Detection of Bacteriuria and Pyuria by URISCREEN, a Rapid Enzymatic Screening Test

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A multicenter study was performed to evaluate the ability of the URISCREEN (Analytab Products, Plainview, N.Y.), a 2-min catalase tube test, to detect bacteriuria and pyuria. This test was compared with the Chemstrip LN (BioDynamics, Division of Boehringer Mannheim Diagnostics, Indianapolis, Ind.), a 2-min enzyme dipstick test; a semiquantitative plate culture method was used as the reference test for bacteriuria, and the Gram stain or a quantitative chamber count method was used as the reference test for pyuria. Each test was evaluated for its ability to detect probable pathogens at $\geq 10^2$ CFU/ml and/or ≥ 1 leukocyte per oil immersion field, as determined by the Gram stain method, or >10 leukocytes per μl, as determined by the quantitative count method. A total of 1,500 urine specimens were included in this evaluation. There were 298 specimens with $\geq 10^2$ CFU/ml and 451 specimens with pyuria. Of the 298 specimens with probable pathogens isolated at various colony counts, 219 specimens had colony counts of $\geq 10^5$ CFU/ml, 51 specimens had between 10^4 and 10^5 CFU/ml, and 28 specimens had between 10^2 and $<10^4$ CFU/ml. Both the URISCREEN and the Chemstrip LN detected 93% (204 of 219) of the specimens with probable pathogens at $\geq 10^5$ CFU/ml. For the specimens with probable pathogens at $\geq 10^2$ CFU/ml, the sensitivities of the URISCREEN and the Chemstrip LN were 86% (256 of 298) and 81% (241 of 298), respectively. Of the 451 specimens with pyuria, the URISCREEN detected 88% (398 of 451) and the Chemstrip LN detected 78% (350 of 451). There were 204 specimens with both $\geq 10^2$ CFU/ml and pyuria; the sensitivities of both methods were 95% (193 of 204) for these specimens. Overall, there were 545 specimens with probable pathogens at $\geq 10^2$ CFU/ml and/or pyuria. The URISCREEN detected 85% (461 of 545), and the Chemstrip LN detected 73% (398 of 545). A majority (76%) of the false-negative results obtained with either method were for specimens without leukocytes in the urine. There were 955 specimens with no probable pathogens or leukocytes. Of these, 28% (270 of 955) were found positive by the URISCREEN and 13% (122 of 955) were found positive by the Chemstrip LN. A majority of the false-positive results were probably due, in part, to the detection of enzymes present in both bacterial and somatic cells by each of the test systems. Overall, the URISCREEN is a rapid, manual, easy-to-perform enzymatic test that yields findings similar to those yielded by the Chemstrip LN for specimens with both $\geq 10^2$ CFU/ml and pyuria or for specimens with $\geq 10^5$ CFU/ml and with or without pyuria. However, when the data were analyzed for either probable pathogens at $<10^5$ CFU/ml or pyuria, the sensitivity of the URISCREEN was higher ($P < 0.05$).

The majority of specimens received in diagnostic microbiology laboratories for culturing are urine specimens. The etiology of urinary tract infections is primarily bacterial, and antimicrobial therapy is recommended to eliminate the infections. In addition, a significant number of patients with infections are bacteriuric but asymptomatic. Therefore, large numbers of urine specimens are sent to clinical microbiology laboratories both for the diagnosis of symptomatic patients and for the screening of asymptomatic patients with an increased risk for urinary tract infections and possible serious sequelae.

Classically, urine specimens have been screened by the semiquantitative plate culture method (2). Although this method provides for the detection of as few as 100 CFU/ml, depending on the inoculum, preliminary results are unavailable until the next day because of the need for overnight

incubation. During the last decade, a number of rapid urine screens have been described and reviewed (1, 5). These rapid screens include a variety of methodologies, and the detection times range from less than 1 min to 13 h. The purpose of a rapid screen is to provide results in a timely manner, allowing prompt patient care, and to eliminate the need to culture specimens that are negative. Although rapid screens are purported to have advantages for both the patient and the laboratory, they are not widely used. Some of the perceived problems associated with rapid screens include the need for instrumentation, the requirement for growth prior to detection, the inability to eliminate a majority of negative specimens because of the high false-positive rates, the need to batch test, the relative high cost per test, and the inability to detect low-level bacteriuria and pyuria.

The purpose of this investigation was to evaluate a 2-min, manual enzyme tube test, URISCREEN (Analytab Products, Plainview, N.Y.), for its ability to detect bacteriuria and pyuria and to compare it with another rapid urine screen, the Chemstrip LN (BioDynamics, Division of Boehr-

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inger Mannheim Diagnostics, Indianapolis, Ind.), an enzyme dipstick test. The results were analyzed for urine specimens with probable pathogens at various colony counts and leukocyte counts.

MATERIALS AND METHODS

Specimens. A total of 1,500 urine specimens, including both clean-voided and catheterized urine specimens from both inpatients and outpatients and submitted to the clinical microbiology laboratories at Erie County Medical Center, Buffalo, N.Y., Mayo Clinic and Mayo Foundation, Rochester, Minn., and University of California Irvine Medical Center, Orange, were included in this study. Patients receiving antimicrobial therapy were not excluded. Upon collection, urine was placed in a sterile tube and processed within 2 h or kept at 4°C and processed within 8 h.

Evaluation of bacteriuria. The semiquantitative plate culture method described by Clarridge et al. (2) was used as the reference method for determining bacteriuria. With a calibrated loop, 0.001- and 0.01-ml samples of well-mixed urine specimens were inoculated onto 5% sheep blood agar plates. In addition, a MacConkey agar plate or an eosin-methylene blue agar plate (BBL Microbiology Systems, Cockeysville, Md.) was inoculated with 0.001 ml of urine. Cultures were incubated overnight at 35°C and examined for the numbers and types of organisms present. Organisms considered contaminants were diphtheroids, lactobacilli, and viridans group streptococci from all specimens and mixed cultures from voided urine specimens.

URISCREEN. Urine specimens were processed in accordance with the instructions in the package insert for URISCREEN. The urine specimen was allowed to come to room temperature prior to being tested. A sample of well-mixed urine (1.5 to 2 ml) was added to a test tube containing URISCREEN reagent powder. Four drops of 10% hydrogen peroxide solution were added to the tube. The tube was mixed gently for 10 s to avoid producing foam and until the reagent dissolved and the solution turned blue. The tube was observed for up to 2 min. The formation of foam within 2 min and to an extent sufficient to form a complete and continuous ring or layer on the surface of the liquid in the test tube was interpreted as a positive test. The resulting foam indicated the presence of catalase originating from bacterial or somatic cells in the urine specimen.

Chemstrip LN. The Chemstrip LN is a plastic strip with reagent papers attached for detecting the presence of leukocyte esterase and nitrite in urine. The urine specimen was allowed to come to room temperature prior to being tested, and the plastic strip was dipped into the specimen and immediately withdrawn along the rim of the container to remove excess urine. Results were read within 2 min in accordance with the manufacturer's instructions. A positive leukocyte esterase test was one that produced a purple color ranging from a trace to a 2+ intensity. For nitrite, any pink color was considered positive.

Evaluation of pyuria. One thousand urine specimens collected at the Erie County Medical Center and the Mayo Clinic and Mayo Foundation were examined by the Gram stain method. A sample (0.01 ml) of well-mixed, uncentrifuged urine was air dried, methanol or heat fixed, Gram stained, and examined for the presence or absence of leukocytes. The criterion for a positive Gram stain was the presence of one or more leukocytes per oil immersion field.

Five hundred urine specimens collected at the University of California Irvine Medical Center were examined for the

TABLE 1. Distribution of positive results obtained by the urine screening methods

Method	No. (%) of specimens found positive for:			
	Probable pathogens[a] and leukocytes[b] (n = 204)	Probable pathogens[a] only (n = 94)	Leukocytes[b] only (n = 247)	No probable pathogens or leukocytes (n = 955)
URISCREEN	193 (95)	63 (67)	205 (83)	270 (28)
Chemstrip LN	193 (95)	48 (51)	157 (64)	122 (13)

[a] $\geq 10^2$ CFU/ml.
[b] 10 leukocytes per μl, as determined by the quantitative count method, or ≥ 1 leukocyte per oil immersion field, as determined by the Gram stain method.

presence of leukocytes by use of a KOVA Glasstic Slide 10 with grids (Hycor Biomedical Inc., Garden Grove, Calif.). With a KOVA Petter, well-mixed uncentrifuged urine was transferred to a notch on the side chamber of the disposable slide. Ten patient samples were tested on each slide. By capillary action, 6.6 μl of the specimen was drawn into a chamber. At a magnification of ×400, a count of >10 leukocytes per μl was interpreted as positive.

Statistical analysis. Predictive values were calculated by the method of Ransohoff and Feinstein (9). The sensitivity, specificity, and predictive values of positive and negative tests were calculated as follows and converted to percentages: sensitivity = TP/(TP + FN), specificity = TN/(TN + FP), predictive value of a positive test = TP/(TP + FP), and predictive value of a negative test = TN/(TN + FN), where TP is true-positive, TN is true-negative, FP is false-positive, and FN is false-negative. Significance was determined by the chi-square test.

RESULTS

Distribution of positive results. A total of 1,500 clean-voided and catheterized urine specimens were evaluated. A summary of the distribution of positive test results is shown in Table 1. These results are based on the presence or absence of probable pathogens at $\geq 10^2$ CFU/ml, as determined by the standard plate culture method, and/or >10 leukocytes per μl, as determined by the quantitative count method, or ≥ 1 leukocytes per oil immersion field, as determined by the Gram stain method.

Detection of bacteriuria. There were 298 (20%) specimens with probable pathogens at $\geq 10^2$ CFU/ml (Table 2). These included the 204 specimens with probable pathogens and leukocytes and the 94 specimens with probable pathogens only (Table 1). Both the URISCREEN and the Chemstrip LN detected 93% (204 of 219) of specimens with $\geq 10^5$ CFU/ml. The distribution of the 219 specimens with probable pathogens at $\geq 10^5$ CFU/ml was 85 at Erie County

TABLE 2. Number (percent) positive test results for probable pathogens

CFU/ml	No. of specimens tested	No. (%) of specimens found positive by:	
		URISCREEN	Chemstrip LN
$\geq 10^5$	219	204 (93)	204 (93)
10^4–$<10^5$	51	36 (71)	30 (59)
10^2–$<10^4$	28	16 (57)	7 (25)

TABLE 3. Probable pathogens detected at various colony counts

Organism (no. of isolates)	No. of isolates detected at the indicated colony count by:								
	Culture			URISCREEN			Chemstrip LN		
	$>10^5$	10^4–10^5	10^2–$<10^4$	$>10^5$	10^4–10^5	10^2–$<10^4$	$>10^5$	10^4–10^5	10^2–$<10^4$
Escherichia coli (136)	110	16	10	104	14	6	105	13	3
Enterococcus spp. (43)	31	7	5	27	5	2	26	4	2
Pseudomonas spp. (34)	24	6	4	24	4	1	22	4	1
Klebsiella spp. (31)	28	2	1	27	0	1	27	0	0
Coagulase-negative staphylococci (16)	11	3	2	11	0	2	11	1	2
Proteus spp. (17)	10	4	3	10	3	1	9	2	0
Other gram-negative bacilli (15)	12	2	1	12	0	1	11	1	1
Candida spp. (15)	7	5	3	7	5	2	6	2	0
Streptococcus agalactiae (9)	4	5	0	3	4	0	4	3	0
Staphylococcus aureus (9)	6	3	0	6	2	0	6	1	0
Enterobacter spp. (6)	5	0	1	5	0	0	5	0	0
Staphylococcus saprophyticus (3)	3	0	0	3	0	0	3	0	0
Total (334)	251	53	30	239	37	16	235	31	9

Medical Center, 48 at Mayo Clinic and Mayo Foundation, and 86 at University of California Irvine Medical Center.

There were an additional 79 specimens with probable pathogens at 10^2 to 10^5 CFU/ml. Of these, the URISCREEN detected 66% (52 of 79) and the Chemstrip LN detected 47% (37 of 79). Overall, the URISCREEN detected 86% (256 of 298) of all specimens with probable pathogens at $\geq10^2$ CFU/ml and the Chemstrip LN detected 81% (241 of 298).

In this evaluation, 334 probable pathogens were isolated at various colony counts from 298 specimens. Of these 334, 264 were pure cultures of one probable pathogen and the remaining 70 were isolated from 35 catheter specimens, each with two probable pathogens. A summary of the probable pathogens detected at the various colony counts by the reference and urine screening methods appears in Table 3. *Enterococcus* spp. showed the highest percentage of false-negative results in both of the urine screening methods: 21% in URISCREEN and 26% in Chemstrip LN.

Of the 251 probable pathogens isolated from the 219 specimens at $\geq10^5$ CFU/ml, the URISCREEN detected 95% (239 of 251) and the Chemstrip LN detected 94% (235 of 251). Of these 251, 55 were gram-positive cocci, 189 were gram-negative bacilli, and 7 were yeasts. Both the URISCREEN and the Chemstrip LN detected 91% (50 of 55) of the gram-positive cocci; the URISCREEN detected 96% (182 of 189) of the gram-negative isolates and 100% (7 of 7) of the yeast isolates, and the Chemstrip LN detected 95% (179 of 189) of the gram-negative bacilli and 86% (6 of 7) of the yeast isolates. There was no significant difference between the two methods in detecting specimens with probable pathogens at $\geq10^5$ CFU/ml ($P > 0.1$).

Of the 53 probable pathogens isolated from the 51 specimens with 10^4 to 10^5 CFU/ml, the URISCREEN detected 70% (37 of 53) and the Chemstrip LN detected 58% (31 of 53). Of the remaining 30 probable pathogens isolated from the 28 specimens with 10^2 to $<10^4$ CFU/ml, the URISCREEN detected 53% (16 of 30) and the Chemstrip LN detected 30% (9 of 30). Although there was no significant difference between the two methods in their ability to detect all specimens with probable pathogens at $\geq10^5$ CFU/ml, the URISCREEN detected significantly more specimens with probable pathogens at $<10^5$ CFU/ml ($P < 0.05$).

There were 955 specimens with no detectable probable pathogens or leukocytes. Of these, 28% (270 of 955) were

found positive by the URISCREEN and 13% (122 of 955) were found positive by the Chemstrip LN.

Detection of pyuria. In this evaluation, 451 specimens were positive for leukocytes by either the quantitative count method or the Gram stain method. These included the 204 specimens with probable pathogens and leukocytes and the 247 specimens with leukocytes only (Table 1). The positive leukocyte parameters for each of the study sites are shown in Table 4. The distribution of positive specimens among the three study sites was 178 at Erie County Medical Center, 74 at Mayo Clinic and Mayo Foundation, and 199 at University of California Irvine Medical Center. Overall, the sensitivity, specificity, and positive and negative predictive values for the detection of pyuria by the URISCREEN were 88, 68, 54, and 93%, respectively, and those of the Chemstrip LN were 78, 85, 70, and 90%, respectively. There was a statistically significant difference between the sensitivities of the two urine screening methods for the detection of pyuria with or without bacteriuria ($P < 0.05$).

Gram stains were performed on 1,000 urine specimens evaluated at Erie County Medical Center and Mayo Clinic and Mayo Foundation to detect the presence of leukocytes.

TABLE 4. Sensitivities, specificities, and predictive values for the detection of leukocytes

Method	Site[a]	Sensitivity (%)	Specificity (%)	Predictive value (%)	
				Positive	Negative
URISCREEN	All	88	68	54	93
	Erie[b]	86	66	58	89
	Mayo[b]	100	67	35	100
	UCI[c]	86	68	64	88
Chemstrip LN	All	78	85	70	90
	Erie[b]	80	71	61	87
	Mayo[b]	88	91	63	98
	UCI[c]	71	93	87	83

[a] Erie, Erie County Medical Center; Mayo, Mayo Clinic and Mayo Foundation; UCI, University of California Irvine Medical Center.
[b] ≥1 leukocyte per oil immersion field, as determined by the Gram stain method.
[c] >10 leukocytes per µl, as determined by the quantitative count method.

TABLE 5. Sensitivities, specificities, and predictive values for the detection of probable pathogens and leukocytes

Method	Parameter (no. of specimens tested)	Sensitivity (%)	Specificity (%)	Predictive value (%)	
				Positive	Negative
URISCREEN	Probable pathogens and leukocytes (204)	95	58	26	99
	Probable pathogens[a] (298)	86	60	35	95
	Leukocytes[b] (451)	88	68	54	93
Chemstrip LN	Probable pathogens and leukocytes (204)	95	72	65	99
	Probable pathogens[a] (298)	84	83	56	96
	Leukocytes[b] (451)	78	85	70	90

[a] Includes the 204 specimens with probable pathogens and leukocytes and the 94 specimens with probable pathogens only.
[b] Includes the 204 specimens with probable pathogens and leukocytes and the 247 specimens with leukocytes only.

Leukocytes were observed in 252 (25%) specimens. Compared with the Gram stain, the URISCREEN detected 227 (90%) and the Chemstrip LN detected 208 (82%).

Of the 500 specimens evaluated at the Erie County Medical Center, 178 (36%) had ≥ 1 leukocyte per oil immersion field; the URISCREEN detected 86% of these, and the Chemstrip LN detected 80%. Of the 500 specimens evaluated at Mayo Clinic and Mayo Foundation, 74 (15%) had ≥ 1 leukocyte per oil immersion field. Of these, the URISCREEN detected 100% of the specimens with leukocytes, with or without bacteria, and the Chemstrip LN detected 88%. A quantitative chamber count method was used to detect leukocytes on the 500 urine specimens evaluated at University of California Irvine Medical Center. Overall, 199 (40%) specimens had >10 leukocytes per μl (Table 4). Of these, the URISCREEN detected 86% and the Chemstrip LN detected 71%. There was a significant difference between the two screening methods when either the quantitative count method or the Gram stain method was used as the reference method for determining the presence of leukocytes, with or without the presence of bacteria ($P < 0.05$).

Detection of bacteriuria and pyuria. The overall sensitivities, specificities, and predictive values for the detection of specimens with probable pathogens at colony counts of $\geq 10^2$ CFU/ml and/or leukocytes at the three study sites are shown in Table 5. When both probable pathogens and leukocytes were present in a urine specimen, the sensitivities of the urine screening methods were the same (95%). These sensitivities decreased for both methods when the data were analyzed either for probable pathogens, with or without leukocytes, or for leukocytes, with or without bacteria. There was a significant difference between the sensitivities of the Chemstrip LN for specimens with both probable pathogens and leukocytes (95%) compared with sensitivities of specimens with leukocytes, with or without organisms (78%) ($P < 0.005$).

DISCUSSION

Although it is a recommended practice to perform urine cultures for all patients with suspected urinary tract infections, this often is not done (4). Until recently, it appeared to be more cost-effective to manage an uncomplicated urinary tract infection on the basis of urinalysis findings because of the time delay before culture results are known (3, 4). An alternative approach to the standard plate culture method has been the use of rapid urine screens (1, 3, 5). Although many of these methods detect a majority (~95%) of the specimens with probable pathogens at $\geq 10^5$ CFU/ml, their sensitivities decrease at lower colony counts. Also, some

screens have high false-positive rates, while others detect only bacterial or somatic cells. Additionally, the complexity of some of the test procedures, the incubation and instrument requirements, and the inability to easily incorporate the screens into the routine workflow seem to be the major reasons for their limited use or lack of use.

Both urine screening methods evaluated in this study provide results within 2 min, require no instrumentation, and are easy to perform. Overall, their sensitivities and negative predictive values compare favorably with those of other screening methods (5). In addition, each has the ability to detect bacteria and leukocytes. It has been reported that the presence of pyuria together with $\geq 10^2$ CFU/ml is a better predictor of a bladder infection than the presence of either $\geq 10^5$ or $\geq 10^2$ CFU/ml without pyuria (10, 11). Therefore, screening tests that take into account both bacteriuria and pyuria theoretically have an advantage in identifying patients with urinary tract infections.

The most important aspect of a urine screening method is its ability to detect positive specimens. Recognizing that the presence of low levels of bacteria along with pyuria may be potentially significant in some patients (10, 11), we analyzed the urine screening methods for their ability to detect low levels ($\geq 10^2$ CFU/ml) of bacteria along with pyuria. In this evaluation, the overall sensitivities of the URISCREEN and the Chemstrip LN for the detection of pyuria together with probable pathogens at $\geq 10^2$ CFU/ml were 95%; however, the URISCREEN detected significantly more specimens with isolates at $<10^5$ CFU/ml ($P < 0.05$), especially *Candida* spp. It should be noted that, although there were only 15 isolates of *Candida* spp., the URISCREEN detected 93% and the Chemstrip LN detected only 53%. It is not surprising that, overall, *Enterococcus* spp. showed the highest percentage of false-negative results in both test methods, because the substrates included in the tests do not detect the enzymes produced by these species. It is also interesting to note that approximately one-half of these false-negative specimens did not contain leukocytes. Overall, 76% of all false-negative results at the various colony counts were from specimens without leukocytes. It has been reported that the absence of leukocytes may cause clinicians to question the diagnosis of a urinary tract infection (4, 10). In one study describing the causes of the acute urethral syndrome in women, 85% of patients with pyuria had a proven infection and patients without pyuria usually had no demonstrable infection (11). In that study, pyuria was definitely a better predictor of infection than bacteriuria.

The URISCREEN and the Chemstrip LN differed in their abilities to detect pyuria ($P < 0.05$). When both methods were compared with the quantitative chamber count

method, the URISCREEN was more sensitive than the Chemstrip LN, 86 versus 71%, respectively ($P < 0.005$). Similar results were obtained when the Gram stain method was used as the reference method for leukocytes; the URISCREEN detected 90%, and the Chemstrip LN detected 82% ($P < 0.025$). It was recently suggested that, although the leukocyte esterase reagent strip method is less sensitive than hemacytometer counting, it can serve as an alternative for the microscopic detection of leukocytes (4, 8). Since the URISCREEN is more sensitive than the Chemstrip LN, it appears to be a better alternative to microscopic examination. However, a positive URISCREEN test is not specific for the presence of leukocytes, whereas the presence of leukocyte esterase can be detected by the Chemostrip LN.

Another important aspect of urine screening is the ability to eliminate a majority of "negative" specimens. However, for some urine screening methods, the percentage of false-positives is higher than that of true-positives (5). This has been a problem associated with urine screening. It appears that methods that detect both bacterial and somatic cells yield more false-positive results than methods that detect only one cell type (5, 7). Both urine screening methods tested in this evaluation detect enzymes present in both bacterial and somatic cells. This fact may account for the low specificities and positive predictive values of these methods in this evaluation and those previously described (1, 7).

In conclusion, the URISCREEN is a rapid, nonautomated urine screening method for the detection of bacteriuria and pyuria. Rapid tests for detecting leukocytes and bacteria in urine permit presumptive identification of urinary tract infections at the time of pretherapy evaluation without the expense and delays associated with urine culturing (3). The URISCREEN meets these requirements as a single test. The two other rapid, nonautomated, simple-to-perform urine screening methods that detect both bacteria and leukocytes are the Gram stain and the Chemstrip LN. Although the Gram stain is one of the most rapid, reliable, and inexpensive methods for estimating bacteria at $\geq 10^5$ CFU/ml and ≥ 1 leukocyte per oil immersion field, it may be difficult to interpret when low numbers of bacteria are present (6). In addition, the procedure can be tedious and time-consuming for the individual examining the specimen, especially because a majority of urine specimens are negative for $\geq 10^5$ CFU/ml and ≥ 1 leukocyte per oil immersion field. In terms of practicality, both the URISCREEN and the Chemstrip LN are easy to perform and interpret. These methods can be easily performed as a single test or in batches and can be readily incorporated into the routine workflow. These methods are equally sensitive for the detection of specimens with probable pathogens and leukocytes. However, in recognition of the importance of pyuria in the diagnosis of a urinary tract infection, the URISCREEN is more sensitive for the detection of specimens with leukocytes.

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