Comparative Evaluation of the Tube and Microdilution Limulus Lysate Techniques for Rapid Presumptive Diagnosis of Gonococcal Urethritis in Men

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Eighty-one men with exudative urethritis were evaluated on initial visit for gonococcal urethritis by using the standard tube and newly developed microdilution Limulus amoebocyte lysate techniques. Serial dilutions of clinical specimens ranging from 1:100 to 1:1,024,400 were each tested, and results correlated with Gram stain and culture. Overall accuracy for predicting culture results was 98% for a dilution of 1:200 and 99% for a dilution of 1:400 for the tube and microdilution techniques, respectively. The microdilution technique predicted culture results in 98% of cases for dilutions ranging from 1:400 to 1:1,600, whereas the tube technique was as accurate for dilutions of only 1:100 and 1:200. The microdilution Limulus amoebocyte lysate technique was a rapid, reliable, sensitive, and economical diagnostic aid in the initial evaluation of exudative urethritis in men.

In a recent report (9), we demonstrated the potential usefulness of the Limulus amoebocyte lysate (LAL) assay as a rapid, reliable, and sensitive technique for the presumptive diagnosis of gonococcal urethritis in men. The assays were performed in test tubes as described by the manufacturer, whereby observation for gelation is simple and only involves inverting the tubes. However, the utilization of the assays in the quantitation of lipopolysaccharide requires a large number of tubes and a considerable amount of lysate and technician time. Despite numerous reports (1, 2, 4–6) describing modifications of the LAL assay which eliminate the need for tubes, most require complex materials and sophisticated equipment. Recently, we described a modification of the standard LAL tube assay in which microdilution plastic plates were used to quantitate lipopolysaccharide (8). The test was easy to perform, and interpretation of results was as definitive as the tube technique. The purpose of this study was to compare the standard tube technique with the microdilution procedure in the initial evaluation of exudative urethritis in men.

MATERIALS AND METHODS

Study population. Eighty-one men with uncomplicated urethritis seen at the Columbus Health Department Venereal Disease Clinic were evaluated. These patients had sought treatment because of urethral discharge or dysuria or both and were selected on a random basis; only patients with a purulent urethral discharge or a discharge obtained after urethral massage were included. Patients receiving antibiotics within 10 days of presentation were excluded. The mean age was 22.8 years with a range of 19 to 26 years. The patients presented 1 to 12 days after initial symptoms with a mean of 3.6 days.

Diagnostic procedures. Standardized patient interviews included demography, sexual and venereal disease histories, present illness, and an examination of the genitals and inguinal lymph nodes.

Urethral exudates (0.05 to 0.1 ml) were collected from the urethral meatus by gentle aspiration with a tuberculin syringe (without needle) and transferred to a pyrogen-free plastic test tube containing 0.9 ml of pyrogen-free water (Travenol Laboratories, Deerfield, Ill.). All specimens were frozen at −20°C before being tested by the LAL assay. For culture of Neisseria gonorrhoeae, a sterile calcium alginate-tipped wire urethro-genital swab (Wilson Diagnostics, Inc., Glenwood, Ill.) or sterile bacteriiological loop was passed 3 to 4 cm into the urethra. The samples were then streaked directly on Thayer-Martin media and incubated for 48 h at 35°C in 5% CO2. The same procedure was repeated to obtain specimens for Gram staining and microscopic examination. Cultures for viruses or chlamydiae were not done.

Laboratory methods. Gram-stained smears of urethral exudate were examined with oil immersion at ×1,000 magnification for the presence of polymorphonuclear cells and gram-negative diplococci. (One microscope was used by one observer.) The results were considered positive if typical gram-negative diplococci were seen, whether located intracellularly or extracellularly. Identification of isolates of N. gonorrhoeae was confirmed as previously described (9). The microscopic diagnosis of nongonococcal urethritis required a mean of 10 leukocytes per ×1,000 field in at least five fields with the absence of gram-negative diplococci.

LAL assay. Specimens of urethral exudate were...
thawed and serially diluted in pyrogen-free water to final concentrations ranging from 1:100 to 1:102,400 of the original sample. The standard tube test involved the addition of 0.2 ml of the diluted specimens to a single-test Pyrotest vial (Difco Laboratories, Detroit, Mich., courtesy of A. L. Lane); the contents were mixed, incubated at 37°C in a water bath for 1 h, and read. A firm opaque gel which remained adherent to the bottom of the tube when inverted was interpreted as a positive test; the absence of firm gelation was interpreted as a negative test.

The microdilution procedure was performed as previously described (8). Briefly, 0.05 ml of the 1:100 dilution used in the tube test was added to wells 1 and 2. Serial twofold dilutions were then made from wells 2 through 11 in water with a 0.05-ml diluter. Then 0.05 ml of lysate (Cape Cod Associates, Woods Hole, Mass.) was added to each well. The plates were covered with plastic lids, mixed, and incubated at 37°C for 1 h. The presence or absence of gelation was then determined by first adding 0.05 ml of a 0.005% aqueous crystal violet stain solution to each well. The bottoms of the plates were viewed at an oblique angle (30 to 45°), and gelation was noted in wells in which the stain did not mix and color the contents; lack of gelation was noted in wells in which the stain mixed and colored the contents.

Known positive and negative controls were tested with both procedures, and the LAL results were read without previous knowledge of the microbiological findings. The minimum sensitivities of the tube and microdilution procedures with soluble *Escherichia coli* endotoxin (lot EC-2, Bureau of Biologics, U.S. Food and Drug Administration) were the detection of 0.25 and 0.03 ng/ml, respectively.

RESULTS AND DISCUSSION

The tube and microdilution LAL test results for urethral specimens from men with gonococcal and nongonococcal urethritis serially diluted from 1:100 to 1:102,400 are shown in Table 1. In accordance with previous findings (9), the tube results correlated with culture-proven cases of gonococcal urethritis with a high degree of accuracy (98%) when specimens were tested at a dilution of 1:200. The LAL test results obtained with the microdilution technique demonstrated similar accuracy (100%) when specimens were tested at a dilution of 1:400. For patients with nongonococcal urethritis, one patient (3%) had a positive LAL test for both the tube and microdilution techniques at dilutions of 1:200 and 1:400, respectively. With the microdilution technique, positive test results were obtained in 33 and 15% of the nongonococcal cases at dilutions of 1:100 and 1:200, respectively. These higher percentages of positive LAL tests for culture-negative urethral specimens were apparently due to the higher sensitivity (0.03 ng/ml) of lysate used in the microdilution technique. However, at dilutions from 1:400 to 1:1,600, overall accuracy in predicting culture results was 98% (79 of 81 patients). The tube technique had comparable accuracy only with dilutions of 1:100 and 1:200. These breakpoints for specimen dilution are dependent on lysate sensitivity and would have to be adjusted if lysate of different sensitivities were used.

A comparison of the results of LAL testing by the tube and microdilution techniques at dilutions of 1:200 and 1:400, respectively, to Gram-stained smears of patients with gonococcal and nongonococcal urethritis is shown in Table 2. The Gram-stained smears correlated with an accuracy of 100% in those cases of nongonococcal urethritis; however, the overall accuracy of the Gram stain in culture-positive gonococcal disease was 95% (40 of 42 cases). The two cases which eluded detection by Gram stain, one of which was also missed by the tube LAL test, were correctly identified by the microdilution LAL technique.

Diagnostic accuracy in the evaluation of urethritis is essential since nongonococcal urethritis in men has been reported to occur as often as gonococcal urethritis by investigators in certain areas of the country (3, 7, 11). Microscopic examination of exudate by a trained technician is a reliable diagnostic method for men with symptomatic urethritis. The finding of typical gram-negative diplococci within polymorphonuclear leukocytes of urethral exudates correlates with positive culture results in 98% of cases. Similarly, when the Gram stain is unequivocally negative, the culture is also negative in 98% of cases.

**Table 1.** Percent positive LAL tests at various dilutions of urethral samples for cases of gonococcal and nongonococcal urethritis in men

| Diagnosis               | No. tested | Test method | % positive at dilution 1:
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Gonococcal urethritis</td>
<td>42</td>
<td>Tube Microdilution</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Nongonococcal urethritis</td>
<td>39</td>
<td>Tube Microdilution</td>
<td>3</td>
</tr>
</tbody>
</table>
However, Gram stains of urethral exudates from as many as 15% of men may not be indicative of either gonococcal or nongonococcal urethritis, commonly showing extracellular atypical pleomorphic gram-negative organisms or small numbers of polymorphonuclear leukocytes (10).

The LAL tube method has been shown to be as reliable as the Gram stain in the initial evaluation of urethritis in men (9). In addition, the test also correlated with subsequent culture results in 99% of the cases even though the LAL assay is not a specific test for *N. gonorrhoeae*. Similar predictability of Gram stain and culture results was obtained by the microdilution technique. The microdilution procedure has the added advantages of requiring a smaller volume of lysate and abbreviated technician time to perform. The need for a trained microbiologist to interpret Gram-stained smears is also eliminated as the LAL assay is easy to perform and interpret. Furthermore, our findings suggest that the microdilution technique provides reliable results over a wide dilution range of clinical specimens.

ACKNOWLEDGMENTS

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LITERATURE CITED


### Table 2. Results of Gram-stained smears and LAL assay for patients with gonococcal and nongonococcal urethritis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
<th>Gram-stained smears</th>
<th>LAL assay</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Gonococcal urethritis</td>
<td>42</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>Nongonococcal urethritis</td>
<td>39</td>
<td>0</td>
<td>39</td>
</tr>
</tbody>
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<sup>a</sup> Original sample diluted 1:200.

<sup>b</sup> Original sample diluted 1:400.