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Microtiter Plate Agglutination Test for *Salmonella* Antibodies

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Similar results were obtained when testing human sera for *Salmonella* antibodies by the tube agglutination test and by the Microtiter plate agglutination test. The plate test was easier to perform and saved time, space, antigen, and serum.

Several serological tests have been adapted to the microtechnique because of its many advantages (1-3). A microtechnique adaptation of the Widal's test for detection of *Salmonella* agglutinins is reported here.

One hundred human sera from suspected cases of salmonellosis were tested by the usual tube agglutination (TA) test and the proposed Microtiter plate agglutination (PA) test. Sera were tested for *Salmonella* agglutinins at dilutions of 1:40 to 1:5,120 in both tests. Each serum was tested with commercially obtained *Salmonella* antigens (febrile antigens, Lederle Laboratories, Pearl River, N.Y.): *Salmonella* group A (somatic, 1, 2, 12), *Salmonella* group B (somatic 1, 4, 5, 12), *Salmonella* group D (typhoid O; somatic, 1, 9, 12, vi), paratyphoid A (flagellar a), paratyphoid B (flagellar b, 1, 2), and typhoid H (flagellar d). Known positive sera and 0.9% saline were used as controls.

In the TA test, sera were serially diluted in test tubes (12 by 75 mm) each containing 0.5 ml of saline. To each serum dilution, 0.5 ml of diluted (1:100) antigen was added. Racks containing the tubes were shaken by hand and incubated in a water bath at 56 C for 18 hr. The tubes were then examined for agglutination in front of a light source and a mirror. A 50% agglutination (2+) was considered as the end point. In the PA test, the same sera were tested with clear, disposable, U-type Microtiter plates, microdiluters, and droppers (Cooke Engineering Co., Alexandria, Va.) Sera were serially diluted in 0.05 ml of saline by means of 0.05-ml microdiluters. An equal volume of diluted (1:100) antigen was added to each serum dilution with a calibrated dropper. Plates were sealed, shaken for 3 min on a Thomas-Boerner shaker (Arthur H. Thomas Co., Philadelphia, Pa.), and placed in an incubator at 56 C for 18 hr. After incubation, the seal was removed from the plate, and the agglutination was read on a Microtiter mirror (Cooke Engineering Co., Alexandria, Va.) without shaking. A positive reaction was indicated by a smooth or irregular mat shape at the bottom of each well in the Microtiter plate, whereas a negative reaction was shown by a settling of the antigen to a button shape (Fig. 1). Each test was read independently, and results were then compared.

Similar results were obtained in both tests, and antibody titers were comparable by TA and PA tests. *Salmonella* antibody titers were identical in 72.8% (average percentage with the six used antigens) of the tested sera; there was a twofold difference in titers in 26.8% and a fourfold difference in only two sera (Table 1).

The PA test presented several advantages over the TA test. It was easier to perform and saved time, space, antigen, and serum. In each plate, two sera can be tested for six *Salmonella* antigens at eight different dilutions. Only 10% of the quantity of serum and antigen used in the TA test was needed in the PA test. Reading of the PA test with the Microtiter mirror was possible only in clear plates but not with opaque plates. Care must be taken to avoid shaking of the plates during and after incubation to allow settling of the antigen. From the above results, it was evident that the Microtiter PA test can replace the TA test with a high degree of confidence.

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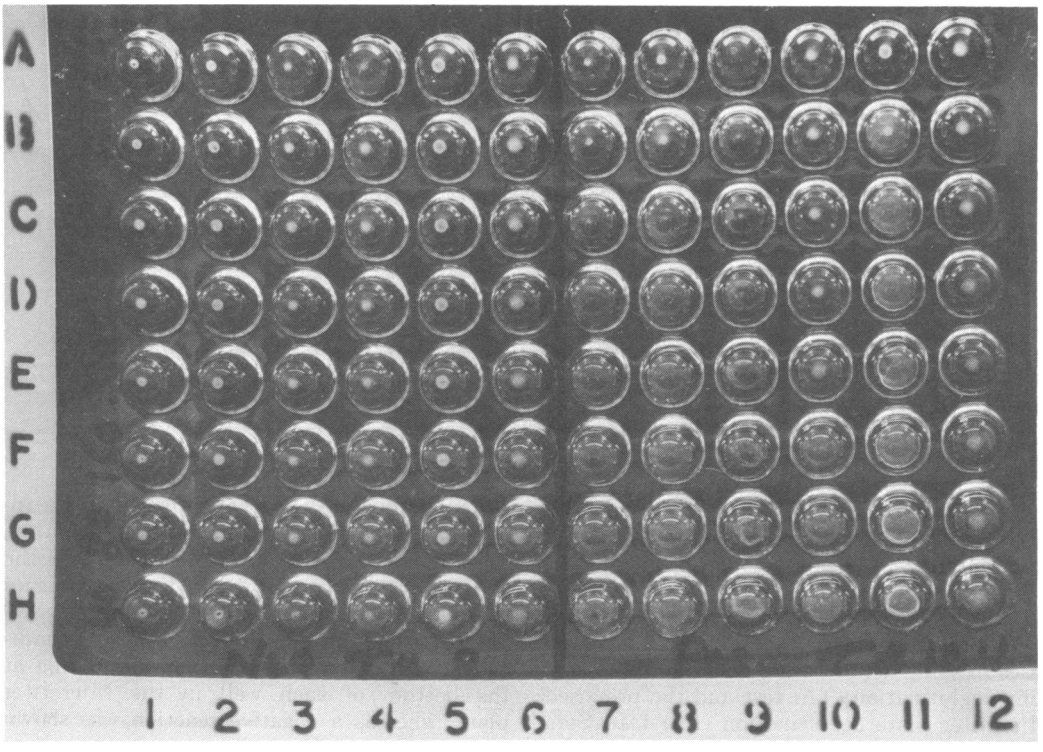

FIG. 1. *Microtiter plate agglutination test for Salmonella antibodies. Two sera are tested in each plate for six Salmonella antigens at 1:40 to 1:5,120 dilutions. A negative reaction is shown by a button shape, and a positive reaction is shown by a smooth or irregular mat shape.*

TABLE 1. *Differences in titers between TA and PA tests on 100 human sera tested for Salmonella agglutinins*[a]

Differences in titers between TA and PA tests	*Salmonella* antigens tested						Avg (%)
	SA	SB	PA	PB	TO	TH	
No difference	69	70	79	77	58	84	72.8
Twofold difference	31	30	21	23	41	15	26.8
Fourfold difference	0	0	0	0	1	1	0.4

[a] SA, *Salmonella* group A antigen; SB, *Salmonella* group B antigen; PA, paratyphoid A antigen; PB, paratyphoid B antigen; TO, typhoid O antigen; TH, typhoid H antigen.

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