Simplified Microscopy for Rapid Detection of Significant Bacteriuria in Random Urine Specimens

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Simplified urine microscopy, nitrite testing, and dipstick culture were compared with urine loop streak culture colony counts in 219 random voided specimens to determine the accuracy of the three rapid screening techniques. Nitrite testing resulted in 65% false negative results, which could not be significantly improved by incubation at 37°C but which could be improved by adding nitrate substrate before incubation. Dipstick culture could not be quantitated until after 18 h of incubation. A new, simplified microscopy technique, using unspun, unstained urine, resulted in 4% false negative results and 4% false positive results in specimens containing over 10^5 organisms per ml and was the best method. Centrifuges, Gram staining reagents, and counting chambers are not necessary for accurate microscopic screening of random urine specimens for the presence of bacteriuria by this technique, and the results are immediately available.

The human urinary tract is normally sterile, with the exception of the distal few millimeters of the urethra. Bacteriuria is abnormal and has been associated with acute urinary tract infections (24), chronic pyelonephritis (28), high blood pressure (10), excess fetal prematurity, and perinatal death (10). Since only 1 to 7% of females and 0.04 to 0.05% of males (11, 15) have bacteriuria, and since the majority are asymptomatic (9), screening methods must be rapid, simple, and accurate. Detection and treatment of persons with bacteriuria may decrease morbidity.

Many tests have been proposed to screen for bacteriuria in voided urine (8), including microscopic examination of urine (centrifuged or uncentrifuged) (3, 13, 16, 18, 22, 23, 26), chemical testing of urine (by nitrite, catalase, tetrazolium, glucose, or enzymes) (2, 12, 17, 19, 25), and rapid culture techniques (dipstick culture, agar cup, or dip slide) (4, 7, 20). The most advantageous test would be both sensitive and specific, utilize a randomly voided urine specimen, and give rapid results while the patient was still present. We have prospectively studied and compared the most promising rapid tests for bacteriuria in random urine specimens: simplified urine microscopy, nitrite test, and dipstick cultures.

MATERIALS AND METHODS

Specimens. Two hundred nineteen unselected urine samples, which had been submitted to the clinical laboratory as clean catch, midstream, voided specimens for culture, were studied. Mean age of patients was 36 years (range 1 to 85); 60 were male and 159

female. No attempt was made to identify treatment at the time of specimen collection. Specimens were refrigerated upon receipt, and all procedures were performed within 24 h. Except for an occasional technical problem, every specimen was quantitatively cultured, examined by simplified microscopy, tested for the presence of nitrite, and cultured by dipstick.

Quantitative urine culture. Each urine specimen was cultured in our laboratory by the calibrated loop (0.01 ml) streak method using eosin-methylene blue agar and sheep blood agar. Colony counts were done from sheep blood agar and were the standard with which other techniques were compared. Bacteria in numbers greater than 10^5/ml were identified to species by using API strips (Analytab Products, Carle Place, N.Y.) and other media and reagents as necessary. No pour plate cultures were performed.

Simplified microscopy of urine. Because of the complexity of reported procedures, we used a simplified technique suggested by in vitro studies of Kunin (13). A drop of manually agitated, uncentrifuged urine from each specimen was placed on a glass slide, covered with a glass slip, and examined for bacteria under oil immersion (magnification ×1,000, field diameter 190 μm) (Fig. 1). The following guidelines established a test as positive: (i) identification of bacteria with smooth surfaces without adherent material, and (ii) demonstration of at least one bacterium per oil field in each of five fields. Care was taken not to misinterpret crystalline material as bacteria.

Nitrite test with incubation. A 2-ml sample of urine from each specimen was pipetted into a sterile tube and tested by dipstick for the presence of nitrite. The urine was then incubated at 37°C and retested after 2, 4, 6, and 24 h. Two different commercially available nitrite indicator dipsticks were utilized simultaneously (N-Uristix, Ames Co., Elkhart, Ind., and Bac-U-Dip, Warner-Chilcott Laboratories, Morris Plains, N.J.).

Nitrite test with supplemental nitrate. Ten urine samples were selected because they contained more than 10^5 organisms per ml but tested nitrite negative. Duplicate 1-ml samples of urine were incubated at 37°C with and without addition of 0.04 ml of a sterile 5% solution of potassium nitrate. Samples were tested immediately for the presence of nitrite, then incubated at 37°C and retested after 2, 4, 6, and 24 h. These patients were comparable to the general test group. Organisms cultured were six *Escherichia coli* and one each of *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*

Urine nitrate concentration and culture. Urine nitrate concentration was measured in paired urine specimens from nine healthy laboratory personnel, four male and five female. Subjects did not alter their normal activities. One specimen was collected at the first voiding after a night's sleep and the second at midafternoon of the same day. All specimens contained less than 10^3 bacteria per ml by quantitative culture. Urine nitrate and nitrite were determined simultaneously in all specimens by utilizing the spectrophotometric method of Wegner (27). A sample containing 10^6 *E. coli* in sterile saline, previously isolated from a nitrite-positive urine specimen, was added to 1 ml of each specimen, and the urine was incubated at 37°C. Nitrite testing was performed by dipstick at 15-min intervals until positive.

Dipstick culture. A commercially available dipstick was used (Microstix, Ames). A strip was dipped in each urine specimen, placed in a plastic pouch, and observed during incubation at 37°C. After 18 h the number of bacteria was estimated by comparing the pads with pictures provided with the strips.

RESULTS

Quantitative urine culture. Two hundred nineteen specimens were processed, and 52 (24%) contained more than 10^5 bacteria per ml. Gram-negative bacteria, especially *E. coli,* predominated. Specimens containing greater than 10^5 bacteria per ml were termed culture positive; those containing less than 10^5 bacteria per ml were termed culture negative.

Simplified microscopy of urine. Two hundred ten specimens were examined microscopically. Microscopy was falsely positive in 7 of 159 culture-negative specimens (4%) and falsely negative in 2 of 51 culture-positive specimens (4%) (Table 1). Cultures from the seven false-positive specimens revealed two specimens with 10^4 to 10^5 *E. coli,* one with 10^4 to 10^5 *S. epidermidis,* and four with less than 10^3 organisms per ml. One falsely negative specimen contained *E. coli* and the other diphtheroids.

Nitrite test with incubation. Two hundred eighteen specimens were tested for nitrite after 0, 2, 4, 6, and 24 h of incubation at 37°C (Table 2). Although chemical formulation was different, results were similar with both nitrite dipstick products. Of the 218, 51 specimens contained

over 10^5 bacteria per ml, but only 18 were initially nitrite positive (35%). Maximum positivity occurred after 4 h of incubation, when 23 (45%) were positive. After incubation for 24 h, only eight of the specimens remained nitrite positive (16%). There was no correlation between the organism cultured and nitrite positivity.

Eighty-five urine specimens contained over 10^3 but less than 10^5 organisms per ml; two were initially nitrite positive, but after incubation for 24 h 18 were positive.

Eighty-two urine specimens contained less than 10^3 organisms per ml; three specimens were positive initially and remained positive throughout the entire incubation. Discoloration of these specimens made interpretation of the test difficult. After incubation for 24 h, six additional specimens converted from nitrite negative to positive.

Nitrite test with supplemental nitrate. Of the 10 culture-positive, nitrite-negative specimens to which nitrate was added, 8 became nitrite positive after incubation for 6 h and the other 2 (*Proteus mirabilis* and *S. aureus*) after 24 h. One control specimen without added nitrate became nitrite positive (*E. coli*) after incubation for 24 h.

Urine nitrate concentration and culture. Nitrate concentration was higher ($P < 0.025$ by paired t test) in first morning than in afternoon urine specimens from nine healthy laboratory personnel (Fig. 2). No nitrite was detected in any specimen. After addition of 10^6 *E. coli* per ml, five first morning and three afternoon specimens first became definitely nitrite positive after incubation for 2.25 h; after incubation for 2.75 h, all specimens were nitrite positive.

Dipstick culture. Forty-five urine specimens containing more than 10^5 organisms per ml were tested by dipstick culture; dipsticks from 39 (87%) were interpreted as indicating 10^5 or more bacteria per ml after incubation for 18 h (Table 3). One hundred sixty-seven specimens containing less than 10^5 organisms per ml were tested; dipsticks from three (2%) of these were interpreted as indicating 10^5 or more bacteria per ml after incubation for 18 h. Although positive dipsticks could be identified after incubation for 4 to 6 h, estimation of the number of organisms was not possible before 18 h, so that dipstick culture was not useful for rapid screening.

DISCUSSION

Quantitative urine cultures are valuable in detecting bacteriuria if properly collected specimens are studied (1, 9). Patients whose voided, clean catch, midstream urine specimens contain over 10^5 bacteria per ml have a high probability
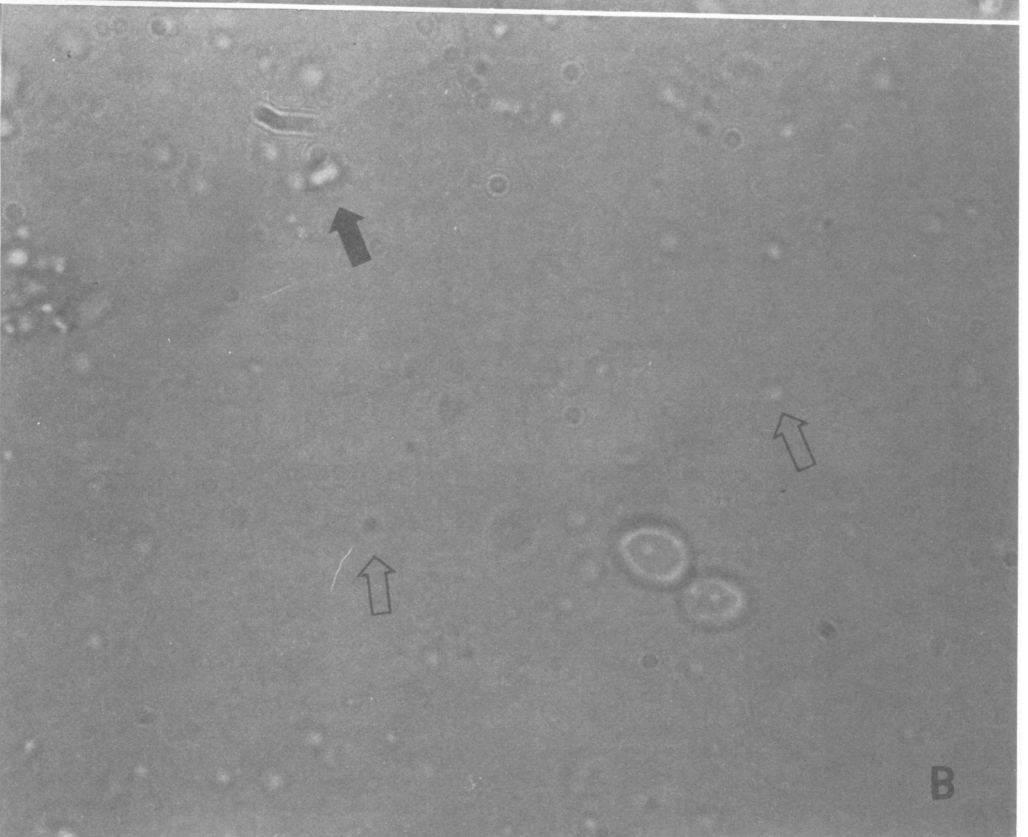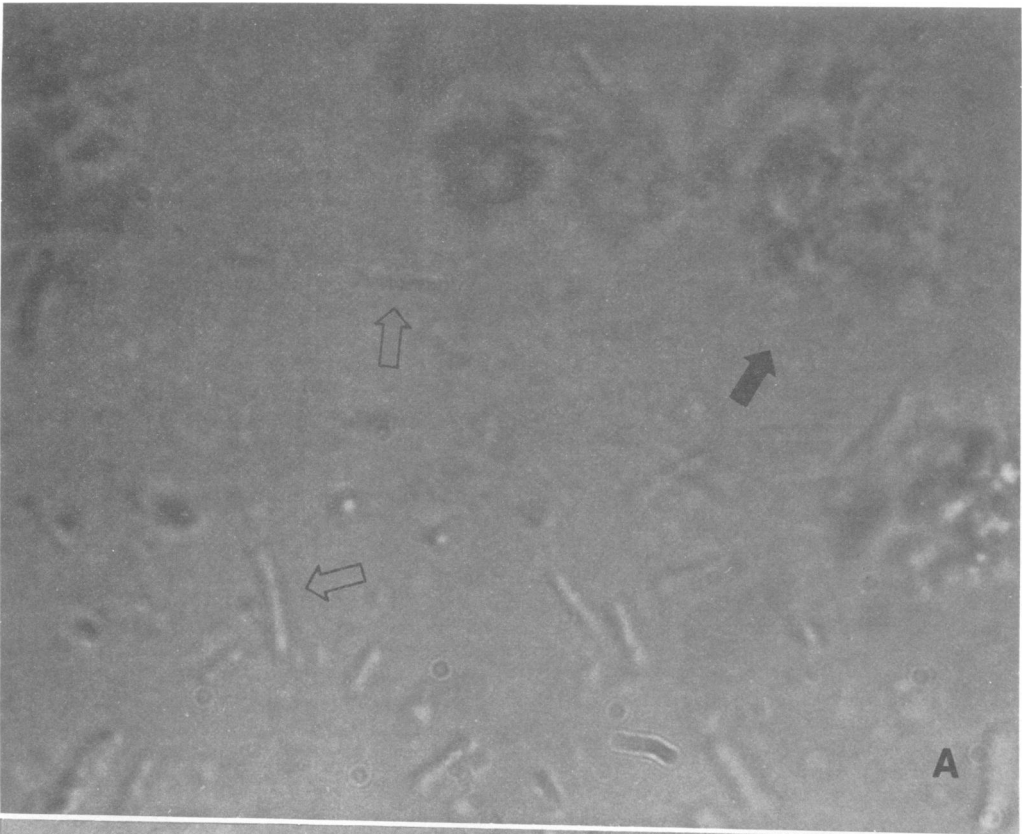
TABLE 1. *Urine microscopy and urine culture*

Bacterial concn (organism/ml) in samples tested	No. of cultures examined	Microscopy results (%)	
		Positive	Negative
$>10^5$	51	49 (96)	2 (4)
$<10^5$	159	7 (4)	152 (96)

TABLE 2. *Presence of nitrite in urine samples of varying bacterial concentrations*

Incubation (h)	No. (%) of nitrite-positive samples by concn:		
	$>10^5$/ml	10^3 to 10^5/ml	$<10^3$/ml
0	18 (35)	2 (2)	3 (4)
4	23 (45)	4 (5)	3 (4)
24	8 (16)	18 (21)	9 (11)

of having significant bacteriuria, whereas those whose specimens contain fewer than 10^5 bacteria per ml do not. However, since 24 h is required to obtain results from quantitative urine culture, more rapid methods have been sought to screen for the presence of urinary bacteria.

The results of urine microscopy have been reported using stained, unspun urine (3, 9), stained sediment (13, 23), or both (16, 26, 28). Robins et al. studied unstained urine utilizing a counting chamber (22). None of these reports states exact criteria for positive microscopy. Using these methods, processing is time-consuming and may complicate interpretation, since it is more difficult to separate bacteria from amorphous debris in sediment, and staining procedures introduce the possibility of false negative results, from lost bacteria if the specimen is inadequately fixed on the slide, and false positive results, from artifacts or contaminated stains. We have demonstrated that a new simplified urine microscopy technique, omitting the use of stains, centrifuges, or counting chambers, is remarkably accurate for rapid screening for bacteriuria in random urine specimens. This method might also be useful for determining response to therapy and could replace the more expensive quantitative urine culture for this purpose.

Nitrite is not normally detected in human urine, but bacteria have enzymes capable of reducing urinary nitrate to nitrite. Cruickshank and Moyes were the first to report urinary nitrite as a marker for urinary bacteria, but found ni-
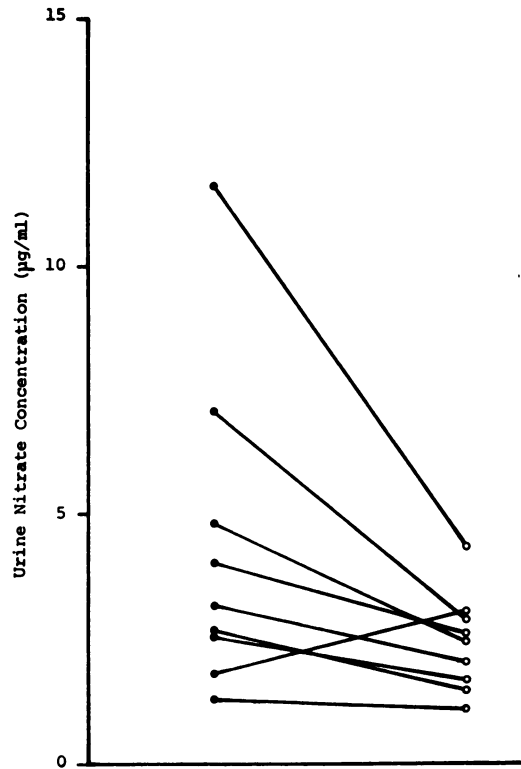

FIG. 2. *Urinary nitrate concentration in paired first morning voided urine (●) and afternoon voided urine (○) from nine healthy individuals.*

trite was not present in all infected urine specimens (5). This may be because not all bacteria reduce nitrate, e.g., enterococci, acinetobacter, or alcaligenes. Sleigh improved the accuracy of the test by adding nitrate and incubating samples for 6 h before testing for nitrite (25). Czerwinski et al. first observed that nitrite testing was more sensitive in infected first morning voided specimens than in random voided specimens (6). This difference in nitrite sensitivity depending upon timing of specimen collection has been confirmed by Craig et al. (4) and explains why some investigators have had good results (21) and others poor results (20) when using urinary nitrite as a marker for bacteriuria.

In an effort to increase the sensitivity of nitrite testing in random voided urine specimens, which are more practical for screening, to the level reported in first morning voided specimens, we

FIG. 1. *Uncentrifuged, unstained urine (×1000) containing (A) E. coli and (B) Streptococcus faecalis. Organisms are marked by open arrows, amorphous crystalline material by closed arrows. Notice multiple planes of focus in wet preparations and smoothness of bacterial outline. Crystalline material is more refractile than bacteria. Note yeast in bottom photograph.*

TABLE 3. *Dipstick culture technique*

Bacterial concn (organisms/ml) in samples tested	Interpreted dipstick results (%):	
	>10⁵/ml	<10⁵/ml
>10^5	39 (87)	6 (13)
<10^5	3 (2)	164 (98)

utilized two techniques, incubation at 37°C and addition of nitrate substrate. Incubation of specimens at 37°C failed to increase the sensitivity of the nitrite test, suggesting that, contrary to the manufacturers' suggestion (package inserts), the difference in sensitivity between random and first morning specimens is not due to duration of incubation of urine within the bladder. Supplemental nitrate converted culture-positive, nitrite-negative specimens to positive during incubation at 37°C, confirming Sleigh's results (25). Although nitrite testing may be useful in selected patients using first morning voided specimens (14), addition of nitrate followed by incubation of random voided specimens is cumbersome and negates the usefulness for office or hospital bacteriuria screening.

Craig et al. (4) and Moffat et al. (20) have reported good results from using the dipstick culture test to screen for bacteriuria. While our results were similar, our purpose in using this test was to determine whether it might be adapted for rapid screening. The manufacturer recommends interpretation of the dipstick after incubation for 12 to 18 h, but we hoped that interpretation might be possible after a shorter incubation. This was not possible. The time required for incubation limits the usefulness of the dipstick culture test for rapid bacteriuria screening.

In summary, we found neither the urinary nitrite test nor the dipstick culture technique useful for rapid detection of significant bacteriuria in random urine specimens. However, simplified microscopic detection of bacteria in unstained, uncentrifuged urine accurately detected bacteriuria. We suggest that when beginning microscopy, and periodically thereafter, a laboratory should compare the results of urine microscopy with simultaneous quantitative cultures. Additionally, including known positives in microscopy studies, such as frozen diluted broth cultures or quantities of positive urine, would serve as a control to ensure that bacteria can be recognized. We suggest that both *E. coli* and enterococci be used, since cocci are more difficult to identify than rods. Since microscopy is quick and inexpensive and can be applied to random, clean catch, midstream urine samples, the new technique of simplified urine microscopy appears to be the best screening technique for bacteriuria currently available.

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