Upper Limit of Normal Titer for Detection of Antibodies to *Legionella pneumophila* by the Microagglutination Test

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An upper limit of normal microagglutination titer for the Knoxville strain of *Legionella pneumophila* was determined by testing 830 sera. The upper limit of normal titer was found to be 8; however, we suggest that when applied to single serum specimens a titer of 32 be considered the lowest titer suggestive of infection (not necessarily disease).

The microagglutination test for the detection of antibodies to *Legionella pneumophila* proposed sp. nov. has been described previously (2), and an upper limit of normal titer was included in that report. Since that report and since the microagglutination test described in the Center for Disease Control manual (3), we have changed the initial serum dilution and now use the Knoxville strain instead of the Philadelphia 1 strain of *L. pneumophila* as the antigen. The purpose of the investigation reported here was to determine the upper limit of normal value for the revised test with more serum specimens than were used in the earlier study.

The initial serum dilution used in this study was 1:4 instead of 1:10 or 1:8 as described in the previous reports (2, 3). The antigen preparation used in this study was similar to that previously described (2, 3) with the following exceptions: the Knoxville strain was used instead of the Philadelphia 1 strain; the bacterium was grown on charcoal-yeast extract agar (4) instead of on enriched Mueller-Hinton agar; the culture was incubated for 4 days instead of for 3 days; and the harvested bacterial cells were killed by autoclaving for 15 min at 121°C instead of steaming for 1 h. Cherry et al. (1) used rabbit antiserum prepared with the Knoxville strain for the direct immunofluorescent test for *L. pneumophila* because they found that it gives better coverage of serogroup 1 strains than does antiserum prepared with the Philadelphia 1 strain. We compared Knoxville and Philadelphia 1 antigens, using 28 human sera from suspected cases of Legionnaires disease, and found that higher titers were obtained with the Knoxville antigen for 15 of the sera (9 2-fold higher, 2 4-fold higher, 3 8-fold higher, and 1 16-fold higher). The Philadelphia 1 antigen gave higher titers with four sera (three twofold higher and one fourfold higher). The remaining nine sera had the same titer with both antigens. The optimal concentra-

tion of cells in the antigen suspension was determined by block titration with reference sera of known titers. The proper antigen concentration is one which produces a well-defined button of sedimented cells in the bottom of the well with a negative specimen and gives the highest endpoint of agglutinated cells with a known positive serum.

A total of 830 sera were tested by the microagglutination test for antibodies to *L. pneumophila*. Of those, 548 were from apparently healthy blood donors who lived in the Knoxville, Cleveland, and Chattanooga areas of eastern Tennessee; 186 sera had been submitted for febrile agglutination tests (primarily brucella and tularemia) but had negative titers. The remaining 96 sera had been submitted for streptococcal antibody tests and had negative titers for these tests. The febrile and streptococcal sera came from several states, covering a wide geographical area.

The procedure for performing the microagglutination test was essentially the same as described in earlier papers (2, 3) with the exception of the antigen preparation and the 1:4 initial serum dilution. A low initial serum dilution should be used so that increases in titer between the acute- and convalescent-stage specimens can be more readily detected. For example, the titer of the acute-stage specimen may be less than 8 and that of the convalescent-stage specimen may be 16, which is a fourfold increase in titer. This increase would have been missed if the initial serum dilution had been 1:8 (1:16 after antigen is added).

A fourfold rise in titer between an acute-stage serum specimen and a convalescent-stage specimen is the most convincing serological evidence suggesting infection and is preferable to the results obtained from a single serum specimen. In the absence of both an acute- and a convalescent-stage specimen, however, an upper limit of

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normal titer may be useful as a base line for indicating the probability of infection with _L. pneumophila_. The upper limit of normal value is defined as the level of antibody titer exceeded by no more than 15% of the sera tested (5).

The microagglutination titers obtained with the Knoxville strain of antigen on the three groups of sera are presented in Table 1. The upper limit of normal titer is 8 for each of the groups because 85% or more of the specimens tested did not exceed this value. This value is for strains of _L. pneumophila_ belonging to the same serotype as the Knoxville strain (e.g., Philadelphia 1) but may differ for other serotypes. Although a titer of 16 is above the upper limit of normal titer, we suggest that it be considered equivocal when applied to single serum specimens and that a titer of 32 is suggestive of infection (not necessarily disease). This value is lower than the titer of 80 in the first report (2) and the titer of 64 in the second report (3). The titer exceeding the upper limit of normal in the three groups of sera tested may be due to either infection with _L. pneumophila_ which did not produce disease (inapparent infection) or residual antibodies from past Legionnaires disease. Although we have not observed cross-reactions with other microorganisms, they may be the cause of some low titers.

We thank Charles F. Peters for the blood donor sera and Robert Weaver for the _L. pneumophila_ cultures.

**LITERATURE CITED**


**Table 1. Distribution of microagglutination titers**

<table>
<thead>
<tr>
<th>Titer</th>
<th>Blood donor sera</th>
<th>Streptococcal sera</th>
<th>Febrile test sera</th>
</tr>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>&lt;8</td>
<td>261</td>
<td>47.6</td>
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<td>8</td>
<td>208</td>
<td>37.9</td>
<td>76</td>
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<tr>
<td>16</td>
<td>53</td>
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<td>8</td>
</tr>
<tr>
<td>32</td>
<td>23</td>
<td>4.2</td>
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<tr>
<td>64</td>
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