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Quantitative Assay for Genus-Specific Leptospiral Antigen and Antibody

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Hemagglutination and hemagglutination inhibition techniques have been developed as quantitative assays for the genus-specific antigen of *Leptospira* and for its antibody.

A genus-specific leptospiral antigen (HL) and corresponding antibody, and their assays by passive hemolysis of sheep erythrocytes (RBC), have been previously described (1-3). Although exceedingly sensitive, these assays required complement and were tedious to perform, and current studies on this antigen required a more convenient but reproducible technique for detection and quantitation. This has been accomplished by modifying the previously described hemolytic assay to one of passive hemagglutination (HA) for quantitating antiserum and passive hemagglutination inhibition (HAI) for quantitating antigen.

Preparation of the HL (or HA) antigen, sensitization of sheep RBC, and assay procedures have been described (1) and were followed, with few modifications. Optimal sensitization of sheep RBC in the HA assay resulted from the use of 64 units of HL antigen, rather than 16 previously reported for the HL procedure. More than 64 units did not result in higher antibody titers. Sensitized sheep RBC were preserved by adding glutaraldehyde in a 1.0% final concentration to a 1.0% suspension of sensitized RBC with slow mixing at room temperature for 3 h. Fixed RBC were then washed three times in Kent triethanolamine buffer (5) containing 1.0 mg of bovine albumin (Pentex fraction V powder) per liter as stabilizer and suspended to 10%, and Merthiolate was added to a final concentration of 1:10,000. This HA antigen was stored in small vials at 4 C and diluted 1:10 for use. Preserved nonsensitized RBC were prepared in the same manner for a control.

The procedure for HA titration is given in Table 1. Sera were inactivated and diluted in the same buffer described above. Occasional heterophilic antibodies were indicated by reaction in tube 9 and were removed by adsorption with nonsensitized RBC. Although four times the amount of antigen used in the HL procedure (1) is used to sensitize RBC for the HA test, antigen may be titrated by a block HA assay using RBC sensitized with double dilutions of antigen and appropriate dilutions of a reference antiserum. The dilution of antigen used in the HA test was the highest one not showing a decrease in antiserum titer, which regularly matched 64 units by the HL assay (1). Although the assay in Table 1 was performed in 13- by

TABLE 1. *Procedure for HA titration of antiserum*[a]

Determination	Tubes[b]									
	1	2	3	4	5	6	7	8	9	10
Antiserum, ml	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	
Dilution (reciprocal)	10	20	40	80	160	320	640	1,280	10	
Sensitized RBC, ml	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1
Nonsensitized RBC, ml									0.1	
Buffer, ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.9

[a] Titer of antiserum is the highest dilution giving a definite positive pattern (flat sediment), compared to negative control tube no. 10 (smooth button).
[b] Tubes were shaken and patterns were read after 4 h at room temperature.

TABLE 2. *Comparative HA and HL titrations*[a]

HL titers (reciprocals)	HA titers (reciprocals)						Mean
	<10	10	40	100	400	1,000	
<100	13[b]						
100		2					10
400			1	2			80
1,000		2	7	6			60
4,000			1	5	4		214
10,000			1	9	6	4	367
40,000					3	1	500
Mean		550	2,140	5,310	15,100	16,000	

[a] Titrations performed on 68 rabbit sera, including 13 from nonimmunized rabbits and 55 from rabbits immunized with pathogenic *Leptospira* and water isolates.

[b] Numbers of sera. All 13 sera were from nonimmunized rabbits.

HA assay would seem to be as qualitatively capable of detecting antibodies.

Quantitation of HA antigen by the HAI assay is shown in Table 3. Sensitivity of the HAI is at least as great as that of the HL assay in quantitation antigen (1). Greatest sensitivity in the HAI is obtained when the antiserum is diluted as close as possible to the exact end point in the HA titration and may require lower than double dilutions near the end point.

Preserved, sensitized RBC have remained stable in appearance and reactivity at 4 C for over 2 years. The HA procedure would seem to offer possibilities for use in serodiagnosis of leptospirosis, because of its genus-specificity, ease of performance, and stability of the antigen. The antigen may be prepared from a wide range of leptospiral serotypes, including nonpathogenic ones. The HAI technique also offers

TABLE 3. *Procedure for HAI titration of antigen*

Determination	Antigen (ml)									
	1[a]	2	3	4	5	6	7	8	9	10
Diluted antiserum	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Antigen	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	
Dilution (reciprocal)[b]	8	16	32	64	128	256	512	1,024	2,048	
Sensitized RBC[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Buffer	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.5

[a] Numbers 1 through 10 across are tube numbers. Tubes were shaken and patterns were read after 4 h at room temperature. Titer of antigen is the highest dilution showing complete inhibition (negative pattern, smooth button), compared to positive control tube no. 10 (flat sediment).

[b] After dilution, mix and incubate for 30 min at room temperature.

[c] Used in the highest dilution which gave a definite positive pattern in the HA titration, Table 1.

100-mm tubes, reducing all volumes by 0.2 permitted an equally sensitive and reproducible assay in disposable trays (Linbro IS-MRC-96).

Comparison of HA with HL titers on 68 rabbit sera is shown in Table 2. Sera included 13 from nonimmunized rabbits, 17 from rabbits immunized with pathogenic *Leptospira* (1, 2) (serotypes bataviae, hyos, ballum, pyrogenes, grippotyphosa, autumnalis, hebdomadis, sejroe, hardjo, pomona Pomona and Wickard, canicola Hond Utrecht and Moulton, icterohemorrhagiae M-20 and RGA, andaman, and semaranga), and 31 from rabbits immunized with *Leptospira* isolated from water (1, 2) (serotypes Patoc, WaZ, Lt430, WaReiden, Sao-Paulo, Ghent, CDC, and 24 antigenically different strains isolated in this laboratory) (4). HA titers were consistently lower than HL titers, but the a convenient, rapid, and sensitive assay for this genus-specific antigen.

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