Evaluation of the Alfred 60/AST Device as a Screening Test for Urinary Tract Infections

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The performance of the Alfred 60/AST device, an automated bacterial culture device which uses laser nephelometry to detect and quantify bacterial growth, was evaluated. The instrument is effective at screening negative samples and is more reliable at detecting bacteria than yeasts. Microscopy can be used to reduce the false-negative numbers.

Urinary tract infections (UTIs) are one of the most common infections diagnosed in community and hospital settings (1, 2). It is therefore not surprising that urine samples constitute the largest proportion of specimens tested in microbiology laboratories (2, 3).

Culture remains the current gold standard for diagnosis of UTI but has limitations. It is time- and labor-intensive. Considering that 70 to 80% of urine samples are proven negative for UTI (2, 4, 5, 6, 7), a rapid screening method could reduce costs and turnaround times. Alternative methods based on chemical and flow cytometry have had mixed results (2, 4, 8). This study was designed to evaluate the utility of a fully automated bacterial culture device (Alfred 60/AST) which utilizes laser nephelometry to detect and quantify growth (CFU/ml) (9). Over a 4-week period, a total of 508 urine samples were randomly selected provided that the volume was >3 ml and that samples did not display extreme turbidity or macroscopic hematuria. Urine samples represented both midstream (MSU) and indwelling catheter specimen urine (CSU) samples from inpatients and outpatients of a tertiary care hospital. All samples were collected in sterile containers and examined within 4 h of receipt with no sample being left at room temperature for >2 h. All selected samples underwent testing using the Alfred 60/AST (Alifax, Padua, Italy) bacterial culture analyzer in parallel with our routine culture method. Prior to testing, all samples underwent phase-contrast microscopy using Vetriplast slides (Thermo Fisher Scientific, Australia).

Alfred 60/AST device. Samples were processed per the manufacturer's instructions using software version 1.05. In brief, 3 ml of urine was aliquoted into a sterile plastic specimen tube and placed in the primary tube sample rack. The instrument automatically inoculates 500 μl of each urine sample into the dedicated vials containing 2 ml of eugonic culture broth and incubates the sample at 37°C for predefined times which correspond to the desired detection threshold. For this study, an incubation period of 240 min was selected for a detection threshold of 800 CFU/ml, although the device can detect a positive result after 45 min of incubation if the bacterial concentration is sufficiently high (9).

Urine microscopy. Mixed unspun urine samples were loaded into Vetriplast slides and examined using a phase-contrast microscope (Carl Zeiss, Germany), which allows high-contrast imaging of unstained material, to establish samples containing any bacteria and/or yeasts and quantitate the presence of leukocytes and epithelial cells.

Culture. All urine samples were inoculated onto horse blood agar/chromogenic UTI split plates (Thermo Fisher Scientific, Australia) using a 1-μl calibrated loop. Plates were examined for significant growth after 18 to 24 h of incubation at 35 to 37°C.

A culture result was considered to be consistent with a UTI if (i) any pure or predominant uropathogen growth (i.e., growth 10-fold greater than other organisms present) was isolated for indwelling catheter specimens, (ii) pure or predominant uropathogen growth at ≥10⁵ CFU/ml for midstream urine specimens was isolated, or (iii) mixed culture growth containing two uropathogens with individual counts of ≥10⁵ CFU/ml was found.

A culture result with (i) no growth; (ii) insufficient CFU/ml; (iii) isolation of nonpathogenic bacteria, such as Lactobacillus species, diphtheroids, coagulase-negative staphylococci (except Staphylococcus saprophyticus), and viridans streptococci; or (iv) mixed growth containing more than 2 types of organisms was considered negative for a UTI.

The Alfred 60/AST device was assessed by comparing the results to a culture gold standard and calculating the sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV). Data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Pearson’s χ² analysis and Fisher's exact test were used to compare proportions. Statistical significance was considered when P was <0.05. Ethics approval was not required for this study.

The 508 urine samples were composed of 76.1% MSU and 23.9% CSU. Our study showed that 80 (15.7%) urine samples were positive for UTI, consistent with previous studies (2, 4, 5, 6, 7). The positive organisms identified correspond well with other reports (3, 5, 10, 11), containing Escherichia coli (26 samples), Klebsiella pneumoniae (2), other Enterobacteriaceae spp. (5), Pseudomonas aeruginosa (7), Candida spp. (15), Enterococcus faecalis or Enterococcus faecium (12), Streptococcus agalactiae (3), Staphylococcus saprophyticus (3), Staphylococcus aureus (1), and mixed organisms (6). The Alfred 60/AST device failed to detect 9 of these 80 isolates. These comprised Candida spp. (5 samples), P. aeruginosa (2), E. faecalis (1), and P. aeruginosa plus E. faecalis (1). On review of the request forms, all 9 patients were reported to have...
TABLE 1 Performance of the Alfred 60/AST device at various detection thresholds

<table>
<thead>
<tr>
<th>Alfred 60 device cutoff (CFU/ml)</th>
<th>n (%)</th>
<th>%</th>
<th>Reduction in culturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>71 (14.0)</td>
<td>9 (1.8)</td>
<td>88.8</td>
</tr>
<tr>
<td>1,000</td>
<td>66 (13.0)</td>
<td>11 (2.2)</td>
<td>86.3</td>
</tr>
<tr>
<td>2,000</td>
<td>60 (11.8)</td>
<td>11 (2.2)</td>
<td>86.3</td>
</tr>
<tr>
<td>5,000</td>
<td>55 (10.8)</td>
<td>12 (2.4)</td>
<td>85.0</td>
</tr>
<tr>
<td>15,000</td>
<td>37 (7.3)</td>
<td>16 (3.1)</td>
<td>80.0</td>
</tr>
<tr>
<td>30,000</td>
<td>32 (6.3)</td>
<td>23 (4.5)</td>
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</tr>
<tr>
<td>100,000</td>
<td>23 (4.5)</td>
<td>27 (5.3)</td>
<td>66.3</td>
</tr>
</tbody>
</table>

a Abbreviations: FP, false positive; FN, false negative; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

a Total number of samples was 508.

Microscopy performed inadequately as a stand-alone diagnostic test (a positive was defined as the presence of bacteria and/or yeast or ≥10 leukocytes/µl) (SN, 83.8%; SP, 57.0%; PPV, 26.7%; and NPV, 94.9%) but has the advantage of being inexpensive and rapid. Similar to other studies (13), using microscopy as an adjunct test for negative Alfred 60/AST samples increased the sensitivity by correctly identifying 8 of the 9 false negatives as positive (one pure growth of yeast at 10^4 to 10^5 CFU/ml was missed) (Table 2).

The Alfred 60/AST device is described as an instrument that detects live bacteria (9). However, in our study 18.8% of all positive cultures were the result of yeast infections. Of further interest, a microbial performed inadequately as a stand-alone diagnostic test (a positive was defined as the presence of bacteria and/or yeast or ≥10 leukocytes/µl) (SN, 83.8%; SP, 57.0%; PPV, 26.7%; and NPV, 94.9%) but has the advantage of being inexpensive and rapid. Similar to other studies (13), using microscopy as an adjunct test for negative Alfred 60/AST samples increased the sensitivity by correctly identifying 8 of the 9 false negatives as positive (one pure growth of yeast at 10^4 to 10^5 CFU/ml was missed) (Table 2). Microscopy performed inadequately as a stand-alone diagnostic test (a positive was defined as the presence of bacteria and/or yeast or ≥10 leukocytes/µl) (SN, 83.8%; SP, 57.0%; PPV, 26.7%; and NPV, 94.9%) but has the advantage of being inexpensive and rapid. Similar to other studies (13), using microscopy as an adjunct test for negative Alfred 60/AST samples increased the sensitivity by correctly identifying 8 of the 9 false negatives as positive (one pure growth of yeast at 10^4 to 10^5 CFU/ml was missed) (Table 2). Although culturing the additional 141 microscopy-positive samples would increase overall need for culture by 27.8%, it would still result in an overall net reduction. However, considering the potential morbidity resulting from missing positive results, the use of microscopy is justified. This reduction in workload is offset, however, by a delay of up to 4 h to the final result. Cost estimates are represented in Table 3, with the Alfred 60/AST device showing cost savings even when used in adjunct with phase-contrast microscopy.

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yeasts were overrepresented in the false-negative group, indicating that the Alfred 60/AST device is less dependable for yeast detection. This has been reported previously with nephelometry and was attributed to the low CFU associated with yeast-induced UTIs (11). All 5 of the false-negative yeast samples identified in our study were from symptomatic patients, with 2 containing <10^4 CFU/ml (CSUs) and 3 containing 10^4 to 10^5 CFU/ml. Low sensitivity to yeasts may be attributed to low growth rates rather than low counts.

In summary, the Alfred 60/AST device is more accurate at screening negative than positive UTI samples. Combined with microscopy, false-negative results were minimized while still reducing culture workload by 44.3%. The low sensitivity to yeasts requires further investigation.

ACKNOWLEDGMENTS
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REFERENCES