).

In the present study, we applied an EIA (Enzygnost RSV (Ag), from Behringwerke AG, Marburg, Germany) for rapid diagnosis of RSV in NPS and compared it with the established method of IFA. This EIA was also evaluated with RSV isolates in Hep2 cell culture representing different groups and subgroups as described recently (9).

MATERIALS AND METHODS

Specimens

Nasopharyngeal secretions (NPS) were obtained from children showing acute respiratory infections within 5 days of onset of symptoms during the 1991 RSV season in Rio de Janeiro. Specimens were processed for viral diagnosis by IFA as described elsewhere (10) and the remainder kept at −70°C or processed immediately for diagnosis by EIA.

Immunofluorescence

All NPS slides were tested by IFA using a guinea-pig RSV antiserum (FIOCRUZ, Brazil) and an anti guinea-pig IgG FITC conjugate (Sigma Chemical Co., St. Louis, MO). All samples were also tested for influenza types A and B, adenovirus, and parainfluenza type 3 by the IFA technique as described previously (11).

EIA

The Enzygnost RSV (Ag) was developed for RSV antigen detection as specified in the preliminary instructions provided by the manufacturer (Behringwerke AG, Marburg, Germany).

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Address reprint requests to Dr. Marilda M. Siqueira, Departamento de Virologia, Fundação Oswaldo Cruz, Av. Brasil, 4365, 21040-360 Rio de Janeiro, Brasil.

The EIA is a sandwich assay of the heterogeneous type with a polyclonal catching antibody from rabbit and a monoclonal detection antibody as described by Mufson et al. (3) conjugated with horseradish peroxidase. Polyclonal catching antibodies are yielded after the immunization of rabbits with complete respiratory syncytial virus as published by Sarkkinen et al. (12). Supplementary reagents for Enzygnost TMB (Product No. OUVP, Behringwerke AG) with Washing Solution POD, Buffer/Substrate TMB, Chromogen TMB, and Stopping Solution POD were used as described in the instruction manual.

Processing of infected cells

Forty-seven RSV isolates from 1990 kept at liquid nitrogen were reinoculated in Hep2 cell culture. After 24 or 36 hr incubation, with or without evident CPE, the medium was removed and replaced by 0.3 ml of sample buffer STD (Product No. OUWO), left for 10 min, and disrupted by pipeting three times before transferring to test plate. Sixty-four RSV isolates from 1982 to 1988 kept at liquid nitrogen were diluted 1:2 in sample buffer STD and prepared as above.

NPS preparation

Whole NPS were diluted 1:2 with sample buffer STD, mixed thoroughly, incubated for 1 hr at 37°C, and spun one minute in an Eppendorf centrifuge. The supernatant was used for EIA testing.

EIA procedure

In short terms test performance is: 0.150 ml of the sample prepared as above was added into each of two wells and the plate (or strips) was incubated for 1 hr at 37°C. After a washing step with Washing Solution POD, 0.1 ml of conjugate was added to each well for 30 min at 37°C followed by an additional washing step. Chromogen TMB working solution (0.1 ml) was incubated at room temperature (18–25°C) for 30 min and the reaction finished with Stopping Solution POD. Photometric evaluation was carried out at 450 nm. In all incubation steps the plate has to be covered by a foil.

Validation of the test

A test plate can be evaluated if the negative and positive controls have absorbance values less than 0.12 A and between 0.5–1.5 A, respectively.

Evaluation of the test

The cut-off value is calculated by adding the mean value of the negative controls to a threshold value of 0.08 A. The retest limit is calculated by adding 0.1 A to the cut-off value. Absorbance readings below the cut-off value are given as "negative" and above the retest limit as "positive." If the absorbance is between the cut-off and retest limits, the sample has to be retested.

RSV Subgroups

RSV subgroups were identified by an EIA performed with MAbs as capture and detector antibodies as described elsewhere (9). Briefly, 3 anti-F and 4 anti-G MAbs were used for grouping and subgrouping, respectively. MAbs were kindly provided by Dr. L.J. Anderson from CDC, Atlanta, GA.

RESULTS

The Enzygnost RSV (Ag), gave negative results with absorbance values below the cut-off value against the following heterologous viruses from our reference virus collection: adenovirus types 1, 7, 11, and 21; polioviruses types 1, 2, and 3; rhinoviruses 1 A and 26; parainfluenza viruses types 1, 2, 3, and 4 A; influenza viruses types A and B; herpes simplex type 1; rotavirus WA; measles; SV-41, coronavirus 229-E, and NDV.

The Enzygnost RSV (Ag) was evaluated with 111 RSV isolates in Hep2 cell cultures representing different subgroups of RSV. It included 64 RSV isolates from 1982 to 1988 whose subgroups results had been recently published and 47 RSV isolates from 1991 (Table 1). The absorbances values in the tests were high ($\geq$ 1.5) and only 4 gave absorbances values between 0.7 and 1.0.

A retrospective evaluation with Enzygnost RSV (Ag) was carried out with 24 samples (NPS) collected between 1986 and 1988 and kept at −70°C. All these samples are RSV positive by IFA and some by isolation in tissue culture (TCI). The Enzygnost RSV (Ag) results were positive in 22 of 24 samples (Fig. 1). The two EIA negative samples were positive only by IFA, and RSV was not isolated from either in tissue culture. Briefly, from these 24, fourteen were isolated in Hep2 cell culture, six had the isolation prevented by bacterial contamination, and four were not isolated.

One hundred and sixty-nine samples from 1991 were tested by EIA and IFA. Of the 31 samples positive by EIA, 25 were

TABLE 1. Subgroups of RSV Groups A and B Isolates Tested by Enzygnost RSV (Ag), Rio de Janeiro[a]

Year	No. tested	Subgroups								
		A1	A2	A3	A4	A6	B1	B2	B3	I[ab]
1982	3						3			
1983	5	2			2			1		
1984	3	3								
1985	6	3			1			2		
1986	3				2		1			
1987	9	1			1		1	4	2	
1988	35	16	13		2	1		2		1
1990	47	16	16	3	11				1	
Total	111	41	29	3	19	1	5	9	3	1

[a]Described in Siqueira et al. (9).

[b]One intermediate isolate (positive for RSV groups A and B).

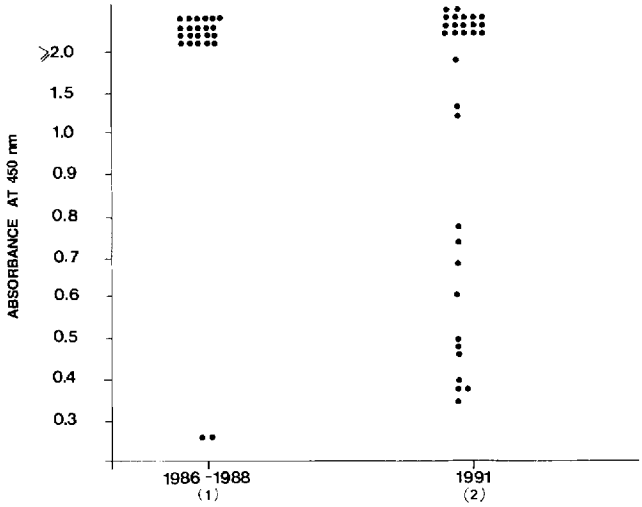

Fig. 1. EIA results expressed as absorbance units of NPS obtained from children with acute respiratory diseases. 1) 24 NPS from 1986–1988, RSV positive by IFA and/or TCI. 2) 31 NPS from 1991, with absorbance units above the retest limit.

positive by IFA (Table 2). The 3 specimens positive only by IFA were retested by EIA, again with negative results. Duplicate slides available of 2 IFA-positive, EIA-negative and 3 IFA-negative, EIA-positive specimens were retested by IFA with another polyclonal antiserum (bovine anti-RSV, Wellcome Diagnostic, England) and the earlier results were confirmed. RSV was isolated in Hep2 cells from 1 of the IFA-positive, EIA-negative and from 1 IFA-negative, EIA-positive specimens. Of the 31 NPS EIA positive samples, 17 gave high absorbance readings (≥ 2.0). For 3 samples the absorbance units lie between 1 and 1.99, for 4 between 0.5 and 0.999, and for seven between 0.309 and 0.499 (Fig. 1). In all positive specimens the absorbance values were above the retest limit. From the 169 samples tested, 11 were positive for adenovirus, 8 for influenza A, and 1 for influenza B. In all these 20 NPS the absorbance values were below the cut-off value. Three bronchial washes collected from immunocompromised patients were tested and showed positive results by both methods. These 3 RSV belong to group A as determined by IFA.

TABLE 2. Comparison of Enzygnost RSV (Ag) and IFA in Nasopharyngeal Secretions Collected During 1991 RSV Season in Rio de Janeiro

	IFA	
	+	−
EIA		
+	25	6
−	3	135

DISCUSSION

The purpose of this study has been to show that a sensitive and specific EIA is suitable as a diagnostic test for RSV of different subgroups. As it is shown in Table 1, 111 RSV isolates representing different subgroups were tested by Enzygnost RSV (Ag) and all reacted, giving high absorbance values. These subgroups represent different epitopes on RSV G protein which was found to be the most prominent antigenic difference between the two groups (13). The finding that Enzygnost RSV (Ag) reacts with all subgroups tested allowed us to have a test with high sensitivity.

In the present study IFA was chosen as the reference method since we earlier established the accuracy of IFA diagnosis of RSV and adenovirus by using it as a routine method in our laboratory (10,14). IFA has been shown to be used as a reference method by others (15,16) and has been recommended by WHO (17).

Comparing the results obtained with 24 NPS RSV positive by TCI and/or IFA in a retrospective study, we found 22 (91.7%) positive by EIA. With 169 NPS from 1991, the results obtained with EIA were better than IFA, as it had been found by Hornsleth et al. (16). The specificity and sensitivity of EIA (Enzygnost RSV (Ag)) in relation to IFA, which is taken as standard, showed 5.3% discrepant results and 94.7% agreement. The sensitivity of EIA compared with tissue culture or immunofluorescence methods has shown to be more than 80% (6,16,18,19) or has ranged from 60–80% (20,8,21).

We detected 6 samples that were EIA positive and IFA negative. All these 6 samples gave absorbance values between 0.309–0.720. This finding as suggested by Hendry et al. (22) comparing washed and whole nasal cells, would indicate that large amounts of extracellular virus or viral proteins are released from infected nasal mucosa during the course of RSV infection and should help optimize conditions for more sensitive immunochemical assays for the diagnosis of RSV infections.

In the present study Enzygnost RSV (Ag) was found to be a sensitive and specific test for the rapid detection of RSV in nasopharyngeal secretions. This test is not time consuming, giving a result around 4 hr in NPS and 3 hr in cell culture. No overnight incubation or sonication of the sample is necessary, and specimen handling is simple.

Another advantage of EIA is its versatility for batch testing and independence of viable virus and intact epithelial cells. Rapid diagnosis of RSV is important for patient management, to avoid hospital cross-infection and for the appropriate use of effective antiviral drugs.

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