**Legionella pneumophila** Serogroup 12 Isolated from Human and Environmental Sources

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A **Legionella**-like organism (strain 570-CO-H [= ATCC 43290]) isolated from the lung tissue of a patient with pneumonia was shown by growth, as well as physiological, serological, and genetic characteristics, to belong to a new **Legionella pneumophila** serogroup, serogroup 12. Two additional strains were detected with antisera specific for strain 570-CO-H. These strains were isolated from environmental sources.

**Legionella pneumophila**, the etiologic agent of Legionnaires disease, was first recognized in 1977 following an epidemic of acute pneumonia in Philadelphia, Pa. (3). Since then, 23 **Legionella** species comprising 37 serogroups have been characterized (1, 4, 6–9). **L. pneumophila**, the most prevalent **Legionella** species in the United States (5), currently contains 11 serogroups (4, 6, 7), all of which cause pneumonia in humans. In this report, we describe a 12th serogroup, the type strain of which was isolated from the abscessed lung of a patient with pneumonia. Subsequently, two isolates from separate environmental water sources were also identified as **L. pneumophila** serogroup 12 strains.

Strains were received from the Colorado Department of Health, Denver (human isolate 570-CO-H), the Laboratory Centre for Disease Control, Ottawa, Ontario, Canada (strain 380-CAN-E), and the University of Melbourne, Parkville, Victoria, Australia (strain 687-AUS-E) for reference identification. Strain 570-CO-H was tested for growth and physiological characteristics, DNA relatedness to the type strain of **L. pneumophila** (Philadelphia 1), and reactivity with direct immunofluorescence assay conjugates as described previously (2, 6). Gas-liquid chromatography was performed with antigens to the 37 **Legionella** serogroups to determine whether the antisera was serogroup specific.

The morphological and growth characteristics of strains 570-CO-H, 380-CAN-E, and 687-AUS-E were consistent with those of **Legionella** species in that these organisms were gram-negative rods that did not grow on blood agar or on buffered-charcoal yeast extract agar without cysteine. Physiological test results for strain 570-CO-H and strain Philadelphia 1, the type strain of **L. pneumophila** (2), were negative for urease, nitrate reduction, glucose fermentation, and autofluorescence and positive for catalase, gelatinase, hippurate hydrolysis, and beta-lactamase. Strain 570-CO-H was oxidase negative and showed minimal browning of tyrosine-supplemented agar, whereas strain Philadelphia 1 (T = type strain) varied between being oxidase positive and oxidase negative and showed heavy browning of the agar. Gas-liquid chromatography profiles of all three strains were consistent with those for strain Philadelphia 1T (W. R. Mayberry, personal communication).

In the direct immunofluorescence assay, strain 570-CO-H gave negative results with all available conjugates. Strain 380-CAN-E reacted (2+ to 3+) with conjugates for **L. pneumophila** serogroups 1 and 4. Strain 687-AUS-E reacted 4+ with **L. pneumophila** serogroup 6 conjugate; however, only a small proportion of the cells were reactive.

In the slide agglutination test all three strains showed minimal agglutination (1+ to 2+) with **L. pneumophila** serogroup 1 antisera, and strain 687-AUS-E also agglutinated with **L. pneumophila** serogroup 6 antisera at a level of 2+. When tested against antigens to the 23 species of 37 serogroups currently recognized, antisera raised in rabbits to strain 570-CO-H reacted only with the **L. pneumophila**

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**TABLE 1.** Slide agglutinating antibody titers of unabsorbed and absorbed rabbit antisera against **L. pneumophila** strains

<table>
<thead>
<tr>
<th>Immunizing strain</th>
<th>Absorbed with:</th>
<th>Lp-1¹</th>
<th>Lp-6</th>
<th>570-CO-H</th>
<th>380-CAN-E</th>
<th>687-AUS-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>570-CO-H</td>
<td>Nothing</td>
<td>NR</td>
<td>16</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Lp-6</td>
<td>NR</td>
<td>&lt;2</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Lp-1T (= Philadelphia 1T)</td>
<td>Nothing</td>
<td>64</td>
<td>NR</td>
<td>36</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>570-CO-H</td>
<td>32</td>
<td>NR</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

¹ NR, Not reactive at the dilution used.

* Reciprocal of dilution giving 2+ or greater agglutination.

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by William R. Mayberry, East Tennessee State University, Johnson City. The slide agglutination test was performed with antisera to all previously named and published **Legionella** species (n = 23) and serogroups (n = 37) (1, 4, 6–9). Antiserum to strain 570-CO-H was prepared as described previously (7), and cross-agglutination tests were performed with antisera to the 37 **Legionella** serogroups 1 and 4. Strain 687-AUS-E reacted 4+ with **L. pneumophila** serogroup 6 conjugate; however, only a small proportion of the cells were reactive.

In the slide agglutination test all three strains showed minimal agglutination (1+ to 2+) with **L. pneumophila** serogroup 1 antisera, and strain 687-AUS-E also agglutinated with **L. pneumophila** serogroup 6 antisera at a level of 2+. When tested against antigens to the 23 species of 37 serogroups currently recognized, antisera raised in rabbits to strain 570-CO-H reacted only with the **L. pneumophila**

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serogroup 6 antigen at a level of 2+. Absorption of the 570-CO-H antiserum with L. pneumophila serogroup 6 cells and L. pneumophila serogroup 1 antiserum with strain 570-CO-H cells resulted in serogroup-specific antiserum in each case (Table 1). The absorbed 570-CO-H antiserum gave slide agglutination test reactions with strains 570-CO-H, 380-CAN-E, and 687-AUS-E at levels of 4+.

The three new strains were shown to belong to the species L. pneumophila by direct or indirect comparison of their DNAs with the DNA of Philadelphia 1, the type strain of L. pneumophila. Labeled Philadelphia 1 DNA was 77% related to DNA from strain 570-CO-H, and labeled 687-AUS-E DNA was 82% related to DNA from both 570-CO-H and Philadelphia 1. Labeled DNA from strain D165, which is not a member of serogroup 12, was 82% related to Philadelphia 1 DNA and 88% related to serogroup 12 strain 380-CAN-E DNA. In the reactions described above, the divergence in related DNA sequences was between 0 and 2%. Since the definition of a genetic species (2) is a group of strains whose DNAs are 70% or more related with 5% or less divergence, the results described above confirm that all three strains are members of the species L. pneumophila and also support the designation of 570-CO-H (= ATCC 43290) as the reference strain of a new L. pneumophila serogroup, serogroup 12.

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LITERATURE CITED


