Detection of Urinary Antigens of *Legionella pneumophila* Serogroup 12 by Broad-Spectrum Enzyme-Linked Immunosorbent Assay

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The first Canadian case of *Legionella pneumophila* serogroup 12 infection is reported. The initial diagnosis was made with a urine specimen by a broad-spectrum enzyme-linked immunosorbent assay; further serogrouping was done by indirect fluorescent-antibody and tube agglutination tests. This case emphasizes the importance of multivalent urinary antigen detection for the diagnosis of legionellosis.

A 52-year-old male was admitted to the hospital in Windsor, Ontario, Canada, with a 5-day history of fever, chills, and headaches. He subsequently developed a scanty productive cough, mild dyspnea, and diarrhea. The chest X-ray showed bilateral alveolar-interstitial disease with almost confluent consolidation in the right lower lobe and lingula. Serum and urine specimens were collected on day 11 after the onset of illness for the diagnosis of legionellosis by an indirect fluorescent-antibody (IFA) test (4) and a broad-spectrum enzyme-linked immunosorbent assay (ELISA) (3), respectively. The ELISA has a sensitivity of >70% and a specificity of >99%. Both capture and detection immunoglobulin reagents were derived from rabbit hyperimmune sera. The ELISA uses polyvinyl chloride U-bottom microdilution plates (Dynatech Laboratories, Inc., Alexandria, Va.). All reagents were added at 100 μl per well unless otherwise stated. Anti- *Legionella* immunoglobulin G at a final concentration of 20 μg/ml in 0.05 M carbonate buffer (pH 9.6) was used to coat the wells. Coating was done at either 4°C for 18 h or 37°C for 1 to 2 h, and the plates were emptied and washed three times with phosphate-buffered saline. Postcoating was done for the same incubation times with 5% bovine serum albumin in carbonate buffer. All test samples were boiled for 1 min in a water bath, centrifuged at 1,200 × g for 10 min, and added to the test wells in duplicate. The plates were incubated for 1 h at 37°C, emptied, and washed six times with phosphate-buffered saline, with a 3-min soaking step after wash three. Enzyme conjugates diluted 1:100 (ca. 4 μg of immunoglobulin G per ml) in phosphate-buffered saline with 2% bovine serum albumin were added, and the plates were incubated at 37°C for 1 h, emptied, and washed as before. A substrate solution of 1 mg of *p*-nitrophenylphosphate per ml in 10% diethanolamine buffer (pH 9.8) was added, the mixture was incubated at 37°C for 1 h, and the reaction was stopped by the addition of 50 μl of 3 M NaOH. The A410 was read with a Microelisa Mini reader MR590 (Dynatech). Six negative urine controls were run per plate. The results of the test duplicates were averaged, and the positive threshold was calculated by using twice the mean of the six negative urine controls (3).

For the diagnosis of legionellosis, our *Legionella* reference laboratory routinely screens respiratory tract specimens by culturing and a direct fluorescent-antibody test (2), serum specimens by an IFA test, and urine specimens by a broad-spectrum ELISA. All reagents are produced in our laboratory. The direct fluorescent-antibody and IFA tests routinely cover a panel of 20 *Legionella* antigens: *Legionella pneumophila* serogroups 1 through 8, *L. bozemanii* serogroup 1, *L. micdadei*, *L. longbeachae* serogroups 1 and 2, *L. feeleii* serogroup 1, *L. wadsworthii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. oakridgensis*, *L. maceachernii*, and *L. anisa*. Tube agglutination is carried out only for IFA-positive specimens as previously described (1).

The urine specimen was positive in the broad-spectrum ELISA, indicating the presence of *Legionella* soluble antigens. The IFA test revealed a titer of <1:32 against the 20 routinely tested antigens. A second serum specimen was requested and collected on day 23 after the onset of illness. No change of the IFA titer was observed in this second serum specimen. Owing to the ELISA-positive results, the IFA test was repeated with both serum specimens by using 22 other *Legionella* antigens which are not routinely tested: *L. pneumophila* serogroups 9 through 14, *L. feeleii* serogroup 2, *L. bozemanii* serogroup 2, *L. hackelliae* serogroups 1 and 2, *L. birminghamensis*, *L. parisiiensis*, *L. rubrilocens*, *L. sainthelenii*, *L. jamestowniensis*, *L. ertyhra*, *L. spirinitis*, *L. cherrli*, *L. steigerwaltit*, *L. santarcus*, *L. israelensis*, and *L. cincimattinensis*. The first serum specimen (11 days after onset) had a titer of <1:32 against all of these supplementary antigens. The second serum specimen (23 days after onset) had a titer of <1:32 against all of the antigens, except for the *L. pneumophila* serogroup 12 antigen, against which the titer was 1:128. To supplement the IFA results, we did a tube agglutination test with both sera. Titers of 1:64 (first serum specimen) and >1:1,024 (second serum specimen) were obtained against the *L. pneumophila* serogroup 12 antigen. This is the first reported case of an *L. pneumophila* infection in Canada involving this serogroup.

*L. pneumophila* serogroup 12 is one of the 28 species and 45 serogroups of *legionellae* (5). The large number of serogroups has made it impractical to routinely screen the newer species and serogroups by direct fluorescent-antibody or IFA tests. Culturing of respiratory tract specimens, the method of choice, has the limitation of a delay of at least 3 days. The broad-spectrum ELISA in this case was the only source for early diagnosis, as the IFA test would have missed the serogroup 12 infection. This case demonstrates the importance of the broad-spectrum ELISA in providing an early diagnosis which could significantly improve the medical management of patients.

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