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

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The performance of the Alfred 60/AST, 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 labour intensive. Considering that 70-80% 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 utilises 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 >3mls and samples did not display extreme turbidity or macroscopic haematuria. Urines 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 hours of receipt with no sample left at room temperature for >2hrs. All selected samples underwent testing using the Alfred 60/AST (Alifax, Padova, Italy) bacterial culture
analysers 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.** Samples were processed as 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 2ml 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 minutes was selected for a detection threshold of 800 CFU/ml, although the device can detect a positive result after 45 minutes of incubation if the bacterial concentration is sufficiently high (9).

**Urine microscopy.** Mixed unspun urine samples were loaded into Vetriplast slides (Thermo Fisher Scientific, Australia) 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 18-24 hrs incubation at 35-37°C.

A culture result was considered to be consistent with a UTI if (i) any pure or predominant uropathogen growth (i.e., growth10-fold greater than other organisms present) were isolated for indwelling catheter specimens; (ii) pure
or predominant uropathogen growth at $\geq 10^4$ CFU/ml for midstream urine specimens was isolated; (iii) mixed culture growth containing two uropathogens with individual counts $\geq 10^5$ CFU/ml were found.

A culture result with (i) no growth or ii) insufficient CFU/ml or iii) isolation of non-pathogenic 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 were considered negative for a UTI.

The Alfred 60/AST was assessed by comparing the results to a culture gold standard and calculating the sensitivity (SN), specificity (SP), positive (PPV) and negative predictive values (NPV). Data was analysed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Pearson’s $\chi^2$ analysis and Fisher’s exact test were used to compare proportions. Statistical significance was considered when P < 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 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), *Klebsiella pneumoniae* (2), other *Enterobacteriaceae* spp. (5), *Pseudomonas aeruginosa* (7), *Candida* spp. (15), *Enterococcus faecalis* or *faecium* (12), *Streptococcus agalactiae* (3), *S. saprophyticus* (3), *S. aureus* (1) and mixed organisms (6). The Alfred 60/AST failed to detect 9 of these 80 isolates. These comprised *Candida* spp. (5), *P. aeruginosa* (2) *E. faecalis* (1), *P. aeruginosa* + *E. faecalis* (1). On review of the request forms all 9 patients were reported to have symptoms consistent with infection and thus these
episodes represented true false negatives. A larger proportion of yeasts were present in the false negative group compared to the true-positive group (55.6% vs. 14.1%, P < 0.01). At the default threshold of 800 CFU/ml the evaluation revealed SN 88.8%, SP 83.4%, PPV 50.0% and NPV 97.5%. Performance characteristics did not markedly improve with increasing instrument detection thresholds (Table 1).

The 428 samples negative for UTI consisted of urine specimens revealing no growth or growth inconsistent with UTI. Of the 226 samples exhibiting no growth, the Alfred 60/AST reported one as positive at 1.5 x 10⁴ CFU/ml of growth. Microscopy revealed only epithelial cells. The sample remained negative on repeat culture. Of the 202 samples displaying growth inconsistent with UTI, 69, 121 and 12 samples had growth characteristics ≤10⁴, between 10⁴-10⁵ and ≥10⁵CFU/ml respectively, with only 5 samples isolating > 2 types of uropathogens. Of these 202 samples, the Alfred 60/AST incorrectly identified 70 as positive of which 77% were MSUs. The Alfred 60/AST identified a larger proportion of positives from the UTI group than from the insignificant growth group (88.8% vs. 34.7%, P < 0.001) which confirms the instrument can to some degree differentiate between uropathogens and contaminants.

The utility of the Alfred 60/AST automated bacterial culturing device is its ability to accurately detect negative urine samples, with a NPV of 97.5% at a threshold of 800 CFU/ml. This would result in a 72.0% reduction in the need for culturing samples. The Alfred 60/AST is less reliable at identifying positive UTI samples. These results are similar to a previous study (PPV of approximately 47%) using the Uro-Quick (currently marketed as HB&L, Alifax,
Padova, Italy), an earlier semi-automatic device employing similar technology as the Alfred60/AST (12). The Alfred 60/AST relies on growth dynamics for detection, presumably making the device more sensitive at detecting uropathogens, since contaminants may initially require a period of adjustment in the urine (12). In our study, 34.7% of insignificant growth was falsely identified as positive. Nirkhiwale et al. reported that when specimens were processed promptly (within 30 minutes of collection) the false positive rate was reduced and the PPV increased from 47% to 96% (12). Prolonged processing times (up to 4 hours) in our study may have contributed to the inflated false positive rate.

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 others (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 10^4-10^5 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 4hrs to the final result. Cost estimates are represented in Table 3, with the Alfred 60/AST showing cost savings even when used in adjunct with phase contrast microscopy.
The Alfred 60/AST 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, yeasts were overrepresented in the false negative group, indicating that the Alfred 60/AST is less dependable for yeast detection. This has been reported previously with nephelometry and was attributed to the low colony forming units 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 contained 10^4-10^5 CFU/ml. Low sensitivity to yeasts may be attributed to slow growth rates rather than low counts.

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

We would like to acknowledge Alifax and Immuno for providing the instrument and the reagents used in the evaluation.
REFERENCES


## TABLE 1. Performance of the Alfred 60/AST at various detection thresholds

<table>
<thead>
<tr>
<th>Alfred 60 cut-off (CFU/ml)</th>
<th>FP(^a) n (%)</th>
<th>FN(^b) n (%)</th>
<th>SN(^c) %</th>
<th>SP(^d) %</th>
<th>PPV(^e) %</th>
<th>NPV(^f) %</th>
<th>Reduction in culturing</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td>800</td>
<td>71 (14.0)</td>
<td>9 (1.8)</td>
<td>88.8</td>
<td>83.4</td>
<td>50.0</td>
<td>97.5</td>
</tr>
<tr>
<td>(n=508)</td>
<td>1,000</td>
<td>66 (13.0)</td>
<td>11 (2.2)</td>
<td>86.3</td>
<td>84.6</td>
<td>51.1</td>
<td>97.1</td>
</tr>
<tr>
<td>2,000</td>
<td>60 (11.8)</td>
<td>11 (2.2)</td>
<td>86.3</td>
<td>86.0</td>
<td>53.5</td>
<td>97.1</td>
<td>74.6</td>
</tr>
<tr>
<td>5,000</td>
<td>55 (10.8)</td>
<td>12 (2.4)</td>
<td>85.0</td>
<td>87.1</td>
<td>55.3</td>
<td>96.9</td>
<td>75.8</td>
</tr>
<tr>
<td>15,000</td>
<td>37 (7.3)</td>
<td>16 (3.1)</td>
<td>80.0</td>
<td>91.4</td>
<td>63.4</td>
<td>96.1</td>
<td>80.1</td>
</tr>
<tr>
<td>30,000</td>
<td>32 (6.3)</td>
<td>23 (4.5)</td>
<td>71.3</td>
<td>92.5</td>
<td>64.0</td>
<td>94.5</td>
<td>82.5</td>
</tr>
<tr>
<td>100,000</td>
<td>23 (4.5)</td>
<td>27 (5.3)</td>
<td>66.3</td>
<td>94.6</td>
<td>69.7</td>
<td>93.8</td>
<td>85.0</td>
</tr>
</tbody>
</table>

\(^{a}\) FP, false positive.

\(^{b}\) FN, false negative.

\(^{c}\) SN, sensitivity.

\(^{d}\) SP, specificity.

\(^{e}\) PPV, positive predictive value.

\(^{f}\) NPV, negative predictive value.
TABLE 2. Distribution of urine samples using a workflow algorithm combining the Alfred 60/AST, direct microscopy and culture method

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of urine samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfred60</td>
<td>Positive: 142, Negative: 366</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Positive: 141, Not required: 225</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive: 71, Negative: 71, Positive*: 8, Negative: 133, Positive: 1, Negative: 224</td>
</tr>
</tbody>
</table>

*False negative Alfred 60/AST results correctly identified as positive by microscopy.

*Represents 1 undiscovered positive culture result.
Table 3. Cost estimates for Alfred 60/AST compared to current testing algorithms for 508 specimens based on observed testing characteristics

<table>
<thead>
<tr>
<th>Test cost</th>
<th>Microscopy</th>
<th>Culture</th>
<th>Alfred60</th>
<th>Total cost*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Algorithm</td>
<td>1001</td>
<td>1017</td>
<td>-</td>
<td>2017</td>
</tr>
<tr>
<td>Culture of Alfred 60/AST positive</td>
<td>-</td>
<td>284</td>
<td>1270</td>
<td>1554</td>
</tr>
<tr>
<td>Culture of Alfred 60/AST positives &amp; selected Alfred 60/AST negatives*</td>
<td>277</td>
<td>300</td>
<td>1270</td>
<td>1847</td>
</tr>
</tbody>
</table>

*Total cost represents the sum of labour (AU$30/hour) and consumables calculated in Australian dollars. Samples positive for both methods require identification and susceptibility costs which have been excluded. Similarly, upfront acquisitions of the Alfred60/AST and phase contrast microscope have been excluded.

* Alfred 60/AST negatives are cultured when microscopy results are positive.