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Rapid Screening of Urine Specimens for Bacteriuria by the Cellenium System

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This study evaluated the performance of the Cellenium 160US urine screening system in comparison to that of the semiquantitative culture method. The performance characteristics of the Cellenium system for all clinically significant uropathogens were 89.5% sensitivity, 94.4% specificity, 97.1% negative predictive value, and 81% positive predictive value.

Urine specimens comprise the largest volume of specimens received for culture in the clinical microbiology laboratory. In the Clinical Microbiology Service of the Columbia University Medical Center, 70% to 80% of urine cultures yield either no growth or clinically insignificant commensal organisms, which is consistent with the findings published in the literature (4, 8). Semiquantitative plate culture is the accepted standard for the laboratory diagnosis of urinary tract infections (UTIs) but does not provide same-day results. This underscores the need for an accurate, automated screening method to eliminate the culture of negative urine samples and to direct efforts into the processing of only true-positive samples.

This study evaluates the Cellenium 160US system (TREK Diagnostic Systems, Cleveland, Ohio), a urine screening system that applies selective molecular fluorescence staining to robot-based computerized microscopic video image analysis to obtain rapid results within 1 to 2 h. The threshold for detection of microorganisms is $\geq 10^4$ organisms/ml urine, and it predicts the Gram reaction and morphology. We evaluated the performance of the Cellenium system in comparison to that of semiquantitative plate culture for the detection of clinically significant bacteriuria.

A prospective study of 1,199 randomly collected urine specimens (voided and catheterized from adult patients) received by the microbiology laboratory of Columbia University Medical Center, was conducted. The specimens were collected in a transport device (BD Vacutainer, Franklin Lakes, NJ) and processed within 1 h of receipt. The urine specimens were inoculated with a 1-μl calibrated loop onto Columbia agar-based agar (5% sheep blood) and MacConkey agar biplates (Becton Dickinson Microbiology Systems, Sparks, MD). The plates were incubated (24 h, 35°C) and examined for significant bacteriuria ($\geq 10^4$ CFU/ml of one or two potentially pathogenic microorganisms). When three or more colony morphologies were detected, mixed commensal flora (MCF), defined as viridans group streptococci, coagulase-negative staphylococci other than *Staphylococcus saprophyticus*, lactobacilli, coryne-bacteria, and *Gardnerella vaginalis*, was reported. Bacterial identification was performed by using the MicroScan system (Dade Behring Inc., Deerfield, IL).

Urine specimens were placed into the Cellenium system in batches of 24, according to the manufacturer's instructions. Using robotics, the instrument dispensed aliquots of the samples into a cassette and mixed them with highly specific fluorescent probes that bind to bacteria, yeast, and other cells. The stained cells were collected through a filtering membrane to produce a monolayer which was illuminated with light of selected wavelengths to excite the fluorescent dyes. The computerized image analysis system employed homogeneous fluorescent beads (1-μm latex spheres) as calibration particles and, using proprietary bacterial recognition algorithms, processed the digital image. A threshold colony count of $\geq 10^4$ organisms/ml urine was reported as a positive result by the instrument within 1 to 2 h.

When the culture and Cellenium system data were discordant, both analysis methods were repeated within 24 h by using the original urine specimens, which had been stored at 5°C. In addition, patient charts were reviewed for urine microscopy and urinalysis results.

Of the 1,199 urine specimens studied, 479 (39.9%) were culture positive and 720 (60.1%) were culture negative. A single bacterial pathogen was recovered from 172 (14.3%) specimens, two pathogens were recovered from 18 (1.5%) specimens, and 289 (24%) specimens had mixed pathogens or grew nonpathogens. Of the culture-positive specimens, 154 (32.2%) were determined to be clinically significant (Table 1).

Overall, 436 (36.4%) urine specimens were positive and 763 (63.6%) were negative with the Cellenium system. Correlations demonstrated that 328 were both culture and Cellenium system positive, 612 were culture and Cellenium system negative, 151 were culture positive and Cellenium system negative, and 108 were culture negative and Cellenium system positive. Of the 151 culture-positive, Cellenium system-negative specimens, 133 (88%) grew mixed or commensal flora. Of the 108 urine specimens that were culture negative and Cellenium system positive, 55 (51%) were Cellenium system negative by repeat testing. The uropathogens missed by the Cellenium system totaled 18 and included 6 group B streptococci, 8 *Enterococcus faecalis* isolates, 1 *Escherichia coli* isolate, 1 *Kleb-*

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TABLE 1. Clinically significant urine culture results

Bacterial group and bacterium	No. of isolates
Gram-negative rods	
E. coli	84
K. pneumoniae	11
P. mirabilis	10
P. aeruginosa	6
Other GNRs	17
Subtotal	128
Gram-positive cocci	
Group B streptococci	18
E. faecalis	17
E. faecium	7
Other GPC (*S. saprophyticus, S. aureus*)	2
Subtotal	44
Total	172

siella pneumoniae isolate, 1 *Klebsiella oxytoca* isolate, and 1 *Proteus mirabilis* isolate. Of a total of 128 gram-negative uropathogens (Table 1), 4 were not detected by the Cellenium system, yielding a 0.6% false-negative rate. Chart review showed that three of the four specimens were positive by urine microscopy or urinalysis. Upon repeat Cellenium system screening, two of four were positive, indicating possible sampling error.

Among 44 clinically significant gram-positive bacteria, 14 (8 *E. faecalis* isolates and 6 group B streptococci) were not detected by the Cellenium system, resulting in a false-negative rate of 2.2% for gram-positive cocci (GPC). Chart review showed that 11 of 14 specimens were positive by urine microscopy or urinalysis. To further investigate the analytical sensitivity for GPC, pooled filtered urine specimens were seeded with serial 10-fold dilutions at 10^3 to 10^6 CFU/ml each of *E. faecalis* and group B streptococci. Cellenium system screens and cultures were performed. *E. faecalis* and group B streptococci were detected only at $\geq 10^5$ CFU/ml, confirming the limitation of the Cellenium system for the detection of these gram-positive bacteria at 10^4 CFU/ml. Upon a repeat of the Cellenium system screen, 5 of the 14 samples with false-negative results tested positive, suggesting sampling error.

The Gram and morphology classifications (as defined by the Cellenium system) of the 53 culture-negative and Cellenium system-positive urine specimens demonstrated the following: cells ($n = 13$); gram-negative rods (GNRs; $n = 16$); GPC ($n = 7$); GNRs and gram-positive rods (GPRs; $n = 4$); GPC and GPRs ($n = 3$); GNRs and yeasts ($n = 3$); GNRs and GPC ($n = 3$); GPC, GNRs, and GPRs ($n = 2$); GPRs and yeasts ($n = 1$); and GPRs, yeasts, and GNRs ($n = 1$). Urinalysis and urine microscopy results were consistent with infection for 36 (68%) specimens reported as "false positive" by the Cellenium system. These specimens may have contained nonviable organisms due to prior antimicrobial therapy, anaerobes, fastidious organisms (e.g., *Chlamydia* or *Ureaplasma*), or preservative-sensitive species. False-negative culture results may also occur if the urine specimen is not mixed thoroughly prior to plating.

By using culture as the reference, the overall sensitivity (SN), specificity (SP), negative predictive value (NPV), and positive predictive value (PPV) for Cellenium system results were 89.5%, 94.4%, 97.1%, and 81.0%, respectively (Table 2). The Cellenium system detected gram-negative rods at 96.9% SN, 96.4% SP, 99.4% NPV, and 84.4% PPV (Table 3) and gram-positive cocci at 68.1% SN, 97.9% SP, 97.8% NPV, and 69.8% PPV (Table 4).

Upon repeat testing, 49% of the specimens with false-positive results converted to negative and 36% of the specimens with false-negative results became positive. The reason for this variability is unclear since several features of the instrument are configured to avoid this problem, i.e., the Cellenium system vibratory urine mixing mechanism, robotic pipetting with new disposable tips for each specimen to minimize cross-contamination, and multiple membrane filter scans to compensate for variations in light intensity and the nonhomogeneous distribution of bacteria. Incorporation of a more rigorous mechanism for mixing of the specimens may yield more consistent data.

In summary, the results of this study indicate that the Cellenium urine screen offers many features favorable for incorporation into a routine clinical microbiology laboratory, i.e., its high-throughput capacity, its ability to distinguish MCF, and its high negative predictive value. Overall, the Cellenium system offers an NPV of 97.1% and is comparable to the previously

TABLE 2. Routine culture versus Cellenium system results (all clinically significant organisms)

Cellenium system result	No. of specimens with the following culture result[a]:	
	Positive	Negative
Positive	154 (TP)	36 (FP)
Negative	18 (FN)	612 (TN)

[a] TP, true positive; FN, false negative; TN, true negative; FP, false positive. Sensitivity = TP/(TP + FN) = 154/(154 + 18) = 89.5%; specificity = TN/(TN + FP) = 612/(612 + 36) = 94.4%; PPV = TP/(TP + FP) = 154/(154 + 36) = 81%; NPV = TN/(TN + FN) = 612/(612 + 18) = 97.1%.

TABLE 3. Routine culture versus Cellenium system results (gram-negative organisms)

Cellenium system result	No. of specimens with the following culture result[a]:	
	Positive	Negative
Positive	124 (TP)	23 (FP)
Negative	4 (FN)	612 (TN)

[a] TP, true positive; FN, false negative; TN, true negative; FP, false positive. Sensitivity = TP/(TP + FN) = 124/(124 + 4) = 96.9%; specificity = TN/(TN + FP) = 612/(612 + 23) = 96.4%; PPV = TP/(TP + FP) = 124/(124 + 23) = 84.4%; NPV = TN/(TN + FN) = 612/(612 + 4) = 99.4%.

TABLE 4. Routine culture versus Cellenium system results (gram-positive organisms)

Cellenium system result	No. of specimens with the following culture result[a]:	
	Positive	Negative
Positive	30 (TP)	13 (FP)
Negative	14 (FN)	612 (TN)

[a] TP, true positive; FN, false negative; TN, true negative; FP, false positive. Sensitivity = TP/(TP + FN) = 30/(30 + 14) = 30/44 = 68.1%; specificity = TN/(TN + FP) = 612/(612 + 13) = 612/625 = 97.9%; PPV = TP/(TP + FP) = 30/(30 + 13) = 30/43 = 69.8%; NPV = TN/(TN + FN) = 612/(612 + 14) = 612/626 = 97.8%.

described Sysmex UF-100 analyzer (NPV, 95%) (7) and the Coral UTI screen system (NPV, 95.9%) (8). Although the sensitivity of detection of gram-negative uropathogens was reliable, the Cellenium system failed to detect some clinically significant gram-positive uropathogens. The setting of a lower threshold for detection at 10^3 CFU/ml may increase the sensitivity of detection for GPC. Additionally, detection of low-count bacteriuria (10^2 to 10^4 CFU/ml) has been reported to be of clinical significance in sexually active women (2, 3).

While automated systems aim at improving uropathogen detection (1, 5) and incorporating algorithms that allow the reporting of results within 1 to 2 h following receipt of the specimen in the laboratory and to minimize the unnecessary use of antibiotics, these cannot be universally recommended. The Cellenium 160US system has recently been voluntarily withdrawn from the market by the manufacturer. To increase marketing success, the system should have a smaller footprint and have the capacity to distinguish between viable and nonviable bacteria to avoid false-positive results, among the other caveats discussed here. Until a reliable system becomes available, culture still remains a "gold standard" for confirmation of a urinary tract infection.

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