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Retrospective Evaluation of the Du Pont Radioimmunoassay Kit for Detection of *Legionella pneumophila* Serogroup 1 Antigenuria in Humans

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We used the Du Pont radioimmunoassay kit for soluble *Legionella pneumophila* serogroup 1 antigenuria (Du Pont Co., Wilmington, Del.) to test 422 urine samples from patients with and without Legionnaires disease (LD). The urine specimens were collected from 23 patients with culture-proven LD and from 346 patients without LD. *L. pneumophila* serogroup 1 was isolated from 14 patients with culture-proven LD, and other *L. pneumophila* serogroups or other *Legionella* species were isolated from 9 patients; 58 urine specimens were tested from these 23 patients. The non-LD group was composed of 75 bacteremic patients (35 gram-negative and 40 gram-positive bacteremias), 7 patients with candidemia, 48 patients with non-LD pneumonia, 90 patients with gram-negative bacteriuria ($>10^5$ CFU/ml), 23 patients with gram-positive bacteriuria ($>10^5$ CFU/ml), 14 patients with candiduria ($>10^5$ CFU/ml), and 89 outpatients with negative urine cultures. All tests were performed in duplicate, including positive and negative controls. Sample results with values ≥3.0 times those of the negative controls were considered positive for *L. pneumophila* serogroup 1 antigenuria. The average sample-to-negative ratios were 19.1 for the *L. pneumophila* serogroup 1 specimens, and 1.0 for both the non-serogroup 1 legionella group and the non-LD specimens. All but one of the patients who were culture positive for *L. pneumophila* serogroup 1 had at least one specimen positive for serogroup 1 antigenuria; none of the non-*L. pneumophila* serogroup 1 patients had a positive urine test. The test was highly specific (100%) and sensitive (93%) for the detection of *L. pneumophila* serogroup 1 antigenuria. Concentration of urine by vacuum evaporation increased test sensitivity without apparently affecting specificity.

Several rapid methods have been described for the laboratory diagnosis of Legionnaires disease (LD). One of the earliest described methods was detection of a soluble *Legionella pneumophila* antigen in urine (1, 3, 15). These antigens have been detected by latex agglutination assays (10), reversed passive agglutination (8, 13), enzyme-linked immunosorbent assays (1, 6, 10, 11, 14, 15), or radioimmunoassay (RIA) (4–7, 10, 11). The sensitivities of these tests range from approximately 70 to 100%, depending on the criteria used to define the disease (3). Assays for *L. pneumophila* antigenuria have been confined to research laboratories because of the unavailability of a commercial system for performing this test. In the present study, we evaluated a commercial RIA for the detection of *L. pneumophila* serogroup 1 antigenuria, using specimens from well-defined patient groups.

MATERIALS AND METHODS

Specimens. A total of 422 urine specimens were tested. Of these, 58 specimens were obtained from 23 patients with culture-proven LD, and the remainder of specimens were obtained from hospital inpatients and outpatients who had urine submitted to the clinical microbiology laboratory for routine urine culture (Table 1). Urine specimens from LD patients were obtained between 1978 and 1984 and were kept at −20°C from the time of collection. Respiratory tract specimens from 13 of the 14 patients with LD caused by *L. pneumophila* serogroup 1 were tested by direct immunofluorescence microscopy (DFA) for serogroup 1; samples from 12 of these patients were positive. A total of 364 urine specimens from 346 patients were used as negative controls. The specimens were taken from patients with well-documented infections and from outpatients being screened for bacteriuria. Pneumonia in this group of patients was defined as the presence of a new radiographic infiltrate and fever. Of the 46 patients with non-LD pneumonia, 13 had pneumonia caused by members of the *Enterobacteriaceae*, 11 had mixed-flora aspiration pneumonia, 4 had *Pseudomonas aeruginosa* pneumonia, 8 had pneumonia of unclear etiology, 4 had *Haemophilus influenzae* pneumonia, 4 had fungal pneumonia, and 2 had beta-hemolytic streptococcal pneumonias. Urine specimens collected from the non-LD patients were stored at 5°C for up to 7 days before being frozen at −20°C, and the specimens were tested within 2 months of the collection date. Urine specimens were thawed only at the time of testing during this trial; none had been previously thawed.

Assay method. The assay is an antigen-capture RIA, using polyclonal monovalent rabbit anti-*L. pneumophila* serogroup 1 antiserum as the capture antibody, and the same antibody, which has been radioiodinated with ^{125}I, as the detection antibody. Testing was performed as recommended by the manufacturer. Urine specimens were allowed to thaw at room temperature (21 to 25°C) before testing. Positive and negative urine specimens were tested in duplicate in each run, as were the supplied negative- and positive-control samples. The sample (100 μl) was pipetted into the supplied antibody-coated tube and incubated for 1 h in a water bath at

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TABLE 1. Average sample-to-negative ratios of urine specimens from different patient groups

Clinical diagnosis	Avg ratio	No. of specimens/ no. of patients
LD		
L. pneumophila serogroup 1	19.1	30/14
Non-*L. pneumophila* serogroup 1	1.0	28/9
L. pneumophila serogroup 4	1.1	5/1
L. pneumophila serogroup 9	1.3	5/1
L. longbeachae serogroup 1	1.0	7/2
L. wadsworthii	1.0	4/1
L. micdadei	0.8	3/2
L. dumoffii	0.9	2/1
L. bozemanii serogroup 1	0.9	2/1
Non-legionella infection	1.0	364/346
Gram-negative bacteremia	1.0	35/35
Gram-positive bacteremia	1.0	40/40
Candidemia	1.0	7/7
Pneumonia	1.0	66/48
Gram-negative bacteriuria	1.0	90/90
Gram-positive bacteriuria	1.0	23/23
Candiduria	1.0	14/14
Negative urine culture	1.0	89/89

37 ± 0.5°C. The tube contents were then discarded by aspiration, and the tubes were washed three times with 1-ml volumes of 0.15% NaCl, aspirating after each wash. Radiolabeled detection antibody (100 μl) was added to each tube; the tubes were incubated for 1 h in a 37°C water bath, after which the tube contents were aspirated and washed as described above. Radioactivity bound to the tubes was determined by using a gamma scintillation counter (Berthold, Nashua, N.H.), counting for 1 min. The gamma counter was calibrated daily, using a sealed-source ^{129}I standard; the average counting efficiency was 79%. The ratio of the radioactivity of each sample to that of the mean of the negative-control samples was calculated automatically. Urine samples with sample-to-negative ratios ≥3.0 were considered positive for *L. pneumophila* serogroup 1 antigenuria. Two falsely negative urine specimens and some truly negative specimens were concentrated fivefold, using a vacuum evaporator (Speed-Vac; Savant Instruments, Inc., Farmingdale, N.Y.) to determine whether test sensitivity could be enhanced without decreasing specificity.

Statistical methods. Descriptive statistics were computed by standard methods (12). Comparison of mean values was by nonpaired two-tailed *t* tests (12), and calculation of type-two error was by the method of Cohen (2).

RESULTS

L. pneumophila serogroup 1 antigenuria was detected in at least one unconcentrated urine specimen from all but one patient with LD caused by *L. pneumophila* serogroup 1 (sensitivity, 93%), whereas there were no positive urine specimens from any other LD or non-LD patient. In the one *L. pneumophila* serogroup 1 patient for whom the antigenuria test was negative (average ratio, 1.9), only one specimen of urine was available. After fivefold concentration of the urine, the ratio rose to 4.2.

Mean sample-to-negative ratios were significantly different in *L. pneumophila* serogroup 1 LD patients as opposed to those of any other group (Table 1). The mean sample-to-negative ratio for the *L. pneumophila* serogroup 1 urine specimens was 19.1, with a median of 21.8, range of 1.9 to 44.4, standard deviation of 12.5, and 99% confidence interval of 12.8 to 25.4. This result was significantly different ($P < 0.0001$) from the mean and median sample-to-negative values of 1.0 for the non-*L. pneumophila* serogroup 1 LD group of urine specimens, with a range of 0.6 to 1.6, standard deviation of 0.2, and 99% confidence interval of 0.9 to 1.1. The mean and median sample-to-negative values for the non-LD group of urine specimens was 1.0, with a range of 0.5 to 1.8, standard deviation of 0.2, and 99% confidence interval of 0.9 to 1.0. No difference was apparent between the ratios of urine specimens from non-*L. pneumophila* serogroup 1 LD patients and those of urine specimens from non-LD patients ($P > 0.5$, $1 - \beta = 0.17$, with $\alpha_2 = 0.05$). None of the duplicate pairs of specimens gave discrepant results for either positive or negative samples.

More than one urine specimen was available from nine patients with *L. pneumophila* serogroup 1 infection. These specimens were collected at various times for up to 15 days after the onset of disease. In all but two of the nine patients with *L. pneumophila* serogroup 1 infections, antigenuria was detected up to the last specimen of the series received. One of the patients whose specimen results were an exception to this result had a series of urine specimens available up to 15 days after onset of disease; antigenuria was detectable until the 14th day. This respiratory secretions of this patient were culture positive for *L. pneumophila* serogroup 1 on hospital admission, 7 days after initiation of symptoms, but were DFA negative. The highest average ratio for this series of urine specimens was only 4.2, and on the 15th day it was 2.3. The other patient had three urine specimens collected during the course of illness; urine specimens collected on hospital days 3 and 5 were both positive, with ratios of 3.7 and 5.6, respectively, whereas a urine specimen collected on day 4 was negative, with a ratio of 2.5. Fivefold concentration of the urine specimen yielding a test ratio of 2.5 resulted in a ratio of 3.8. The respiratory tract secretions from the second patient were culture positive for *L. pneumophila* serogroup 1 on admission and negative thereafter; DFA results for the same secretions were positive for *L. pneumophila* serogroup 1 for all specimens, including one specimen examined on day 6. Except for urine specimens from the two patients discussed above, the values for the mean sample-to-negative ratios within each patient series did not vary significantly among specimens during the period of observation.

In the subgroup of patients with non-*L. pneumophila* serogroup 1 LD, a series of urine specimens was available from eight of nine patients. All 28 specimens tested were negative for *L. pneumophila* serogroup 1 antigen, with ratios ranging from 0.6 to 1.6. The highest values were found in the *L. pneumophila* serogroup 9 subgroup (range 0.9 to 1.6, mean 1.3), which were not significantly different from the ratios of the other non-*L. pneumophila* serogroup 1 group urine specimens.

Fivefold concentration of three truly negative urine specimens yielded ratios below 3.0. One specimen collected from a patient with LD caused by *L. pneumophila* serogroup 9 had a preconcentration ratio of 1.6; the value was 1.4 postconcentration. Another specimen collected from a patient with a urinary tract infection had preconcentration and postconcentration ratios of 1.8 and 1.1, respectively. The preconcentration ratio of a urine specimen collected from a patient with non-LD pneumonia was 0.9; the postconcentration value was 1.2.

The assay took about 3 h to complete for processing 25

samples, excluding controls, of which 1 h was actual hands-on time; this includes the time needed for tube labeling, pipetting samples and reagents, washes, and counting.

DISCUSSION

In this study, we have demonstrated, like others, that the detection of *L. pneumophila* serogroup 1 antigenuria by RIA is highly sensitive and that the results obtained with a commercial kit appear to be as good as those obtained by using research laboratory techniques. Unlike previous reports (10, 11), we had no false-positive results (specificity, 100%). Sathapatayavongs and colleagues found three false-positive results in 178 control urine samples tested with an enzyme-linked immunosorbent assay, all of which became negative after the specimens were boiled; the same specimens were negative when tested by RIA even before being heated (11). In a separate study, the same group of investigators found one false-positive specimen in 161 negative control urine specimens tested by latex, enzyme-linked immunosorbent assay, and RIA; all three assay types produced a false-positive test with this specimen. In this case, culture and DFA results were negative, but serological tests were not done (10). In an earlier evaluation of RIA, Kohler and colleagues (7) found one probable false-positive specimen among 241 negative-control urine specimens that they did not include in the study due to lack of diagnostic data. In a retrospective study performed by the same group, no false-positive results were obtained among 498 negative urine specimens tested by RIA (3). In their evaluation of the Du Pont test for *L. pneumophila* serogroup 1 antigenuria, Kohler and colleagues found that six of 287 previously negative urine samples were falsely positive (R. Kohler, L. Wheat, and C. Bentsen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C50, p. 340). This was found to be due to freeze-thawing by an unknown mechanism. Since none of our specimens had been freeze-thawed previously, this phenomenon, which we did not observe, would have been unexpected. It is unlikely that prolonged freezing made the test apparently more sensitive in the *L. pneumophila* serogroup 1 specimens, because none of the non-*L. pneumophila* serogroup 1 LD specimens were falsely positive, despite having been stored identically.

Since none of the specimens in the non-*L. pneumophila* serogroup 1 LD group had average ratios above 2.0, we propose that values between 2.0 to 3.0 be considered highly suggestive of true antigenuria and that a new specimen be assayed for confirmation. It is possible that diuresis may render borderline-positive tests negative, so that repeat testing of concentrated urine may be diagnostic, as demonstrated in this study. However, since we tested few truly negative specimens after concentration, the specificity of testing concentrated specimens should be regarded as undefined until further studies clarify this issue.

We included in our study a patient population with LD not caused by *L. pneumophila* serogroup 1. Previous studies have included samples from patients with *L. pneumophila* serogroups 1, 3, 4, 6, 8, and 10, *Legionella micdadei*, and *Legionella longbeachae* serogroup 1 infections (3, 4, 14). We did not detect positive reactions with specimens from patients with serogroup 4 infections, in contrast to a previous report (4), but we tested few such samples. All the non-*L. pneumophila* serogroup 1 specimens tested negative by this assay, demonstrating its high specificity.

We used only culture-confirmed cases of LD to determine the sensitivity of this assay. It is possible that this case selection method overestimates the true sensitivity of the assay, as demonstrated by Kohler and colleagues in earlier studies of a similar assay system (4, 5). Since there is no good non-culture-based standard for detection of LD, such a bias is inevitable. The exceptional specificity of this assay for *L. pneumophila* serogroup 1 makes it very useful for ruling in disease, because the positive predictive value is 100% regardless of disease prevalence. However, this high specificity also means that the test will miss all patients with legionella infections caused by non-*L. pneumophila* serogroup 1 legionellae. Since the majority of LD cases appear to be caused by *L. pneumophila* serogroup 1 (9), this test would likely be of benefit in most settings. Although large prospective studies have yet to be performed, this test could probably be used to replace DFA, serological testing, DNA probe testing, or all three. However, in those regions in which non-*L. pneumophila* serogroup 1 legionella infections are common, other diagnostic methods might be better used. In all cases, culture should be used in addition to urine assay, for epidemiological purposes and for optimal sensitivity. Since antigenuria may persist for prolonged periods after treatment, as shown in this study and by others (6), it will always be possible that a positive test represents remote pneumonia rather than an acute illness. Regardless, the test will be of considerable benefit in patients not able to produce sputum for culture and for those with negative cultures because of antimicrobial therapy.

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