Rapid Detection of Significant Bacteriuria by Use of an Automated Limulus Amoebocyte Lysate Assay

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Previous studies have demonstrated that significant gram-negative bacteriuria can be detected by using the Limulus amoebocyte lysate test. A series of 580 urine specimens were tested in parallel with the automated MS-2 (Abbott Laboratories) assay and with quantitative urine bacterial cultures. The overall ability of the MS-2 Limulus amoebocyte lysate test to correctly classify urine specimens as containing either \( \geq 10^5 \) organisms or \(< 10^5 \) organisms per ml during a 20-min test period was 92.6%.

Previous methods for the rapid detection of significant bacteriuria included chemical tests for the presence of bacterial enzymes or by-products in urine, such as the Griess nitrate reductase and tetrazolium reductase tests (6, 8), examination of urine sediment by either phase-contrast microscopy (1) or Gram stain of uncentrifuged urine sediment (2), and more recently, the use of automated instruments for rapid detection of bacterial growth, e.g., Autobac (General Diagnostics), AutoMicrobic system (Vitek Systems), and MS-2 (Abbott Laboratories) (7). The Limulus amoebocyte lysate (LAL) endotoxin assay has been shown previously to be a novel approach to the rapid detection of significant gram-negative bacteriuria (4). The LAL test has recently been made faster and more sensitive through automation by the Abbott MS-2 Microbiology System (3). This communication describes the testing of a series of 580 urine specimens in parallel with the automated MS-2 LAL test and standard quantitative urine bacterial cultures.

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The LAL used in this study was obtained from Associates of Cape Cod, Inc., Falmouth, Mass., and had a sensitivity of \( \leq 0.25 \) ng/ml of EC2 reference endotoxin by gelation testing. This lysate could detect between 0.001 and 0.005 ng of endotoxin per ml with the automated MS-2 LAL test (3). The Abbott MS-2 Research System was used in this study, which employed disposable glass Ampvettes and specially modified Ampvette holders to minimize reagent volumes. The MS-2 LAL test on urine was performed with 400 \( \mu l \) of urine which had been diluted 1:2,000 in sterile pyrogen-free water. The diluted urine was placed in Ampvettes and then 100 \( \mu l \) of LAL was pipetted into each Ampvett immediately before its insertion into an MS-2 analysis module. Ampvettes were incubated at 35.5°C without shaking (3) for a 20-min test period. For the purposes of this study, a positive MS-2 LAL test was defined as a change equal to or greater than 100 MS-2 transmission units occurring in any 1- to 5-min reading interval during the 20-min test period. Likewise, a urine was declared negative if this predefined threshold was not achieved within 20 min.

As a method for comparison of MS-2 LAL results, quantitative bacterial cultures were performed by the calibrated loop method (0.001-ml loop). Well-mixed urine samples were streaked onto the surfaces of sheep blood and MacConkey agar plates with the calibrated loop. The plates were incubated at 35°C for a total of 48 h. Significant bacteriuria was defined in this study as a urine colony count equal to or greater than \( 10^3 \) organisms per ml.

The isolates recovered from patients in this study with significant bacteriuria and the MS-2 results are shown in Table 1. The majority of isolates were members of the family Enterobacteriaceae. The MS-2 LAL test detected 94.0% (78 of 83) of those urine samples which contained \( \geq 10^5 \) organisms of gram-negative bacteria per ml. The MS-2 LAL test could not detect urines which contained \( \geq 10^5 \) gram-positive bacteria per ml. Thus, if all of the urine samples which contained \( \geq 10^5 \) organisms of any species per ml are considered, 84.8% (78 of 92) were detected by the MS-2 LAL test. The overall ability of the MS-2 LAL test to correctly catego-
rize a urine specimen as containing either $\geq 10^5$ or $<10^5$ organisms per ml was 92.6% (537 of 580).

Table 2 shows the results of the MS-2 LAL test on those urine samples which contained less than $10^5$ organisms per ml. The MS-2 detected 40% (10 of 25) of those urine samples which contained between $10^4$ and $10^5$ gram-negative bacteria per ml. Likewise, urine samples which contained $10^3$ to $10^5$ mixed gram-positive and gram-negative bacteria per ml were not consistently detected by the MS-2 (5 of 12). Approximately 3.3% (14 of 426) of the urine samples which contained fewer than $10^4$ viable bacteria or which produced no growth were interpreted as positive by the MS-2 LAL test criteria. No effort was made to determine whether any of these latter specimens were obtained from patients who were either receiving antibiotics or who had just completed antibiotic therapy.

The MS-2 LAL test was found to be a very objective and rapid method for the detection of urine samples likely to reflect significant gram-negative bacteriuria. The MS-2 printout of changes in light transmission values between each 5-min reading were easily interpreted as either positive (a change equal to or greater than 100 units in any 5-min interval) or negative (lacking a significant change in optical density). Most often, urine samples which contained $\geq 10^5$ gram-negative bacteria per ml produced a significant change in light transmission values within the first or second 5-min reading period. Thus, most urine samples representative of significant gram-negative bacteriuria were detected within the first 10 min of the test. In only a few instances were positive readings obtained in the third or fourth 5-min reading interval. Therefore, the test provided a very rapid indication of positive samples with very few false positives observed.

The overall ability to correctly classify urine specimens as positive or negative compared favorably with the rapid culture techniques employed by the Autobac or the growth-screening method currently used with the MS-2 (7). However, the MS-2 LAL test required only 20 min, as compared with approximately 5 hours for either of the automated growth-screening techniques (7). The definition of significant bacteriuria employed for this study was a colony count of $\geq 10^5$ bacteria per ml. The results of preliminary experiments were employed to arrive at the urine dilution ratio of 1:2,000 which was incorporated into this study. It is possible that a lesser dilution of urine, such as 1:200 or 1:500, might allow better detection of those specimens which contain $10^4$ to $10^5$ organisms per ml. It is also possible that LAL of lesser sensitivity (and therefore lower cost) could be readily applied for MS-2 LAL testing of urine. The use of less sensitive LAL would not require as large a dilution of the urine before testing and would undoubtedly lower the reagent cost of the test. The objective indication of a positive LAL test by the MS-2 was found to be very useful for urine screening. Instead of interpretation of gelation endpoints of varying degrees (5), an objective determination of a predefined optical density threshold was employed (3). The MS-2 LAL test may prove to be a rapid, automated approach for the detection of significant gram-negative bacteriuria.

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LITERATURE CITED


