Use of the Bac-T-Screen to Predict Bacteriuria from Urine Specimens Held at Room Temperature

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Results from the Bac-T-Screen (BTS) of fresh urine specimens were compared with the BTS results obtained when the same urine specimens had been held at room temperature for 24 h. Of the 246 specimens studied, 43 were initially BTS positive, 11 were false-negative, and 39 had $\geq 10^5$ CFU/ml. After 24 h at room temperature an additional 60 specimens had $\geq 10^5$ CFU/ml, of which only 16 were BTS positive; 10 specimens still gave false-negative results, and the number of false-positive specimens increased by only 6.5% of all specimens. For significant specimens (containing $\geq 10^6$ CFU of probable pathogens per ml), the predictive value of a negative test changed by only 0.1% (99.5 to 99.4%), whereas the sensitivity of the test remained at 96.4% for incubated specimens. Of those specimens that developed $\geq 10^5$ CFU/ml in vitro, 85% contained gram-negative bacilli. Neither bacteria grown in vitro nor urine specimens from normal females containing $\geq 10^6$ CFU/ml were positive with the BTS. For reasons not entirely understood, the BTS system may be unique in its ability to discriminate between bacteria which represent true bacteriuria and those which are present because of contamination, possibly due to other cellular elements present in infection-related bacteriuria, namely leukocytes and sloughed bladder epithelial cells.

Kass (7) defined significant bacteriuria in asymptomatic females as the presence of $\geq 10^5$ CFU of bacteria per ml in clean-catch urine, whereas subsequent studies showed that for different patient populations significant bacteriuria may be associated with a much smaller number of bacteria in the urine (16; W. E. Stamm, Clin. Microbiol. NewsL. 5:15–17, 1983). Efforts to increase cost effectiveness in the laboratory detection of bacteriuria have led to the development and use of several approaches for urine screening (4–6, 9–13, 15). Although differing in methodology, most of the currently used screening approaches depend on the quantitative or semiquantitative detection of the bacteria present in urine specimens (4, 5, 9–12, 14, 15), and basic to most of these systems is the concept of significant bacteriuria suggested by Kass (7), i.e., $\geq 10^5$ CFU/ml. Thus, although many factors such as specimen collection and culture procedures are important to the reliability of the laboratory determination of bacteriuria, without a procedure for distinguishing between contaminating and significant bacterial flora the prevention of in vitro bacterial growth in the specimens is critical. Various methods, such as refrigeration and urine transport kits, have been recommended to prevent changes in the number of the bacterial CFU per ml initially present in clinical urine specimens (1, 3, 8, 17).

The Bac-T-Screen (BTS) instrument (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) is designed to provide a rapid, semiquantitative measurement of the bacteria present in urine specimens. The principle of the screen is that the amount of bacteria from bacteriuria patients containing $\geq 10^5$ CFU of bacteria per ml give a positive BTS result and those containing $< 10^5$ CFU/ml are usually negative (12–14). We noted, however, that bacteria grown in vitro to concentrations $>10^5$ CFU/ml frequently failed to produce a positive result when tested by the BTS. This raises questions about the impact on this system of in vitro bacterial growth occurring between collection and delayed processing due to postponed transport and also raises the possibility that the BTS may distinguish between significant bacterial flora and that which is present because of contamination or poor specimen processing. This paper reports the results of an investigation of these questions.

MATERIALS AND METHODS

Seven species of bacteria (Enterobacter cloacae, enterooccus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus saprophyticus) and one yeast (Candida albicans) were grown overnight at 35°C in fresh filter-sterilized urine, in fresh clean-catch urine, and in brain heart infusion. After growth, the organisms were diluted to concentrations of $10^3$, $10^4$, and $10^5$ CFU/ml in the growth media. The dilutions of each culture were then processed with the BTS (model 400) according to the recommendations of the manufacturer. One BTS instrument and a single lot of reagents were used throughout the study.

Fresh urine specimens submitted to the University of Utah Medical Center Clinical Microbiology Laboratory were examined with the BTS. Immediately after each of the 246 fresh specimens was processed with the BTS, 0.001 ml of each specimen was quantitatively cultured on sheep blood and MacConkey agar plates by a standard procedure (1).

After the urine specimens were screened and cultured, they were held without preservatives at room temperature (RT) for 24 h. They were then processed again with the BTS and cultured as before. After an 18- to 24-h incubation period, the cultures were examined, the number of bacteria per ml of urine was determined based on the number of colonies present on the agar (1), and the organisms were identified.

The data obtained with the BTS, the quantitation, and the organisms identified for each fresh urine specimen were compared with the data obtained for the same specimens after they had been held at RT for 24 h. Statistical analysis
was performed by the procedures recommended by Galen and Gambino (2).

Urine specimens which were positive with the BTS were examined microscopically by a procedure reported by Barry et al. (1). Thirty of these specimens which appeared to contain a microorganism in pure culture at concentrations \(\geq 10^5\) CFU/ml were subcultured overnight in tryptic soy broth. The overnight culture was diluted such that it contained approximately \(5 \times 10^5\) CFU/ml and was processed with the BTS. Cultures which remained positive with the BTS were transferred to fresh tryptic soy broth every 24 h until the BTS results became negative.

An additional group of 34 urine specimens was obtained from normal, healthy asymptomatic women. These urine specimens were not obtained by a clean-catch procedure but were collected in sterile containers which were immediately refrigerated and remained refrigerated until processing. Each of these urine specimens was processed with the BTS and cultured on sheep blood agar within 2 h of collection.

**RESULTS**

Only two of eight microbial isolates grown in vitro (C. albicans and enterococcus) gave a positive BTS result, and they were positive only at a concentration of \(10^7\) CFU/ml (the isolates were also tested at \(10^8\) and \(10^9\) CFU/ml); these data were for organisms grown in fresh urine. Organisms grown in sterile urine or brain heart infusion gave similar results, and none was positive with the BTS at a concentration less than \(10^7\) CFU/ml.

Concern that this lack of BTS sensitivity for bacteria grown in vitro may have been a reflection of the source of the organisms tested and that it may not have been representative of results for microbial strains directly associated with bacteriuria led us to examine the stability of the BTS results as a function of in vitro culture when organisms freshly isolated from urine specimens were tested (Table 1). Of the 30 microbial isolates tested, all became BTS negative when subcultured in vitro. Conversion from a positive BTS result to a negative BTS result usually occurred within three or four subcultures. However, the two C. albicans isolates were not BTS positive after the first subculture, and only one (3.3%) bacterial isolate was positive for as many as nine passes.

Interpretation of the BTS results relies on the Kass definition of bacteriuria (\(\geq 10^5\) CFU/ml); this was applied to the data in this study. Significant bacteriuria was defined by the presence of one but not more than two pathogenic or potentially pathogenic bacteria at concentrations \(\geq 10^5\) CFU/ml in the same specimen. Of the 246 urine specimens screened, 43 were BTS positive, and of these, 39 contained \(\geq 10^5\) CFU of bacteria per ml on the first examination (Table 2).

It is apparent from the data in Table 2 that although there was a 153.8% (24.4% of all specimens) increase in the number of urine specimens that contained \(\geq 10^5\) CFU/ml after incubation at RT for 24 h, the increase in the number of BTS-positive specimens was only 37.2% (6.5% of all specimens). One previously false-negative specimen was BTS positive after 24 h, but the number of false-negative specimens containing a significant pathogen remained at only 2.5% of specimens.

The results of an analysis of the data as a function of all specimens and of only those specimens containing significant bacterial flora are presented in Table 3. Holding urine specimens at RT for 24 h decreased the specificity of the BTS test only slightly, reducing an already poor positive predictive value by 16%, and had little or no effect on the predictive value of a negative test. The sensitivity of the BTS remained essentially unchanged by the incubation of urine specimens at RT.

The distribution of BTS values for fresh urine and urine held at RT for 24 h is shown in Table 4. Most of the BTS readings on fresh urine were either 1+ (73%) or 4+ (20%); after 24 h, there was an almost uniform distribution of BTS results from 1+ through 4+. After incubation at RT, there was a marked change in the distribution of bacteria which gave positive BTS results, as compared with the distribution in fresh urine. This was demonstrated by a change in the percentage of positive BTS specimens containing gram-positive bacteria, mixed flora, or fewer than \(10^3\) CFU/ml; 85% of the urine specimens which were BTS positive after 24 h contained gram-negative flora exclusively (Table 4).

Of the 34 urine specimens collected from normal females, 11 contained fewer than \(10^4\) CFU of bacteria per ml, 9 had between \(10^4\) and \(10^5\) CFU/ml, and the remaining 14 contained \(\geq 10^5\) CFU/ml. All of the cultures were mixed, and most of them contained staphylococci, diptheroïdes, and lactobacilli. Only three contained E. coli. Of the specimens containing \(\geq 10^5\) CFU/ml, one was marginally positive with the BTS; two other specimens, containing \(< 10^5\) CFU/ml,
were also positive. None of the specimens which contained *E. coli* was positive with the BTS, and none were pyuric (>5 leukocytes per high-power field).

**DISCUSSION**

Efforts to discriminate between urine specimens from patients with bacteriuria, and therefore of legitimate clinical concern, and urine specimens which contain only contaminating or associated microorganisms have produced a plethora of urine screening and testing systems (4, 6, 9–11, 15). Recent studies have focused on significant bacteriuria in patients other than asymptomatic females in whom concentrations <10^5 CFU/ml are of significance and are associated with true bacteriuria (16; W. E. Stamm, Clin. Microbiol. Newsl. 5:15–17, 1983). Even though it is well understood that in these patients bacteriuria may be represented by as few as 10^2 microorganisms, the design and sensitivity of many urine screening systems, including the BTS, have persisted at a level consistent with 10^5 CFU/ml.

Our observation that bacteria grown in vitro did not in general give a positive BTS result (see Results and Table 1) at a sensitivity similar to that for bacteria present in specimens collected from patients with evidence of urinary infection prompted our interest in the possibility that such a screening system could be used to discriminate between urine specimens which were properly collected and processed and those which contained insignificant bacteria either because of poor specimen collection procedures or inappropriate delay in laboratory processing of the specimens.

The value of a urine screen that gives excellent predictive negative results and could also be used to screen urine specimens that may have been contaminated during collection or delayed in transport or processing is obvious. A procedure with the ability to discriminate between bacterial populations that do not represent infection and those that represent infection, as well as bladder sampling, now appears to be available with the BTS. The number of urine specimens containing ≥10^4 CFU/ml increased 153.8% when the specimens were allowed to stand at RT for 24 h; however, the corresponding increase in the number of BTS-positive results for the same group of urine specimens was only 37.2% (Table 2).

The accuracy of the BTS as a screen for bacteriuria, as represented by ≥10^3 CFU/ml, does not seem to be seriously affected by the rapidity with which the specimen is processed by the laboratory (Table 3). With a planned, excessive delay in processing, there was only a 6.5% increase in the number of false-positive specimens that would require further laboratory processing and there were no increases in the number of false-negative results. Moreover, although the predictive value of a positive test was considerably decreased, this parameter is not particularly useful, even for fresh urine specimens. As anticipated, most of the specimens that became false-positive after 24 h contained gram-negative bacilli; one of the two specimens which contained gram-positive bacteria, which was BTS positive only after 24 h, was in fact a true-positive that was falsely negative when the fresh urine specimen was examined.

It appears from the data that the BTS can reliably discriminate between infection-related bacteriuria and non-infection-related or contamination-overgrowth bacteriuria, as defined, in urine specimens that may be contaminated during collection or because of excessive delays in handling of up to 24 h. These data, along with the negative BTS results obtained for urine specimens containing ≥10^5 CFU/ml of contaminating bacterial flora per ml from normal females, support the concept that a BTS result is probably a valid indicator of infection-related bacteriuria regardless of contamination which may occur during collection or as a result of growth during specimen handling and processing.

At this time, we can only speculate about the reasons for the reported observations. It is apparent that in vitro cultivation of organisms isolated from significant urine specimens leads to a rapid loss of their capacity to produce a positive BTS result (Table 1). These data suggest that either the positive BTS result is a function of some bacterial characteristic that is not readily reproduced in vitro or that the positive BTS result may be in part a function of the presence of other cellular elements associated with urinary tract infection, such as leukocytes and sloughed bladder epithelial cells. In fact, a positive BTS result may be a reflection of microbial virulence factors, such as adherence phenomena, leukocyte response, and the impact on epithelial cells, that are expressed only in vivo and are rapidly lost on subculture or are not present in vitro. The BTS is able to detect host factors, such as leukocytes, which occur with bacteria and accept the safranin stain. Although these cells may frequently contribute to a positive BTS, as suggested by J. S. Hibbard (personal communication), this does not explain why it may take several in vitro subcultures for bacteria isolated from specimens of patients with bacteriuria to become BTS negative. The fact that this loss of BTS

**TABLE 4. Distribution of false-positive BTS results for fresh urine specimens and specimens held at RT for 24 h**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fresh urine (%)</th>
<th>New positive specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BTS values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>73.0</td>
<td>28.0</td>
</tr>
<tr>
<td>2+</td>
<td>7.0</td>
<td>17.0</td>
</tr>
<tr>
<td>3+</td>
<td>0.0</td>
<td>22.0</td>
</tr>
<tr>
<td>4+</td>
<td>20.0</td>
<td>33.0</td>
</tr>
<tr>
<td><strong>Bacteria present</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram positive</td>
<td>24.4</td>
<td>10</td>
</tr>
<tr>
<td>Gram negative</td>
<td>42.2</td>
<td>85</td>
</tr>
<tr>
<td>Mixed</td>
<td>17.7</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>15.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Positive after 24 h at RT.
* Semi-quantitative value assigned on the basis of results obtained from BTS analysis. 

**TABLE 3. Specificity, sensitivity, and predictive value data obtained from BTS testing of fresh urine specimens and specimens held at RT for 24 h**

<table>
<thead>
<tr>
<th>Test characteristic</th>
<th>All specimens</th>
<th>Specimens with significant flora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh 24 h at RT</td>
<td>Fresh 24 h at RT</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>69.2</td>
<td>71.0</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.2</td>
<td>85.0</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>62.7</td>
<td>46.5</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>94.0</td>
<td>91.4</td>
</tr>
</tbody>
</table>

* Assigns the number of false-negative results based on the type and number of bacterial species present.
sensitivity occurred with every microbial isolate studied supports the idea that the BTS adherence process is related in part to a microbe and not totally to host response functions. Additional studies directed toward providing an understanding of these observations are in progress.

LITERATURE CITED