

## Use of Enzyme-Linked Immunosorbent Assay Techniques with Cross-Reacting Human Sera in Diagnosis of Murine Typhus and Spotted Fever

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**Enzyme-linked immunosorbent assay (ELISA) techniques for the determination of immunoglobulin G to rickettsial lipopolysaccharides were developed. These techniques provide a simple and convenient way to serodiagnose Mediterranean spotted fever and murine typhus with a single serum dilution. The results of the ELISAs correlated with the indirect immunofluorescence assay titers of cross-reacting sera.**

Mediterranean spotted fever (MSF) and murine typhus (MT), two endemic diseases in Israel, are caused by *Rickettsia conorii* and *Rickettsia typhi*, respectively. Serological diagnosis is made by indirect immunofluorescence assays (IFA) by using the homologous somatic antigen. Since spotted fever group and typhus group rickettsiae have common protein antigens (3), cross-reactivity exists in some of the sera examined (1). The diagnosis, in these cases, must rely on the differences between the IFA titers of immunoglobulin G (IgG) to both rickettsia species.

Since rickettsial lipopolysaccharides (LPS) were shown to be group specific (3), enzyme-linked immunosorbent assay (ELISA) techniques were developed to detect antibodies to the LPS of *R. conorii* and *R. typhi* in human sera and to avoid cross-reactivity. Sixteen IFA cross-reacting sera were tested in the enzyme immunoassays. The results of the tests confirm the IFA serodiagnosis, and cross-reactivity was significantly reduced.

**Rickettsiae.** *R. conorii* (Moroccan strain) and *R. typhi* (Wilmington strain) were used for the preparation of somatic antigens and LPS extraction. Rickettsiae were grown in chicken embryo yolk sacs and were partially purified by three differential centrifugation cycles and a Renografin gradient density centrifugation as described previously (4). Rickettsial suspensions were inactivated in 0.1% formalin prior to their use in the tests.

**IFA.** Spots of somatic antigen of either *R. conorii* or *R. typhi* were prepared. Drops of the suspensions were applied onto slides, air dried, and fixed in acetone for 15 min. Drops (ca. 5  $\mu$ l) of serum dilutions were applied on the spots and incubated for 30 min at 37°C. After being washed with tap water, the spots were incubated with anti-human IgG-fluorescein conjugate (Sigma) solutions. The spots were observed by fluorescent microscope.

**LPS preparation.** LPS preparations were done as described previously (2). Rickettsial somatic antigen suspensions (450  $\mu$ l; 0.8 mg of protein per ml) were treated with 20  $\mu$ l of 10% sodium dodecyl sulfate and 24  $\mu$ l of proteinase K (10 mg/ml) for 90 min at 56°C and then for 5 min at 95°C (to inactivate the proteinase K). The disintegration of the rickettsial protein was

demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**ELISA.** LPS solutions of either *R. typhi* or *R. conorii* diluted 1:100 in TS buffer (0.05 M Tris, 0.85% NaCl [pH 7.6]) were used to sensitize polyvinyl chloride microplates (Dynatech). Each well was sensitized by the addition of 0.15 ml of the LPS solution and an incubation of 2 h at 37°C. Human sera diluted 1:50 or 1:100 with TSAT buffer (TS buffer with bovine serum albumin [1 mg/ml] and 0.05% Tween 20) were applied to the wells, 0.1 ml per well, and incubated for 1 h at 37°C. Then,

TABLE 1. IFA titers and LPS ELISA values for sera of suspected MSF or MT patients

Serum no. <sup>a</sup>	IFA IgG titers to:		ELISA values for IgG to LPS of <sup>b</sup> :	
	<i>R. conorii</i>	<i>R. typhi</i>	<i>R. conorii</i>	<i>R. typhi</i>
1 <sup>c</sup>	<100	<100	0.018	0.012
2	<100	200	0.052	0.101
3	<100	800	0.035	0.116
4	<100	3,200	0.000	0.125
5	<100	1,600	0.033	0.155
6	800	<100	0.908	0.024
7	200	<100	0.123	0.019
8	1,600	200	0.459	0.132
9	1,600	200	0.697	0.076
10	3,200	100	0.976	0.028
11	1,600	200	0.329	0.112
12	200	800	0.049	0.456
13	400	1,600	0.019	0.363
14	200	1,600	0.131	0.299
15	100	1,600	0.072	0.118
16	400	800	0.016	0.191
17	400	800	0.068	0.158
18 <sup>d</sup>	400	3,200	0.067	0.214
19 <sup>d</sup>	400	3,200	0.000	0.180
20 <sup>d</sup>	800	3,200	0.000	0.131
21 <sup>d</sup>	800	3,200	0.007	0.309
22	100	800	0.017	0.361
23	400	100	0.145	0.010

<sup>a</sup> Serum samples 1 to 14, 16 to 17, and 20 to 23 were tested by ELISA at a dilution of 1:50, and serum samples 15, 18, and 19 were tested at a dilution of 1:100.

<sup>b</sup> ELISA values are expressed as optical density units at 630 nm. Results are the averages of duplicate tests. Standard deviations did not exceed 20%.

<sup>c</sup> Similar results were obtained with 15 other negative serum samples.

<sup>d</sup> Sera 18 and 19 and 20 and 21 are paired sera.

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plates were washed three times with TST (TS buffer with 0.05% Tween 20) and incubated with rabbit anti-human IgG-peroxidase conjugate solution (1:1,000) (0.05 ml per well). After a similar incubation step the plates were washed four times with TST buffer, and soluble TMBLUE substrate solution (TSI-CD, Milford, Mass.) was added to the wells (0.05 ml per well). Plates were incubated for 20 min at room temperature and read at 630 nm.

Sixteen cross-reacting sera were found in a group of 58 positive sera to MSF or MT rickettsiae as determined by IFA (i.e., IgG and IgM titers of  $\geq 100$ ). The presence of IgG to LPS of MSF and MT rickettsiae was determined by ELISA. The results of the ELISAs and the IFA IgG titers for both rickettsia species are presented in Table 1. Sera with IFA titers of  $< 100$  for both rickettsiae were found to be negative in the LPS ELISAs (i.e., serum sample 1). Homologous sera for MT and MSF rickettsiae reacted only with the appropriate LPS in the ELISAs (sera 2 to 7). Out of 16 IFA cross-reacting sera, 11 were found by ELISA to be seropositive to MT (sera 12 to 22)

and 5 were found to be seropositive to MSF (sera 8 to 11, 23). The LPS ELISA was found to be a convenient way to differentiate serologically between MSF and MT patients whose sera cross-react in the IFAs. These techniques can also serve as diagnostic tools in laboratories which lack the equipment needed to perform IFAs.

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