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Ruling out False-Positive Urinary *Legionella pneumophila* Serogroup 1 and *Streptococcus pneumoniae* Antigen Test Results by Heating Urine

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We report here false-positive urinary *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* antigen test results due to rabbit antilymphocyte serum treatment and provide a simple and fast solution to rule them out by heating urine.

Lower respiratory tract infection is the most common infectious cause of death worldwide and represents a major public health issue (1). The management strategies developed to reduce mortality and costs (1) include the identification of the microorganisms causing severe community-acquired pneumonia and health care-acquired pneumonia. This is based on microbial cultures from blood, respiratory tract, or pleural fluid specimens and detection of urinary antigens for *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1 (1, 2, 3). The BinaxNOW urinary *Streptococcus pneumoniae* and *Legionella* antigen tests (Alere, Jouy-en-Josas, France) are *in vitro* immunochromatographic membrane assays designed to detect the cell wall C polysaccharide of *S. pneumoniae* and the specific lipopolysaccharide of *L. pneumophila* serogroup 1 in human urine specimens (4, 5). Each test uses specific capturing and conjugated rabbit antibodies adsorbed on a nitrocellulose membrane. The specificities of these tests are high (>95%), but microbiologists and practitioners should be aware of the possible causes of false-positive results. In this article, we report false-positive BinaxNOW urinary *Legionella* and *Streptococcus pneumoniae* test results due to rabbit antilymphocyte serum (i.e., rabbit serum with antibodies to thymocytes) treatment and provide a simple and fast solution to rule them out.

A 16-year-old male patient with T-cell acute lymphoblastic leukemia underwent an allogeneic bone marrow transplant (BMT) after three lines of unsuccessful treatment. At chemotherapy induction, he received thiotepa, cyclophosphamide, etoposide phosphate, and rabbit antilymphocyte serum (Thymoglobulin [Genzyme, France], 175 mg at day −3 and day −2 before the BMT). At day 9 after the BMT, he was transferred to an intensive care unit due to hypovolemia caused by diarrhea in the context of a digestive graft-versus-host reaction, confirmed by examination of a digestive tract biopsy specimen. At day 16 after the BMT, the patient experienced fever, requiring mechanical ventilation, and septic shock, requiring norepinephrine. Bronchoalveolar lavage (BAL) fluid and blood specimens were weakly positive for human herpesvirus 6 (654 copies/10^6 blood cells) by PCRs. Blood specimens were negative for *Aspergillus* and *Toxoplasma gondii*, and BAL fluid specimens were negative for *Pneumocystis*. Urinary *L. pneumophila* serogroup 1 and *S. pneumoniae* antigen tests performed at day 17 after the BMT were both positive. However, BAL fluid samples cultured on sheep blood agar aerobically and anaerobically at 37°C for 2 days and on chocolate blood agar in 5% CO_2 at 37°C for 2 days for bacteria, on buffered charcoal-yeast extract medium in 2.5% CO_2 at 37°C for 10 days for *Legionella*, and on Sabouraud-chloramphenicol-gentamicin agar aerobically at 37°C for 10 days for fungi only yielded *Pseudomonas aeruginosa* at a concentration of >10^4/ml (strain susceptible only to ceftazidime, amikacin, tobramycin, and colistin). Urinary *S. pneumoniae* and *Legionella* antigen tests performed at day 21 after the BMT on new samples remained positive, whereas respiratory sample cultures remained negative for *S. pneumoniae* and *L. pneumophila*. The *Legionella* serology tests, performed twice 2 weeks apart, were negative.

The initial therapy included ceftazidime, levofloxacin, azithromycin, and cotrimoxazole. Since *P. aeruginosa* was still isolated in BAL fluid at >10^4/ml 6 days later, intravenous amikacin, intravenous, and aerosolized colistin were added to ceftazidime, whereas levofloxacin and cotrimoxazole were discontinued and azithromycin was maintained for a total of 14 days. The deterioration of gas exchange and rapid progression toward refractory acute respiratory distress syndrome led to the patient's being given veno-venous extracorporeal membrane oxygenation. In the following days, the cutaneous and digestive graft-versus-host disease involvement worsened, and gastrointestinal bleeding occurred despite additional immunosuppressive treatment (increase in corticosteroids and cyclosporine dosages). Eventually, despite aggressive resuscitation, the patient progressed to refractory shock, which evolved into intractable multiorgan failure, leading to death 43 days after the BMT.

The BinaxNOW urinary *L. pneumophila* and *S. pneumoniae* antigen tests are rapid and simple assays used to help in the diagnosis of pneumonia. The urinary *Streptococcus pneumoniae* antigen test has sensitivity of 75% (6) (from 77% to 89% in bacteremic community-acquired pneumonia and from 44% to 64% in non-bacteremic pneumonia [7]) and a specificity of 95% (6). False-positive results can be due to infection by *Streptococcus viridans* species which share the same antigen or asymptomatic nasopharyngeal colonization by pneumococci or previous pneumococcal

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infection (8) (the positivity of the test is not suppressed by 1 week of antibiotics and a positive result persists for several weeks [7]). The BinaxNOW *Legionella* test has a sensitivity of 75% and a high specificity (99%) for the detection of *L. pneumophila* serogroup 1 (9). False-positive results can occur in patients with serum sickness or those who had a previous *Legionella* infection (7, 10). The excretion of *Legionella* antigens in urine samples can be detected 1 to 3 days after the onset of the disease and can last for 1 year (7). The false-positive results for these two tests could also be associated with rheumatoid-like factors or with previous pneumococcal and *Legionella* infections in the past few months; however, tests for rheumatoid factor in serum specimens were negative, and the patient had had no infections, in particular no respiratory infection, in the last 6 months. In this context, false-positive results for these tests represent an important clinical issue. To our knowledge, this is the first report of false-positive BinaxNOW *Streptococcus pneumoniae* test results in the urine specimens of a patient receiving rabbit antilymphocyte serum. Different arguments favor the false-positive tests in the present case. The urinary *L. pneumophila* serogroup 1 and *S. pneumoniae* antigen tests were both positive, whereas cultures of respiratory samples (including those collected before antibiotic treatment) remained negative for these bacteria. A false-positive result for the urinary antigen test for *L. pneumophila* serogroup 1 was described previously in a patient receiving rabbit serum with antibodies to thymocytes and having serum sickness, in the context of renal transplantation (10), whereas *Legionella* serology was negative and an alternative cause was identified to explain the degradation of the respiratory condition of the patient. The false-positive results described here may have been due to the presence of interfering antibodies that developed secondary to rabbit antilymphocyte serum administration and were directed against rabbit antibodies in the urine of the patient (11). These interfering antibodies might react with the capturing and conjugated rabbit antibodies used in the BinaxNOW tests, leading to false-positive results. In their case report, Deforges et al. demonstrated that the interfering molecule was a polypeptide since the test became negative after urine pretreatment by proteinase K, whereas true-positive tests, confirmed by positive cultures, remained positive after this treatment (10). Since proteinase K pretreatment is not available in all laboratories, here we propose an alternative easier and faster procedure. Urine samples were heated at 95°C for 5 min in order to remove interfering antibodies (and rheumatoid-like factors, if present), without affecting heat-stable urinary bacterial antigens (11, 12). As expected, the results of the BinaxNOW tests performed after heating centrifuged urine samples (5 min at 8,000 rpm) became negative. This procedure was also tested on urine samples from patients with *S. pneumoniae* ($n = 7$) or *L. pneumophila* serogroup 1 ($n = 4$) pulmonary infections, confirmed by positive respiratory cultures. In each case, the BinaxNOW test results remained positive after heating.

We also tested urine specimens collected on the same day or before the injection of rabbit antilymphocyte serum (Thymoglobulin [Genzyme, France] or ATG-Fresenius [Fresenius Medical Care, France]) from 5 patients and urine specimens collected at least 3 weeks after an injection (but less than 3 months) from 22 patients who received an injection of rabbit antilymphocyte serum. Interestingly, these 22 patients received a low mean cumulative dose of antilymphocyte serum of 131 mg per patient. All urinary *Legionella pneumophila* serogroup 1 and *Streptococcus pneumoniae* antigen tests were negative. This is not surprising since serum sickness, a type III hypersensitivity, is an immune complex-mediated illness that can occur in patients after polyclonal antibody therapy, and serum sickness after administration of rabbit (or horse) serum with antibodies to thymocytes occurs in 1% to 87% of cases according to the literature. In most publications, patients received several doses (13–18), particularly in those that reported a high incidence of serum sickness (19, 20). Conversely, in a study by Büchler et al. (21) including patients receiving a mean cumulative dose of 560 mg during 9.6 days, the incidence of serum sickness was low (7.5%, 18/240 patients receiving renal transplants), and, interestingly, all patients who developed serum sickness, except one, had at least 7 days of treatment with Thymoglobulin. The patient with the false-positive urinary antigen tests described in our article received 350 mg of Thymoglobulin. Currently, there are no methods for predicting future serum sickness development; however, previous exposure to antilymphocyte serum, significant rabbit or horse exposure, concomitant immunosuppression, and antilymphocyte serum dosing may be predisposing factors (13, 14). It has not been demonstrated that patients receiving higher doses of antilymphocyte serum have a higher probability of developing serum sickness. However, we hypothesize that the additional patients receiving antilymphocyte serum had negative urinary antigen tests because they had received a low dose of antilymphocyte serum, in contrast to the patient with positive urinary antigen tests, who had received a higher dose of antilymphocyte serum.

The risk of false-positive results with the BinaxNOW urinary *Legionella* and *Streptococcus pneumoniae* tests in patients receiving rabbit antilymphocyte serum is not mentioned in the manufacturer's recommendations. Altogether, our findings indicate that since patients receiving antilymphocyte serum (transplant patients) are at high risk for legionellosis, microbiologists and physicians must be made aware of the possibility of false-positive results of the BinaxNOW urinary *Legionella* and *Streptococcus pneumoniae* tests in patients who have been treated with rabbit antilymphocyte serum, although the occurrence of false-positive urinary antigen tests is rare.

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