JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1913–1915
0095-1137/88/091913-03$02.00/0

Vol. 26, No. 9

Solid-Phase Immunosorbent Technique for Rapid Detection of Rift Valley Fever Virus Immunoglobulin M by Hemagglutination Inhibition

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Received 16 November 1987/Accepted 3 June 1988

A solid-phase immunosorbent technique (SPIT) was adapted to detect Rift Valley fever (RVF) virus-specific immunoglobulin M (IgM) in serum samples from humans vaccinated with Formalin-inactivated RVF vaccine. Microdilution plates coated with goat anti-human IgM were successively incubated with serum samples from human vaccinees, RVF virus hemagglutinating antigen, and goose erythrocytes. The RVF virus-specific IgM in the serum samples from vaccinees bound to the RVF virus antigen and inhibited hemagglutination of goose erythrocytes. SPIT was compared to the IgM capture enzyme linked immunosorbent assay (ELISA) and the indirect immunofluorescent-antibody (IFA) assay and was found to be sensitive in detecting RVF virus-specific IgM antibody, with high correlations between SPIT and the other two tests (Pearson's correlation coefficient [r] = 0.9 and 0.6, respectively). Results of SPIT were obtained within 5 h, offering speed over ELISA (8 h). In addition, SPIT does not require sophisticated equipment or expensive reagents. Serum rheumatoid factor did not produce false-positive reactions in SPIT as in the indirect immunofluorescent-antibody assay and IgM capture ELISA.

Since the Egyptian outbreak in 1977, Rift Valley fever (RVF) has become a zoonotic disease of global concern (4). This epizootic resulted in several hundred human fatalities and many thousand animal deaths (5). During the epidemic, several virus isolates were obtained, but the identification of the etiological agent was not available until 40 days after the first reported case. During epidemics, rapid diagnosis is of great significance for control measures. Whereas virus isolation ensures a firm diagnosis of the etiological agent, the procedure is tedious and time-consuming. The demonstration of specific immunoglobulin M (IgM) antibody provides a rapid presumptive diagnosis and indicates recent exposure to the agent. A solid-phase immunosorbent technique (SPIT) to detect specific IgM for rubella in human sera has been described (3). In this assay, anti-IgM antibody is adsorbed to polystyrene plates. Upon addition of serum samples to the coated plates, specific IgM in the serum sample binds to the system and subsequently to added antigen, inhibiting hemagglutination of erythrocytes. The assay is reported to be simple, sensitive, and specific. In the present study, SPIT was developed and applied to measure specific RVF virus serum IgM in people immunized with RVF-inactivated vaccine. The sensitivity and specificity of SPIT was compared to those of the IgM capture enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent-antibody (IFA) assay.

Antigen. A beta-propiolactone-inactivated, sucrose-acetone-extracted, mouse liver RVF virus antigen was used for SPIT and ELISA. The antigen was produced for hemagglutination inhibition serology by the Government Services Division, Salk Institute, Swiftwater, Pa., by using the Entebbe strain of RVF virus (11).

Vaccine. The RVF vaccine used for human immunization was produced for the U.S. Army Medical Research and Development Command by the National Drug Co., Swiftwater, Pa. The Formalin-inactivated vaccine was prepared from the Entebbe strain of RVF virus propagated in primary African green monkey kidney cell cultures (1).

Immune reagents. RVF hyperimmune mouse ascitic fluid, prepared by using sarcoma 180/TG as previously described (8), was used as positive RVF virus antibody control. Affinity-purified, μ chain-specific, goat anti-human IgM, peroxidase-labeled, affinity-purified, goat anti-mouse IgG, and fluorescein-labeled, affinity-purified goat anti-human IgG (heavy and light chain) and IgM (μ chain) were obtained commercially (Kirkegaard and Perry Laboratories, Gaithersburg, Md.).

Human sera. A total of 31 serum samples from 12 people previously immunized with the RVF vaccine were analyzed. The vaccinees received three injections of the vaccine on days 0, 10, and 28 and were subsequently bled at different intervals after vaccination. A total of 60 serum samples from unimmunized people were used as negative controls.

SPIT. SPIT, as described by Krech and Wilhelm (3) for rubella, was followed with some modifications. Polystyrene 96-well microdilution plates were filled with 100 μl of anti-human IgM per well diluted 1:500 in carbonate-bicarbonate buffer, pH 9.6, and were left overnight at 4°C in sealed plastic bags. Coated plates were washed three times with washing buffer (0.01 M phosphate-buffered saline, pH 7.4, 0.05% Tween 20). Two-fold serially diluted (in 0.4% bovine albumin borate saline, pH 9.0) test serum (100 μl) was added to each well. A dilution of 1:10 of the serum served as serum control (without antigen). With each run, a positive and a negative human serum control was included. The plates were again put in sealed plastic bags, incubated in a humidified incubator for 2 h at 37°C, and then washed three times with 0.01 M phosphate-buffered saline, pH 7.4, and once with 0.4% bovine albumin borate saline. Pre-titrated RVF virus antigen containing 1 U of hemagglutinin in 25 μl was then added to each serum dilution, and the plates were incubated for 2 h at room temperature. Each assay included a titration of the antigen. Following incubation, 50 μl of goose erythrocytes diluted to an optical density (OD) of 0.75

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in adjusting buffer (0.15 NaCl, 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, pH 6.0) was added to each well and mixed, and the plates were read after 1 h of incubation at room temperature. Serum samples that inhibited hemagglutination at a dilution of ≥1:20 were considered to contain RVF virus-specific IgM.

IgM capture ELISA for RVF virus. Plates coated with goat anti-human IgM were washed three times with washing buffer. Serum (100 μl) diluted 1:200 in serum diluent (SD; phosphate-buffered saline, pH 7.4, 0.05% Tween 20, 0.5% bovine serum albumin) was then added, and the plates were incubated for 1 h at 37°C. After three washes, 100 μl of RVF virus antigen diluted 1:40 in SD was added, and the plates were incubated for 1 h at 37°C. After three washes, 100 μl of RVF virus hyperimmune mouse ascitic fluid diluted 1:1,000 in SD was added. The plates were incubated for 1 h at 37°C and washed three times, and 100 μl of peroxidase-labeled goat anti-mouse IgG diluted 1:1,000 in SD was added to each well. After incubation for 1 h at 37°C, the plates were washed three times and 100 μl of 2.2′-azino-di(3-ethyl-benzthiazoline sulfonate) substrate (Kirkegaard and Perry) was added to each well. The reaction was read after 20 min at room temperature with a spectrophotometer (Titertek Multiskan MC; Flow Laboratories, Inc., McLean, Va.) set at 405 nm. A serum sample was considered positive if the difference in OD between the serum sample with specific antigen and that without antigen (with normal mouse liver) was more than the mean background of negative control sera (mean, 10) plus 3 standard deviations. The technique was evaluated by three replicates of a panel of five RVF virus IgM-positive and five IgM-negative serum samples. Serum samples positive by IgM capture ELISA were tested for rheumatoid factor (RF) by a latex slide agglutination test (bioMérieux, Charbonnieres, France) (10).

IFA. Specific IgM titers to RVF virus antigen were determined by using fluorescein-labeled goat anti-human IgM or IgG as previously described (2). Gamma-irradiated RVF virus spot slides (provided by the Centers for Disease Control, Atlanta, Ga.) were used as the antigen. Two-fold dilutions of tested serum were added to the antigen spots, and the slides were incubated in a humid chamber for 30 min at 37°C. After the slides were washed, spots were stained with the fluorescein-conjugated antiserum, and the slides were incubated again, washed, and then examined with a fluorescence microscope. A bright-green granular cytoplasmic fluorescence of the infected cells, compared with no fluorescence of the uninfected cells, was considered a positive reaction. Serum IgG or IgM titers ≥10 were considered positive for RVF virus.

A total of 91 serum samples (31 from immunized and 60 from unimmunized individuals) were tested by IFA for IgG and IgM and by capture ELISA and SPIT for IgM antibody to RVF virus. Of the 31 serum samples from immunized individuals, 21 were negative by the three tests. On the other hand, all of the 10 samples that were positive by capture IgM ELISA were also positive (≥20) by SPIT but only 6 samples were positive (≥10) by IFA for IgM antibody (Table 1). Seven samples were strongly positive (OD, >0.2) by ELISA, of which six were strongly positive (>40) by SPIT, while only two samples were strongly positive (>40) by IFA (IgM). All of the 10 samples positive by ELISA and SPIT were RF negative.

Of 60 serum samples obtained from unvaccinated people, 2 were positive by capture ELISA (OD, 0.280 and 0.413) and by IFA (titers, 10 for both serum samples) for IgM antibody. They were found to be RF positive when tested by the latex slide agglutination test, and their IFA IgG titers were both 80. These two serum samples were consistently negative by SPIT (Table 2).

TABLE 1. Comparison of results obtained by IFA (IgM), IgM capture ELISA, and SPIT with serum samples from RVF vaccinees

Sample no.	RVF-specific IgG by IFA	RVF-specific IgM by:		
		ELISA[a]	IFA	SPIT
1	20	0.119	<10	40
2	20	0.148	<10	20
3	20	0.187	<10	20
4	80	0.230	40	20
5	20	0.435	20	320
6	80	0.520	10	160
7	40	0.592	<10	160
8	20	0.837	80	80
9	160	1.096	80	640
10	160	1.688	20	640

[a] Cutoff value, Mean + 3 × SD = 0.0336 + 3 × 0.0272 = 0.115.

The overall agreement between the results of IgM capture ELISA and SPIT was 83.3% (r, 0.9; P, <0.001), while the overall agreement between the results of IgM capture ELISA and IFA (IgM) was 80% (r, 0.6; P, <0.001). Ten RVF virus-positive serum samples and seven negative samples were coded and tested by SPIT on four different occasions by two investigators. Results obtained were in agreement for at least 15 of the 17 (88%) tested samples.

Rapid diagnostic techniques which can detect recent exposure to viral agents are greatly needed. The detection of specific IgM and rising IgG titers are useful in supporting presumptive diagnoses and possibly in confirming a clinical diagnosis of recent infection. ELISA capture IgM has been applied for the detection of specific IgM to RVF virus (7); however, high background due to nonspecific binding of sticky serum is a well known problem in most ELISA systems (12). The presence of RF in human serum may also cause false-positive reactions in IgM capture ELISA (6).

SPIT appears to be an easy and rapid method for the detection of specific IgM to rubella (3). In the present study, SPIT was adapted, with modifications, to detect specific IgM to RVF virus, and results obtained indicate that the test is appropriate and reliable. In our experience, more stable coating of the plates with anti-human IgM was obtained after overnight incubation at 4°C rather than after 2 h at 37°C. We successfully used coated plates stored up to 7 days at 4°C. Longer storage was not tried; however, the shelf life of the plates coated for the detection of rubella antibody was reported to be at least 2 months (3). Comparing results obtained by using known positive and negative sera treated and untreated for the removal of nonspecific hemagglutinin

TABLE 2. Comparison of results obtained by IFA (IgM), IgM capture ELISA, and SPIT with serum samples from 31 RVF-vaccinated and 60 unvaccinated humans

Expt (cutoff)	No. of SPIT results	
	Positive	Negative
IFA (titer, ≥10)	6	2[a]
IFA (titer, <10)	4	79
ELISA (OD, ≥0.115)	10	2[a]
ELISA (OD, <0.115)		79

[a] RF positive serum sample.

inhibitors demonstrated that with SPIT there is no need for removal of hemagglutination inhibitors or heteroagglutinins as recommended in the hemagglutination inhibition test (9). There was no difference in results obtained when RVF virus antigen was incubated for 2 h at room temperature or overnight at 4°C. Therefore, when coated plates are available, the assay may be completed in a single day.

The sensitivity, specificity, and reproducibility of SPIT compare well with those of IgM capture ELISA. In both tests of 91 serum samples, 10 were positive and 79 were negative. Two were positive by IgM capture ELISA and negative by SPIT. These two serum samples were found to be RF positive.

Titers obtained by SPIT (mean value, 210.0) were much higher than those obtained by IFA (IgM) (mean value, 27.0), but the overall sensitivity in terms of positive and negative is comparable.

SPIT has several advantages. (i) Results are obtained within 5 h. (ii) Several hundred serum samples can be tested daily. (iii) Reagents are inexpensive, and there is no need for an ELISA reader or spectrophotometer for interpretation of results, thus reducing cost per test. (iv) There is no cross-reaction with IgG leading to false-positive results. (v) RF does not give false positive results. (vi) No special serum treatments (extraction and/or absorption) are required before screening. (vii) Only 1 U of antigen (8 U for the hemagglutination inhibition test) is needed for the test. In conclusion, SPIT can be used in the field for screening of acute-phase sera (recent exposure to RVF virus) and may be applicable for the detection of specific IgM to other viral agents.

We deeply thank Adel W. Salib for his valuable directions in regard to the evaluation of the hemagglutinin antigen used. We also thank Andrew J. Main for his critical evaluation and review of the manuscript.

This study was supported by the Naval Research and Development Command, Naval Medical Command, National Capital Region, Bethesda, Md., Work Unit no. 3M162770A870.AQ.126 and Independent Research Project no. MR0000101.3074.

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