Effect of Centrifugation and Microagglutination Techniques on
Brucella Agglutinin Titors

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The microagglutination technique without centrifugation was more effective
than centrifugation of the standard tube test for increasing Brucella agglutinin
titors 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 agglutini-
tin titers. We did not know which specimens
were from documented cases of brucellosis and
which were from brucellosis-free patients; how-
ever, 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 consid-
ered 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 $= 8$;
$6 = 20, 19 = 40, and 16 = 80$. 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 pro-
dure used was described by Schubert and Colvin
(5). After the titer of the routine TAT was
recorded, the tubes were centrifuged at 850 x 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) 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 at 37°C for 24 h.

Several of the microagglutination plates were
centrifuged after the titers were recorded at the
top of the 124-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 Specimen Group</th>
<th>Geometric Mean Titers by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAT</td>
</tr>
<tr>
<td></td>
<td>CTAT(^a)</td>
</tr>
<tr>
<td></td>
<td>MAT(^b)</td>
</tr>
<tr>
<td>47 TAT &lt;160</td>
<td>39</td>
</tr>
<tr>
<td>46 TAT ≥160</td>
<td>395</td>
</tr>
<tr>
<td></td>
<td>1,581</td>
</tr>
<tr>
<td></td>
<td>3,903</td>
</tr>
</tbody>
</table>

\(^a\) CTAT, Centrifuged tube agglutination test.
\(^b\) MAT, Microagglutination test.

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