Three Techniques Compared for Detecting Bacteruria in Symptomatic Patients

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We wanted to determine whether the microscopic evaluation of urinary sediment could be replaced by either a biochemical determination (Chemstrip-9) or a colorimetric staining procedure (Bac-T-Screen), and to evaluate the feasibility of omitting from urinalyses attempts to culture urines. Cultures were considered positive when colony counts were \( \geq 10^3 \) for catheterized patients and \( \geq 10^4 \) for noncatheterized patients. The results of three separate studies on symptomatic patients showed a progressive decline in the sensitivity of the Chemstrip-9, which is a test for leukocyte esterase activity, and a difference in the sensitivity of the Bac-T-Screen between two of the studies. Neither test was consistently more sensitive or more predictive of a positive culture than was urine microscopy. By the end of the third study, we were convinced that the three methods are comparably sensitive and specific. Because 13 to 36% of positive cultures would be missed by these techniques, urine from symptomatic patients should routinely be cultured.

Additional Keyphrases: bacteria · urinary tract infection · screening · economics of laboratory operation

The literature regarding the relevance of urine microscopy to the practice of medicine is extensive and often confusing (1–4). This is understandable because of the various collection procedures used and the subjectivity involved in the actual performance of the test. This lack of standardization contributes significantly to differences in interpretation of results.

We compared the "Bac-T-Screen," "Chemstrip-9," and urine microscopy for detecting bacteria in urine, using bacterial identification and colony count as a reference, according to state-of-the-art microbiological techniques. We believed this would help us evaluate sensitivity, specificity, and predictive value in diagnosing urinary tract infection in symptomatic patients, in contrast to studies (5–8) in which (e.g.) the number of leukocytes present was compared with results for leukocyte esterase or nitrite readings with bacteria, without use of any such reference. The ideal reference for all such studies would be the actual presence or absence of clinical urinary tract infection and its response or lack of response to an appropriate antibiotic directed against the infecting microorganism(s).

From various reports (9–13), we concluded that cultures producing no fewer than \( 10^6 \) colony-forming units per milliliter indicate infection. We set different cutoff limits, depending on whether or not the patient was catheterized. We wanted to determine whether any of the three procedures clearly and consistently outperformed the other. We expected that the Chemstrip-9 and Bac-T-Screen approaches to urinalysis would be more reproducible and less labor intensive than subjective evaluations of urine sediment by microscopy.

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Materials and Methods

Procedures

Bac-T-Screen (Model 400; Marion Scientific, Kansas City, MO 64114). This procedure is a colorimetric staining procedure.

Chemstrip analysis. We tested samples for leukocyte esterase activity, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, and hemoglobin with the Chemstrip TM-9 (Boehringer Mannheim, Indianapolis, IN 46250).

Microscopic examination of urinary sediment. After centrifuging 10 mL of the well-mixed urine sample for 10 min at 327 \( \times g \), we discarded the supernate, and resuspended the sediment in the residual urine for microscopic examination. Bacteria were reported as negative, slight, moderate, or heavy; crystals, casts, and epithelial cells (squamous or renal) were reported as negative, occasional, frequent, or many; yeast as negative or present; erythrocytes as negative, few, moderate, or full; and leukocytes as number per high-power field, based on an average of 10 high-power fields examined. The results were read by a series of trained medical technologists.

Routine culture analysis. We plated cultures on TSA plates (BBL, Cockeysville, MD) with sheep blood (50 mL/L) and on McConkey agar plates (BBL), using 10-\( \mu L \), sterile disposable A/S Nunc loops. Results were observed after 24 and 48 h. All organisms observed were considered pathogenic except for yeast, lactobacilli, and mixed bacterial flora (three or more organisms).

Statistical analysis. For calculating the sensitivity, specificity, and predictive values in predicting a positive culture result, we used an ad hoc computer program. Culture results were considered positive with colony counts \( \geq 10^3 \) and \( \geq 10^4 \) for catheterized and noncatheterized patients, respectively.

Patients

In Study A (summer of 1983) we evaluated urine specimens from 240 patients with the Chemstrip-9 and by microscopic examination. In Study B (February 1984), 102 patients were evaluated with the Chemstrip-9 and the Bac-T-Screen. In Study C (summer of 1984) we evaluated 95 patients with the Chemstrip-9, the Bac-T-Screen, and by microscopic examination. All specimens were refrigerated until plated, and the tests were all done at the same time or just before plating. All patients, ages from newborn to 96 years (65% women), were clinically suspected to have urinary tract infection.

Results

The sensitivity of the Chemstrip-9 in detecting leukocyte esterase activity, for the purpose of predicting a positive culture, progressively decreased in Studies A, B, and C from 83% to 55% to 40%, respectively (Table 1). This decline was paralleled by the number of samples that were detected by leukocyte esterase activity, and subsequently cultured for
Various Escherichia coli examined could remain and of 95% count (seven culture) was positive. When we set the cutoff value for predicting a positive culture at three leukocytes per high-power field, the sensitivities were 81% and 80%, respectively, for studies A and C (Table 1). Thus the sensitivity of the microscopic analysis remained fairly constant, in contrast to the decreasing sensitivity of the Chemstrip-9.

The sensitivity of the Chemstrip-9 for nitrite detection was 57% in Study A, 36% in Study B, and 47% in Study C. Various combinations of tests improved the sensitivity of the Chemstrip-9 (Table 1). The sensitivity of moderate or heavy bacteria in urinary sediment was 87% in Studies A and C, in contrast to the Bac-T-Screen, where values of 96% and 80% were observed for Studies B and C, respectively. For Study B, using 4 instead of 5 as a cutoff for the Bac-T-Screen improved both the sensitivity (96% to 100%) and the predictive value (98% to 100%) of a negative result. Urine is passed through a filter on the test card and the residue that remains on the card is stained for microorganisms with a safranin dye and subsequently read in a colorimetric card-reading device.

To assess whether or not any of these procedures could predict which urines did not need to be cultured we examined the predictive values of negative results, PV(-). When the Chemstrip-9 was evaluated for the presence of leukocyte esterase activity, and (or) the presence of nitrite, and (or) the presence of protein at 300 mg/L, the PV(-) was 98% for Study A, 90% for Study B, and 96% for Study C. The Bac-T-Screen yielded a PV(-) of 98% and 95% for Studies B and C with a cutoff value of 5, and 100% and 95% when the cutoff value was 4. For the microscopic test, when the leukocytes exceeded 3 per high-power field or the bacteria count was high or the erythrocyte field was full—or any combination of these conditions—the PV(-) was 97% and 95% for Studies A and C, respectively. The best combination of observations in the microscopy test would have missed as many as 13% of positive cultures, as many as 20% with the Bac-T-Screen results, and as many as 36% with the Chemstrip-9 results (Table 1).

Discussion

Previous work in evaluating Chemstrip as a screening tool for urinary tract infection in asymptomatic, healthy individuals has shown them to be as effective as microscopy (3, 7, 10, 14, 15). Physicians also use the strips in symptomatic individuals to rule out urinary tract infections. We wanted to evaluate the use of Chemstrip-9, Bac-T-Screen, and microscopy of urinary sediment to detect the presence of urinary tract infections in clinically asymptomatic individuals, so that appropriate treatment could be initiated. Because results may vary according to patient population, case mix, culture methodology, etc., the results of this study may not be directly applicable to other groups of subjects in other studies.

In the literature the quantitative definition of significant bacteruria varies anywhere from 10^2 to >10^5 microorganisms per milliliter of urine (8–13). Stark and Maki (10) concluded that fewer than 10^5 colony-forming units per milliliter could be clinically significant although, traditionally, more than 10^6 have been considered to represent infection (2–5). Although Stamm (11) showed that results ≥10^5 for enteric Gram-negative bacilli represent lower urinary tract infection, we used here values of ≥10^3 for catheterized and ≥10^2 for noncatheterized patients as indicative of urinary tract infections. However, recalculating our results with respective cutoffs of 10^2 and 10^3 for the two groups produced similar results.

An unexpected finding was the progressive decrease in sensitivity of the strip for detecting leukocyte esterase activity, paralleled by a loss of sensitivity in detecting Escherichia coli, a Gram-negative pathogen. In addition, however, we also observed a decrease in the percentage of total cultures showing Gram-negative bacteria, which could have been partly responsible for the observed loss in sensitivity. All of our results for leukocyte esterase were obtained by visual determination; perhaps the use of a reading device would minimize subjectivity as a source of variation. The same individuals read the strips in Studies A and C, so the possibility of technologist-to-technologist differences should not have been involved. Further to evaluate the validity of this sensitivity loss, we looked at comparable results for leukocytes in Studies A and C. As mentioned above, the consistent sensitivity of microscopy contrasts with the loss of sensitivity by Chemstrip-9. Increased amounts of protein in urine can be produced false-negative results for leukocyte esterase (16). Although there was some correlation between

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Table 1. Sensitivity and Specificity of Some Urine Test Procedures in Predicting a Positive Urine Culture

<table>
<thead>
<tr>
<th>Test Results</th>
<th>Study A</th>
<th>Study B</th>
<th>Study C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemstrip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte esterase ≥ trace</td>
<td>83</td>
<td>64</td>
<td>55</td>
</tr>
<tr>
<td>Nitrite positive</td>
<td>57</td>
<td>94</td>
<td>36</td>
</tr>
<tr>
<td>Leukocyte esterase ≥ trace and (or) nitrite positive</td>
<td>92</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>Leukocyte esterase ≥ trace and (or) nitrite protein &gt;300 mg/L</td>
<td>94</td>
<td>61</td>
<td>64</td>
</tr>
<tr>
<td>Microscopic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes &gt;3/HPF*</td>
<td>81</td>
<td>64</td>
<td>—</td>
</tr>
<tr>
<td>Bacteria detectable</td>
<td>87</td>
<td>41</td>
<td>—</td>
</tr>
<tr>
<td>Leukocytes &gt;3/HPF* and (or) many bacteria and (or) erythrocytes</td>
<td>92</td>
<td>61</td>
<td>—</td>
</tr>
<tr>
<td>Bac-T-Screen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reading &gt;4</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Reading &gt;5</td>
<td>—</td>
<td>—</td>
<td>96</td>
</tr>
</tbody>
</table>

*HPF: high-power field.
increased protein and false-negative leukocyte esterase activity in the present study, this does not entirely explain the difference. Another possibility for the loss of sensitivity is deterioration of the reagents on the strip, either because of inadequate storage or instability of the chemicals involved in the color reaction. A suitable control for leukocyte esterase activity should help to recognize this problem.

The sensitivity of the nitrite strip for the three studies varied from 36% to 57%, and appears to correlate with that reported (3, 17). In addition, among the individual Chemstrip tests, the nitrite test yielded the highest specificity. However, the Chemstrip-9 vial should be recapped immediately after strips are taken from it, because exposure to air for as little as 15 min will cause a color change equivalent to a false-positive result. We have also noted a false-positive result when we removed the strips for 5 min, put them back in the container for 20 min, took them out again for 5 min, etc., for a total time outside the container of 25–30 min.

The Bac-T-Screen sensitivities of 80 and 96% were comparable with the 87% and 92% values for urine microscopy when counts for leukocytes, erythrocytes, and bacteria were used in combination, and somewhat better than the 64%, 80%, and 94% when the Chemstrip-9 results for leukocyte esterase activity, nitrite, and protein were utilized. Davis et al. (13) reported a somewhat higher sensitivity of the Bac-T-Screen (98%) when compared with colony counts ≥10⁴/ml.

The predictive value of a negative result is important when one considers the benefit of ruling out urinary tract infection two days before culture results can be available. None of the three procedures appeared to offer any clearcut advantage for doing this, however.

We conclude that neither the Chemstrip-9 nor the Bac-T-Screen is consistently superior to microscopy of urine for ruling out urinary tract infection. The false-negative rates are similar and the sensitivities are comparable, except for the inconsistent results with the Chemstrip-9. If these latter problems can be identified and solved, use of the strips could be substituted for microscopy in most cases. Nonetheless, all three methods yield about 5% false-negatives, a figure we do not consider acceptable. Therefore we recommend that urine cultures should be routinely performed on all symptomatic patients, regardless of urinalysis results.

We thank Drs. Robert S. Galen and Sholom Weiss, for their computer support in the computerized analysis of our data; Boehringer Mannheim, for supplying the Chemstripes; and Marion Scientific, for the use of the Bac-T-Screen.

References

Ed. note: cf. the paper (and comment) on pp 448–450 of our March 1985 issue.