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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 $\geq 10^4$ CFU/ml for midstream urine specimens was isolated, or (iii) mixed culture growth containing two uropathogens with individual counts of $\geq 10^5$ 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 χ^2 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

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TABLE 1 Performance of the Alfred 60/AST device at various detection thresholds[a]

Alfred 60 device cutoff (CFU/ml)	*n* (%)[b]		%				
	FP	FN	SN	SP	PPV	NPV	Reduction in culturing
800	71 (14.0)	9 (1.8)	88.8	83.4	50.0	97.5	72.0
1,000	66 (13.0)	11 (2.2)	86.3	84.6	51.1	97.1	73.4
2,000	60 (11.8)	11 (2.2)	86.3	86.0	53.5	97.1	74.6
5,000	55 (10.8)	12 (2.4)	85.0	87.1	55.3	96.9	75.8
15,000	37 (7.3)	16 (3.1)	80.0	91.4	63.4	96.1	80.1
30,000	32 (6.3)	23 (4.5)	71.3	92.5	64.0	94.5	82.5
100,000	23 (4.5)	27 (5.3)	66.3	94.6	69.7	93.8	85.0

[a] Abbreviations: FP, false positive; FN, false negative; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.
[b] Total number of samples was 508.

symptoms consistent with infection, and thus, these episodes represented true false negatives. A larger proportion of yeasts were present in the false-negative group than in the true-positive group (55.6% versus 14.1%, $P < 0.01$). At the default threshold of 800 CFU/ml, the evaluation revealed SN of 88.8%, SP of 83.4%, PPV of 50.0%, and NPV of 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 device reported one as positive at 1.5×10^4 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 of $\leq 10^4$, between 10^4 and 10^5, and $\geq 10^5$ CFU/ml, respectively, with only 5 samples isolating >2 types of uropathogens. Of these 202 samples, the Alfred 60/AST device incorrectly identified 70 as positive, of which 77% were MSU specimens. The Alfred 60/AST device identified a larger proportion of positives from the UTI group than from the insignificant growth group (88.8% versus 34.7%, $P < 0.001$), which confirms that 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 an 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 device 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, Padua, Italy), an earlier semiautomatic device employing technology similar to that in the Alfred60/AST device (12).

The Alfred 60/AST device 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 min of collection), the false-positive rate was reduced and the PPV increased from 47% to 96% (12). Prolonged processing times (up to 4 h) in our study may have contributed to the inflated false-positive rate.

TABLE 2 Distribution of urine samples using a workflow algorithm combining the Alfred 60/AST device, direct microscopy, and culture method

Test result	No. of urine samples (total, 508)
Alfred 60 positive	142
Microscopy positive	—[c]
Culture positive	71
Culture negative	71
Alfred 60 negative	366
Microscopy positive	141
Culture positive	8[a]
Culture negative	133
Microscopy negative	225
Culture positive	1[b]
Culture negative	224

[a] False-negative Alfred 60/AST results correctly identified as positive by microscopy.
[b] Represents 1 undiscovered positive culture result.
[c] —, confirmation not required.

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.

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,

TABLE 3 Cost estimates for Alfred 60/AST device compared to current testing algorithms for 508 specimens based on observed testing characteristics

Test cost	Cost in AU$			
	Microscopy	Culture	Alfred 60 device	Total[a]
Current algorithm	1,001	1,017		2,017
Culture of Alfred 60/AST-positive specimens only		284	1,270	1,554
Culture of Alfred 60/AST positives and selected Alfred 60/AST negatives[b]	277	300	1,270	1,847

[a] Total cost represents the sum of labor (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 Alfred 60/AST device and phase-contrast microscope have been excluded.
[b] Alfred 60/AST negatives are cultured when microscopy results are positive.

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.

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