Effect of Centrifugation and Microagglutination Techniques on Brucella Agglutinin Titers

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The microagglutination technique without centrifugation was more effective than centrifugation of the standard tube test for increasing Brucella agglutinin titers of specimens with a titer $\geq 160$ but was less effective than centrifugation of the standard tube test for specimens with a titer $<160$.

The Brucella agglutinin titer of many serum specimens can be increased by the use of a Coombs antiglobulin agglutination test, presumably because so-called incomplete or blocking antibodies are more effectively detected (3). Centrifugation alone, however, has been shown to be as effective as the Coombs technique for increasing Brucella agglutinin titers when blocking antibodies are present (2). In a previous report, we noted that higher Brucella agglutinin titers are obtained with a microagglutination test than with the standard tube agglutination test on sera with titers $\geq 160$ (1). The purpose of the present study was to compare centrifugation of the standard tube test with the microagglutination test as a means of increasing Brucella agglutinin titers.

Serum specimens. The human sera used in the study were sent to the Centers for Disease Control through various state health departments for the determination of Brucella agglutinin titers. We did not know which specimens were from documented cases of brucellosis and which were from brucellosis-free patients; however, an agglutinin titer $\geq 160$ with an unpaired specimen is usually considered to suggest a brucella infection (4). Therefore, the specimens were divided into two groups, those with a tube agglutination test (TAT) titer $<160$ were considered to be negative, and those with a TAT titer $\geq 160$ were considered to be positive. There were 47 specimens with a TAT titer $<160$ and 46 specimens with a TAT titer $\geq 160$. The titers of the first group were distributed as follows: $6 = <20; 6 = 20; 19 = 40$, and $16 = 80$. The titers of the second group were distributed as follows: $13 = 160; 12 = 320; 16 = 640; 4 = 1,280$, and $1 = 2,560$.

Antigen. The antigen used in all of the serological tests was a 1:50 dilution of a stock suspension of Brucella abortus (strain 1119-3) which was prepared and standardized by the National Animal Disease Laboratory, U.S. Department of Agriculture, Ames, Iowa.

TAT. The procedure used was described by Spink et al. (6). Doubling dilutions of serum were made in 0.85% saline in tubes (13 by 100 mm), starting with a 1:10 dilution and ending at 1:5,120. An equal volume (0.05 ml) of a 1:50 dilution of the stock antigen suspension was added to each tube, making the final dilutions of serum range from 1:20 to 1:10,240. The tubes were incubated in a 37°C water bath for 48 h.

Centrifuged tube agglutination test. The procedure used was described by Schubert and Colvin (5). After the titer of the routine TAT was recorded, the tubes were centrifuged at 850 × g for 15 min, and the titer was recorded again.

Microagglutination test. The procedure has been described in detail by Brown et al. (1). Briefly, doubling dilutions of serum were made in phosphate-buffered saline (pH 7.2) in conical (V) bottom polystyrene microtitration plates with the use of an automatic diluter (Dynatech). The serum dilutions ranged from 1:10 to 1:20,480. An equal volume (0.05 ml) of a 1:50 dilution of the stock antigen suspension was added to each well, making the final dilutions of serum range from 1:20 to 1:40,960. The plates were incubated in a 37°C water bath for 24 h.

Several of the microagglutination plates were centrifuged after the titers were recorded at the end of the 24-h incubation period; however, the procedure was discontinued because the titers from both groups of specimens were unchanged by centrifuging.

The geometric mean Brucella agglutinin titer obtained with the TAT, the centrifuged tube agglutination test, and the microagglutination test are given in Table 1. There was a distinct difference in the way that the two groups of specimens, those with a TAT titer $<160$ and those with a TAT titer $\geq 160$, were influenced by the centrifugation technique and by the mi-
TABLE 1. Comparison of geometric mean titers

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Specimen group</th>
<th>Geometric mean titers by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TAT</td>
</tr>
<tr>
<td>47</td>
<td>TAT &lt;160</td>
<td>39</td>
</tr>
<tr>
<td>46</td>
<td>TAT ≥160</td>
<td>395</td>
</tr>
</tbody>
</table>

<sup>a</sup> CTAT, Centrifuged tube agglutination test.
<sup>b</sup> MAT, Microagglutination test.

croagglutination technique. The geometric mean titer of specimens with a TAT titer <160 was increased twofold (87) by centrifugation of the TAT but was not increased by the microagglutination technique. However, the geometric mean titer of specimens with a TAT titer ≥160 was increased fourfold (1,581) by centrifugation and more than eightfold (3,903) by the microagglutination technique without centrifugation.

The titer increases resulting from centrifugation of the TAT or by the microagglutination technique without centrifugation were not influenced by how high the individual TAT titer was but only by whether the individual TAT titer was <160 or ≥160. Specimens with a TAT titer of 640 did not have microagglutination test titer increases proportionately greater than specimens with a TAT titer of 160. Sixteen specimens with a TAT titer of 640 had titer increases due to the microagglutination technique ranging from twofold to 128-fold. Thirteen specimens with a TAT titer of 160 had increases due to the microagglutination technique which also ranged from twofold to 128-fold. In general, the titer increases due to centrifugation were not as high as those due to the microagglutination technique without centrifugation. The increases ranged from none to 16-fold for the 16 specimens with a TAT titer of 640 and from twofold to 32-fold for the 13 specimens with a TAT titer of 160.

The results indicate that the microagglutination technique without centrifugation is more effective than centrifugation of the tube test for increasing Brucella agglutinin titers of specimens with a TAT titer ≥160 but is less effective than centrifugation of the tube test for specimens with a TAT titer <160.

LITERATURE CITED