Evaluation of Leukocyte Esterase Activity as a Rapid Screening Technique for Bacteriuria

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Microscopy and leukocyte esterase activity, both employed as screening techniques for urine cultures, were evaluated with respect to two distinct populations, male and female. When 424 urine specimens from males were examined, 95% of the Gram-stained smears and 91% of the leukocyte esterase tests correctly correlated with culture results, indicating significant bacteriuria. There were no statistical differences between these two screening procedures. Likewise, there were no significant differences between the Gram stain and leukocyte esterase activity in predicting a negative culture: 99% and 98%, respectively. Neither microscopy nor esterase activity proved as sensitive or as efficient in predicting a negative culture with the female population.

Increasing importance has been placed on rapid screening tests for bacteriuria. Since most urine specimens are negative, screening reduces time, effort, and expense of more traditional quantitative methodologies. Additionally, it is a necessary step in directing rapid susceptibility testing on those urine specimens that are positive.

Numerous techniques and instruments have been introduced to facilitate the elimination of unnecessary cultures and to reduce the processing time of clinical specimens. Rapid methods such as microscopy (1, 3, 7, 14) and colorimetric means (13) have been utilized effectively for urine screening. More recently, automated direct disk elution susceptibility testing of urine specimens has been performed after screening by light-scatter photometry (2, 4). Cost of instrumentation or additional technical time needed to perform some screening procedures may make them prohibitive to all but the larger laboratories. In the present study, we assessed the feasibility of rapidly screening for bacteriuria by utilizing leukocyte esterase, a biochemical marker of pyuria (8).

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MATERIALS AND METHODS

Urine. Urine specimens were collected from two distinct populations. A total of 424 consecutive urine samples collected from males (midstream, 67%; catheterized, 15%; and unspecified, 18%) were processed from the VA Medical Center. An all-female population was surveyed by processing 371 consecutive urine specimens, all midstream, received by Statlab, Inc.

Cultures. Urine specimens were plated within 2 h of collection or held at 4°C until processed. A 0.01-ml calibrated platinum loop was used to inoculate a sheep blood agar and MacConkey agar plate for quantitative culture (6). Plates were incubated at 36°C for 18 h in an air incubator. Estimates of colony counts were made by visually examining the blood agar plates and correlating growth with photographs of plates seeded with known concentrations of bacteria distributed by the same inoculation technique. Bacterial isolates were identified by conventional procedures.

Significant bacteriuria was defined as ≥10⁵ colony-forming units of one clearly predominating organism per ml. Secondary organisms were accepted in this definition only if they occurred in concentrations less than 10⁴. A culture with higher counts of secondary organisms or any ratio of three organisms was discarded from the study.

Gram stain. Smears were made by applying 1 drop of well-mixed, unspun urine from a Pasteur pipette to a clean glass slide (14), allowing the drop to air-dry without spreading, and then heat-fixing and Gram staining the smear (6). Smears were examined under an oil-immersion objective (×1,000) for at least 20 fields. A positive Gram stain was defined as one with an average of two or more organisms of the same morphological type per oil-immersion field.

Leukocyte esterase activity. Leukocyte esterase activity was determined by dipping a Chemstrip L (Bio-Dynamics, a Division of Boehringer-Mannheim, Indianapolis, Ind.) for 1 s into urine. The strip was drawn over the rim of the specimen cup to remove excess urine and then visually compared to a color chart on the strip container after 15 min of incubation in room air. The formation of a light blue color signified a positive esterase test and has been demonstrated to correlate well with significant pyuria (8). These investigators reported the esterase test to give a sensitivity and specificity of 87.9% and 94.3%, respectively, with
TABLE 1. Summary of microscopic examination, esterase activity, and culture results of urine specimens from two distinct populations

<table>
<thead>
<tr>
<th>Culture results</th>
<th>Gram-stained smear</th>
<th>Esterase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAMC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>STAT&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Pos&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Neg</td>
</tr>
<tr>
<td>Positive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>74</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>340</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>344</td>
</tr>
</tbody>
</table>

<sup>a</sup> VAMC, 424 specimens collected from VA Medical Center patients.  
<sup>b</sup> STAT, 371 specimens from female patients, received by Statlab, Inc.  
<sup>c</sup> Pos, ≥2 organisms of one morphological type per oil-immersion field.  
<sup>d</sup> Positive culture is defined as ≥10<sup>2</sup> colony-forming units of one clearly predominating organism per ml.

The number of specimens, which were examined on an oil-immersion field, is given in Table 1. The presence of Leucocytes per cubic millimeter in urine and the time of appearance of a positive reaction on the dip stick show the higher number of leukocytes, the more rapid the appearance of a positive esterase test.

RESULTS

Microscopic examination and leukocyte esterase activity are compared with culture results in Table 1. The percentage of positive cultures for the male and female populations was 18 and 26%, respectively. The indexes of efficacy of the two screening methods compared to culture results are shown in Table 2 and are based on methods described by Ransohoff and Feinstein (11).

DISCUSSION

The Gram-stained smear of uncentrifuged urine from a male population is both a sensitive and specific test in detecting significant bacteriuria. Similar results have been reported for female patients attended by trained personnel during specimen collection (14). The females studied in this report submitted random specimens collected without the benefit of such experienced staff. It is obvious (Table 2) that the accuracy of microscopic examination of urine for bacteria is dependent upon the method and time of specimen collection and is most probably biased as to the sex of the patient.

Leukocyte esterase activity utilized as a predictor of significant bacteriuria in males produced no statistical differences in sensitivity or predictive value of a negative culture from that of the Gram stain. This was not the case for the female population studied. Although both groups showed a marked increase in false-positives with the esterase test, the female population produced 16% false-negatives compared to only 2% for the male patients. The significantly higher false-positive rate in females could be attributed, in part, to the fact that we had no knowledge of when these patients had last voided or if they were indeed symptomatic at the time of specimen collection.

Esterase activity does not require intact cells and is not affected by urinary pH, protein,
bacteriuria, underlying disease, renal function, or a receipt of a variety of drugs (8). This esterase appears to be quite specific for leukocytes and thus is a useful marker of pyuria. Pyuria and its relationship to "urethral syndrome" in women has been studied, but a correlation with bacteriuria of $\geq 10^5$ colony-forming units per ml has not been conclusively found (5, 9, 12). It is apparent that pyuria occurs in women with and without urinary tract infections; thus esterase activity is neither a sensitive nor a specific screening test for detecting significant bacteriuria in midstream urine from females. Leukocytes in the urine of females can arise from vaginal secretions or may persist for several days after a true bacteriuria has been successfully treated (7). Leukocyte counts have been shown to be elevated in 65% of female midstream specimens growing $\geq 10^5$ colony-forming units per ml, but counts were also increased in 57% of the sterile specimens (9). Pyuria may be frequently found in female populations in the absence of bacteriuria, due to extrarenal infection and inflammation (10, 12). The present study appears to support the evidence of frequent pyuria, as detected by leukocyte esterase, without bacteriuria in women (Table 1). Conversely, pyuria was not a common finding in the asymptomatic males studied in this report, and esterase activity correlated well with positive culture results.

Symptomatic male patients are the source of many urine specimens in VA Medical Centers. Those institutions with semiautomated instruments may use them to screen these specimens and may subsequently proceed with rapid direct susceptibilities and identification. Other institutions lacking such instrumentation, or those not wishing to wait the 3 to 6 h necessary to achieve maximum accuracy, may screen specimens microscopically, thereby expediting the process of direct susceptibility testing. The present study suggests that leukocyte esterase activity of urine in a male population is an excellent screening technique for significant bacteriuria, comparable with the Gram stain. It should, however, be used with caution when evaluating midstream specimens collected from females.

Subject to the quality of the specimen received, negative urines can be accurately eliminated from more costly, time-consuming screening procedures by this inexpensive, rapid test. When the specimen is positive for esterase activity, a Gram stain can be performed to yield information regarding morphology and Gram reaction to facilitate subsequent testing.

LITERATURE CITED

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