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Comparison of Binax Legionella Urinary Antigen EIA Kit with Binax RIA Urinary Antigen Kit for Detection of *Legionella pneumophila* Serogroup 1 Antigen

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The Legionella Urinary Antigen EIA kit (Binax, Portland, Maine) was compared with the EQUATE RIA Legionella Urinary Antigen kit (Binax) for its ability to detect the presence of urinary antigens to *Legionella pneumophila* serogroup 1. Urine specimens from patients without Legionnaires' disease ($n = 33$) were negative by both methods (specificity, 100%). Twenty (77%) of 26 urine specimens from patients with Legionnaires' disease positive by the radioimmunoassay kit were also positive by the enzyme immunoassay (EIA) kit. If the cutoff for a positive EIA result were lowered to a ratio of ≥2.5, 23 of 26 (88%) urine specimens would have been positive by EIA and the specificity would remain 100%. Use of the EIA kit is an acceptable method for detecting *L. pneumophila* serogroup 1 urinary antigens by laboratories that do not want to handle radioactive materials.

Although population-based studies suggest that 10,000 to 25,000 cases of Legionnaires' disease occur annually (6), only 1,300 suspected cases are reported to the Centers for Disease Control and Prevention each year (5). The definitive diagnosis of Legionnaires' disease is made by culture of respiratory secretions, which requires 3 to 5 days of incubation (3, 7). Many laboratories do not or are unable to culture *Legionella* spp. (2). Direct fluorescent-antibody staining of respiratory secretions is a rapid test, but it has a low level of sensitivity in the clinical laboratory (3). Indirect fluorescent-antibody testing of paired serum specimens is useful in epidemiologic studies (10) but is of little use in patient management. The EQUATE RIA Legionella Urinary Antigen kit (Binax, Portland, Maine) is a rapid test that detects *Legionella pneumophila* serogroup 1 antigen, which is excreted early in the course of the illness (4). Legionnaires' disease is most commonly (80 to 85% of cases) caused by *L. pneumophila* serogroup 1 (4, 9). The EQUATE RIA Legionella Urinary Antigen has a sensitivity of >90% and a specificity of 100% in patients with Legionnaires' disease from whom *L. pneumophila* serogroup 1 is isolated (1, 4, 8). An enzyme immunoassay (EIA) kit recently became available from Binax. We compared the EQUATE RIA Legionella Urinary Antigen kit and the Binax Legionella Urinary Antigen EIA kit for their abilities to detect *L. pneumophila* serogroup 1 antigens in urine.

Twenty-six frozen urine specimens from patients with Legionnaires' disease and positive for *Legionella* antigen by radioimmunoassay (RIA) were available for retrospective testing. For 14 patients, *L. pneumophila* serogroup 1 had been isolated from cultures of respiratory specimens or patients had had a fourfold rise in indirect fluorescent-antibody titers in an epidemiologic study of community-acquired pneumonia. Twelve patients had pneumonia and fulfilled the case definition of Legionnaires' disease during an outbreak of Legionnaires' disease. Thirty-three frozen control urine specimens previously negative by RIA for *Legionella* urinary antigens were either from pneumonia patients with negative *Legionella* cultures and no fourfold rise in serology or from healthy controls from an outbreak investigation.

RIA. Urine specimens were tested for the presence of urinary antigens of *L. pneumophila* serogroup 1 by using the EQUATE RIA Legionella Urinary Antigen kit (Binax) and following the manufacturer's instructions. All solutions required for the assay are contained in the kit. Urine samples, including a positive and a negative control, were pipetted in duplicate into tubes coated with polyclonal rabbit antibody specific for *L. pneumophila* serogroup 1. The tubes were incubated at 37°C for 60 min and were then washed three times. Detector antibody labeled with ^{125}I was added to the tubes, which were incubated at 37°C for 60 min and again washed three times. The average counts per minute of bound detector antibody was determined on a gamma counter. Ratios were determined by dividing the mean counts per minute for test samples by the mean counts per minute for the negative control. Samples with ratios of ≥3 were considered to be positive.

EIA. Urine specimens were tested by using the Legionella Urinary Antigen EIA kit (Binax) and following the manufacturer's instructions. All solutions required for the assay are contained in the kit. Urine samples, including positive and negative controls, were pipetted in duplicate into microtiter wells coated with polyclonal rabbit antibody specific for *L. pneumophila* serogroup 1 antigen. Rabbit anti-*L. pneumophila* horseradish peroxidase conjugate was added. The microtiter plate was incubated at room temperature for 2 h and was then washed three times. Color developer was added, and after a 15-min incubation, stop solution was added to all wells. The A_{450} was determined with a microtiter plate reader. Samples with an absorbance greater than or equal to three times the absorbance of the negative control were considered positive.

Results. The results were compared by Fisher's exact test. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Figure 1 shows the correlation of the results from the two assays. For the 26 patients with Legionnaires' disease, the ratios ranged from 3.1 to 47.2 (mean ± standard deviation [SD], 8.6 ± 9.0) with the RIA kit, whereas the ratios ranged

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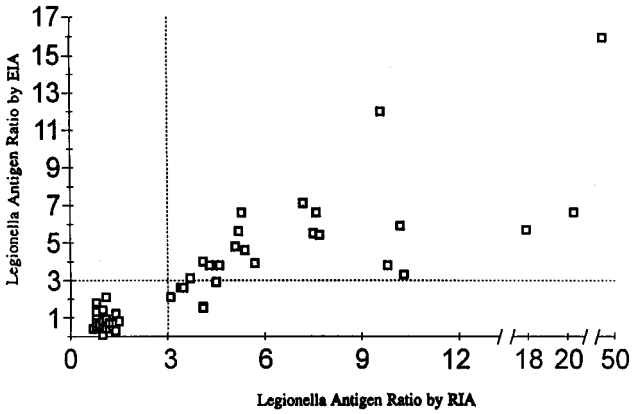

FIG. 1. Comparison of ratios (mean counts per minute for test samples/mean counts per minute for negative controls) obtained with two Legionella urinary antigen detection kits; EQUATE RIA Legionella Urinary Antigen kit (Binax) and Legionella Urinary Antigen EIA kit (Binax). The dashed lines indicate the cutoffs for a positive result.

from 1.5 to 16.0 (mean ± SD, 5.1 ± 3.1) with the EIA kit. Twenty of 26 (77%) urine specimens positive by RIA were positive by EIA. The concordance between the two methods in defining positive and negative results was 89.8% ($P < 0.0001$). The positive predictive value of the EIA kit was 100%, and the negative predictive value was 85%. Three of the six urine specimens false negative by EIA had ratios of 2.5 to 2.9. The other three false-negative urine specimens had ratios of 1.5, 1.6, and 2.1, respectively. Thirty-three urine specimens from patients without Legionnaires' disease were negative by RIA (range, 0.7 to 1.5; mean ± SD, 1.0 ± 0.21) and EIA (range, 0.4 to 2.1; mean ± SD, 0.8 ± 0.45).

The EQUATE RIA Legionella Urinary Antigen kit (Binax) has been proven to be a specific and sensitive method for detecting the presence of urinary antigens of *L. pneumophila* serogroup 1 (1, 4, 8). Unfortunately, this assay has been underutilized (3, 8). In both of our laboratories, the EIA urinary antigen kit showed 100% specificity compared with the RIA kit, although the sensitivity was only 77%. Discrepant results were seen when the ratio by RIA was near 3 and the ratio by EIA was between 2 and 3. If the ratio for a positive result by the EIA kit were lowered to 2.5, the sensitivity of the EIA kit would increase to 88% and the specificity would remain 100%, with 23 specimens having a ratio of ≥2.5 by EIA and 3 specimens having a ratio of <2.5 by EIA (positive predictive value, 100%; negative predictive value, 92%; correlation, 95%; $P < 0.0001$). All 33 specimens with a ratio of <3 by RIA had a ratio of <2.5 by EIA.

The EQUATE RIA Legionella Urinary Antigen kit was introduced in Franklin County, Ohio, in 1991 as a research tool in a study of community-acquired pneumonia (6). Because of physician requests, the assay was made routinely available through the Medical Center Reference laboratory of Ohio State University. The results were reported within 1 working day. Since 1992 more than 2,000 urinary antigen tests have been requested annually by community-based physicians. The rate of positivity by the urinary assay has been approximately 2% among all samples tested. Ideally, sensitive and specific assays for the detection of *Legionella* urinary antigens from non-*L. pneumophila* serogroup 1 isolates will be developed and marketed.

Tests with both the EQUATE RIA Legionella Urinary Antigen kit and the Legionella Urinary Antigen EIA kit are easy to perform and results can be obtained on the same day (less than 3 h from beginning to end by either assay). The RIA kits are less expensive and more sensitive and, because of a shorter shelf-life, should be used by any laboratory having the capability of using radioactive isotopes and a demand of 25 tests per month. For other laboratories, the EIA kit is an acceptable option. The costs of the reagents will vary depending on how many samples are tested in each assay. If only one sample were tested each day, the cost of reagents with the RIA kit would be approximately $22 and the cost of reagents with the EIA kit would be approximately $44. The reagent costs would be halved if four samples were tested per day. Timely results from the hospital laboratory will provide a valuable diagnostic tool to clinicians caring for patients with pneumonia. As physicians become aware of the clinical utility of the Legionella Urinary Antigen assay, the volume of tests requested will increase.

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