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Capture-ELISA for serum IgM antibody to respiratory syncytial virus

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SUMMARY

A four-component solid-phase capture enzyme immunoassay was set up to test for serum IgM antibody to respiratory syncytial (RS) virus and was compared with immunofluorescence assay (IFA).

A total of 128 young children with acute respiratory infections were studied. Thirty-six were shown to be RS virus-positive by the detection of RS virus in nasopharyngeal secretions and 92 were RS virus-negative. A serum specimen was collected after admission to the hospital (days 0–4) and a further specimen was obtained during days 10–14. Out of 36 RS virus-positive patients, 28 (77·7 %) were found to be positive for IgM by both capture-ELISA and IFA. Out of 92 RS virus-negative patients 5 (5·4 %) were IgM-positive. Four false-positive results were obtained by IFA due to the presence of rheumatoid factor.

The capture-ELISA was shown to be a reliable technique in detecting specific IgM antibody to RS virus.

INTRODUCTION

The diagnosis of respiratory syncytial virus infection in young children is usually based on virus isolation or on the detection of viral antigens in nasopharyngeal secretions (NPS) by immunofluorescence (Gardner & McQuillin, 1980), ELISA (Chao *et al.* 1979), radioimmunoassay (Sarkkinen *et al.* 1981) or enzyme immunoassay (Cevenini *et al.* 1983). The serological diagnosis of RS virus infection by the detection of serum IgM to RS virus is less sensitive than antigen detection. However, in some instances, it may be useful.

Immunofluorescence and ELISA techniques have been previously described for detecting serum IgM to RS virus (Meurman *et al* 1984a; Hornsleth *et al.* 1984). In this paper the results obtained by using a solid-phase capture antibody assay to detect IgM immunoglobulins to RS virus by a four-phase enzyme immunoassay are reported and compared with immunofluorescence.

MATERIALS AND METHODS

Sera and patients

All the patients studied ($n = 128$) were young children (6 months–4 years of age) hospitalized for acute respiratory illness. RS virus infection was diagnosed by identifying viral antigens in the cells from NPS by an indirect immunoperoxidase technique as previously described (Cevenini *et al.* 1983). The diagnoses were later confirmed by recovery of virus in tissue culture using HEp-2 cells also as previously described (Cevenini *et al.* 1983).

From 36 RS virus-positive and from 92 RS virus-negative patients a serum specimen was collected after admission to hospital (days 0–4). A further specimen was obtained on days 10–14.

Sixty-five additional sera were available from healthy subjects (aged 1–4 years). These served as negative controls for IgM in the capture-ELISA.

Antigens for the capture-ELISA

Antigen for RS virus was from cultures of Vero cells infected with RS virus (Ramp strain) isolated in our laboratory. When culture showed + + to + + + viral cytopathic effect the culture fluid was collected, centrifuged at 4000 rev./min and the supernatant stored at −70 °C until used. Control antigen was prepared in the same manner from uninfected Vero cell cultures.

Capture-ELISA for detection of RS virus IgM antibody

Polyvinylcholoride microplates (Flow Laboratories, Irvine, UK) were sensitized by addition of anti-human IgM (Dako, Copenhagen, Denmark) diluted 1/500 in carbonate–bicarbonate buffer, pH 9·6, in a volume of 0·1 ml per well. After overnight incubation at 4 °C, the contents of the wells were aspirated and the wells washed five times with 0·01 M phosphate-buffered saline, pH 7·2 (PBS) containing 0·05 % Tween 20 (PBS-Tween).

The test sera and viral antigen were diluted in PBS-Tween with 0·5 % bovine serum albumin and the conjugate was diluted in PBS-Tween with 1 % foetal calf serum. Each serum was diluted 1/100 and added in duplicate to 'antigen' and 'control' wells in a volume of 0·1 ml per well. After incubation for 1 h at 37 °C, the plates were washed five times with PBS-Tween. Optimal dilutions of viral antigen or control antigen in a volume of 0·1 ml were then added to the appropriate wells. The final protein concentration of the RS virus antigen or control antigen preparations was 20 μg/ml. After incubation for 1 h at 37 °C, the plates were washed five times and bovine antiserum to RS virus (Wellcome, Dartford, UK) diluted 1/50 was added in 0·1 ml volumes. The plates were then incubated for 1 h at 37 °C and washed five times with PBS-Tween, and 0·1 ml of a 1/1000 dilution of peroxidase-conjugated goat anti-bovine globulin (Kirkegaard and Perry laboratories, Gaithersburg, MD) was added. After incubation for 1 h at 37 °C, the plates were washed five times and 0·1 ml of a citric acid-phosphate buffer (pH 5·6) containing 40 mg 1,2-phenylenediamine-dihydrochloride (Merck, Darmstadt, West Germany) and 0·002 ml of 3 % hydrogen peroxide per 100 ml buffer was added per well. The enzymic reaction was stopped by the addition of 0·05 ml of 2N H_2SO_4 and the results of the test were read at 492 nm in a Dynatech Micro-ELISA

autoreader, model MR 580. All readings were made against a blank well which had received all the reagents except that human serum was replaced by PBS-Tween.

Immunofluorescence assay for RS virus IgG and IgM antibody

RS virus-infected Vero cells were used as antigen. The human serum dilutions were incubated on the antigen at 37 °C over 45 min and 2 h, when IgG and IgM respectively were assayed. After washing, fluorescein-conjugated rabbit anti-human globulins G or M (Dako) were added for 45 min at 37 °C and then given a final wash.

Complement fixation

The test was performed by a standard microtitre procedure (Welliver *et al.* 1980), using an RS virus antigen prepared in HEp-2 cells.

Absorption of sera with heat-aggregated human IgG

Human immunoglobulin G, provided by the blood donor service of S. Orsola Hospital, Bologna, was heated to 73 °C in a water bath for 10 min to aggregate the globulins. They were then washed three times in PBS by centrifugation at 3000 rev./min for 10 min. The pellet was resuspended in PBS, sonicated briefly, and stored at 4 °C. To 0·3 ml of aggregated globulins, 0·1 ml of each serum to be examined was added, mixed thoroughly and incubated at 37 °C for 60 min with occasional shaking. The mixture was then transferred to 4 °C overnight. After the addition of 0·6 ml of PBS the suspension was centrifuged at 3000 rev./min for 10 min. The supernatant (absorbed serum) was removed for testing.

RESULTS

Standardization of the capture-ELISA

The optimal dilution of the RS virus antigen used in the test was found to be a 1/10 dilution of an RS virus stock which had an infectivity titre of 10^6 $TCID_{50}$/0·2 ml. Serial twofold dilutions (from 1/50 to 1/25600) of sera with high, medium and low positive levels of antibody were tested with this antigen and showed a linear relation between dilution and absorbance values (Fig. 1). The cut-off in absorbance value chosen to discriminate between positive and negative sera at a dilution of 1/100 was an OD of 0·095. This value was the mean plus five standard deviations of the OD values given by the sera from 65 healthy controls tested three times in three separate assays for IgM. The value for each control serum as well as the value for each test serum was the net absorbance obtained by subtracting the absorbance readings on control antigen-coated wells from those detected on RS virus antigen-coated wells. As a rule, the OD values for positive sera obtained in the RS virus antigen-coated wells were 3–6 times higher than those observed in the control antigen-coated wells.

To estimate the effect the rheumatoid factor on the capture-ELISA for IgM antibody, a serum known to be strongly positive for RF by Rheuma-Wellcotest (Wellcome Diagnostic, Dartford, UK) was mixed with an equal volume of five sera known to be positive for IgM to RS virus by IFA. After incubation for 10 min at room temperature, the mixtures and the original untreated sera were either

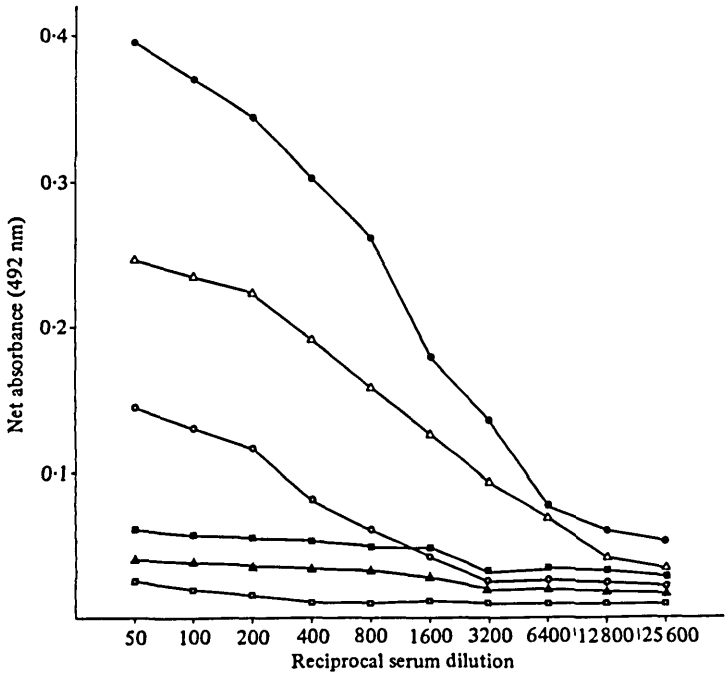

Fig. 1. Titration curves for serum RS virus IgM antibody by capture-ELISA. The results from a high positive (●), an intermediate positive (△), a low positive (○) and three negative (● ▲ □) sera are shown.

Table 1 *Effect of rheumatoid factor (RF) and heat-aggregated human IgG (HA-IgG) on capture-ELISA for RS virus IgM antibody*

	Net absorbance at 492 nm*			
Serum	Untreated	Absorbed with HA-IgG	After addition of RF-positive serum	As in column 3 but after absorption with HA-IgG
1	0·204	0·177	0·210	0·119
2	0·267	0·276	0·250	0·128
3	0·153	0·132	0·170	0·115
4	0·297	0·243	0·310	0·203
5	0·139	0·155	0·135	0·105

* The mean value plus five standard deviations from sera of 65 healthy controls tested in triplicate in three separate assays was taken as the cut-off value (0·095 OD) in the IgM capture assay.

treated with heat-aggregated human IgG (HA-IgG) or tested without treatment, all at a final serum dilution of 1/100. The net absorbance values obtained are shown in Table 1. The presence of RF increased the OD values of the sera in both the virus–antigen and control antigen containing wells to the same extent. Therefore the net values obtained by subtracting the absorbance readings on the control antigen-containing wells from those obtained on the RS virus-containing wells were the same as those obtained without the addition of RF (Table 1). In addition, nine RF-positive sera were tested at a 1/100 dilution. None was positive in

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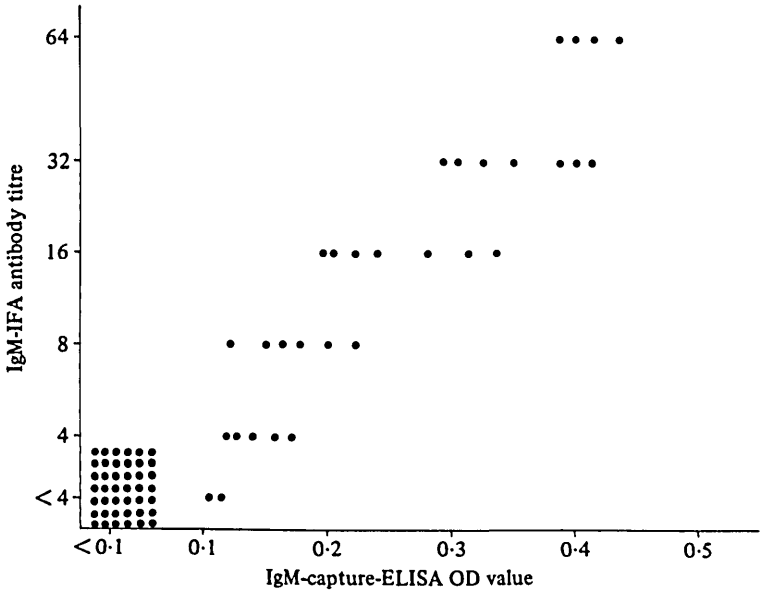

Fig. 2. Comparison of the detection of IgM antibody by capture-ELISA and by immunofluorescence assay (IFA) in 72 serum samples from 36 patients with virus infection.

the test. However, because the presence of RF raised all the values in the assay, all sera were absorbed with heat aggregated human IgG.

Assays for RS virus antibody

Twenty-eight (77·7 %) out of 36 patients with RS virus infection were positive for IgM antibody to RS virus either in the first or in the second serum specimens by both capture-ELISA and IFA. By IFA, four more specimens were found to be positive for IgM but this was due to the presence of RF. These sera were negative when re-tested after treatment with aggregated human IgG. The results obtained by capture-ELISA and IFA are compared in Fig. 2.

Seroconversion detected by the capture-ELISA was found in 25 (89·2 %) of the 28 IgM-positive patients. Seroconversion by IFA was found in 23 (82·1 %) of the patients and increases in titre were found in two patients in whom IgM antibody was already present in the first serum specimen available. Thirty-four (94·4 %) of the 36 patients showed a diagnostically significant fourfold or greater rise in IgG antibody to RS virus by IFA, whereas only 19 (52·7 %) patients showed seroconversion by complement fixation.

Of 92 children with acute respiratory infection in whose NPs no RS virus antigen was demonstrated, 5 (5·4 %) were positive for IgM by the capture-ELISA. These five positive patients also showed fourfold rises in IgG titres in paired serum specimens.

DISCUSSION

This study shows that serum IgM antibody to RS virus may be detected by our capture-ELISA. With our reagents we did not encounter background reactivity when using either fetal calf serum or bovine serum albumin in the diluents. This assay system differed from other capture assays in being a four- rather than a

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three-phase system. This provides greater versatility in assays for IgM antibodies because the only labelled immunoglobulins necessary are those against the species in which the viral antibodies have been produced, and such labelled immunoglobulins are commercially available. Also we found that unpurified antigen was satisfactory in the capture assay for RS virus IgM antibody. To monitor the effect of RF on the specificity of the test, we found it important to test each serum against an uninfected control antigen. Non-specific reactivity in the test sera was easily recognized and false positive results were consequently avoided.

The capture-ELISA proved to be as sensitive as IFA for the detection of IgM antibodies. It was, however, more specific than IFA since false positive results, due to the presence of RF, were avoided. As others have reported (Hornsleth *et al.* 1984; Meurman *et al.* 1984*a*) we could detect IgM serum antibody to RS virus in only a few (three) of the serum specimens obtained on days 0–4 from patients with known virus infections. By contrast 28 (77·7 %) patients out of 36 with proven infection had detectable IgM in sera taken on days 10–14.

The most sensitive method for the serological diagnosis of RS virus infections is the demonstration of significant rises in IgG antibody titre in paired serum specimens (Richardson *et al.* 1979; Hornsleth *et al.* 1984; Meurman *et al.* 1984*b*). However, paired serum specimens are not always available, particularly from infants. Therefore, the demonstration of IgM antibody in a single serum sample collected 10–14 days after the onset of disease is a practical serological tool for the diagnosis of RS virus infection in at least some instances. IgM detection may also be helpful when antigen is not detected in NPS and where the clinical picture does suggest RS virus as a possible cause. In such cases a retrospective serological examination of a single serum specimen may be made by IgM capture-ELISA or IFA. Nevertheless, the results described here show that it is important to combine serological tests with techniques for virus detection to be sure of diagnosing all RS virus infections. In our study five (5·4 %) RS virus infections would have been missed without IgM detection.

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