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Quantitative Urine Culture Method Using a Plastic "Paddle" Containing Dual Media

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A new dip-inoculum method for quantitative urine culture is described which utilizes a dual-chambered plastic "paddle" housing both a general purpose and differential medium. Comparative bacterial counts of 1,000 clinical specimens using the pour plate and this device were identical in 82.9% and within a factor of five in 95.6%. The "paddle" detected all but 19 of 258 specimens (92.6%) with 100,000 or greater colonies per ml. This simple, convenient method should allow more extensive use of quantitative urine culture in the diagnosis and follow-up of patients with urinary tract infections in office practice. It should not be considered as a substitute for the more definitive pour plate method or for standard methods for characterization of bacteriological species when more exact information is required.

The importance of quantitative urine cultures in defining significant bacteriuria has been well established (3, 5, 6). The standard methods of obtaining bacterial counts have been by serial dilution pour plate or calibrated loop streaking procedures. These methods, although quite simple, are of sufficient complexity not to be readily adaptable to routine office practice.

The Wisconsin Regional Medical Program is sponsoring an extensive program to provide physicians in office practice with a simple, inexpensive, reliable method to detect significant bacteriuria. The dip slide culture method has been shown to be quite effective for this purpose (1, 2), and numerous physicians and clinics throughout the state of Wisconsin have adopted this method. More recently, a culture pipette method was also found to provide reliable quantitative information. Both of these methods require minimal training of personnel, and the culture units are compact with good storage qualities and are readily transported by mail.

The Bacteriuria Screening Test (Bioquest, Division of Becton, Dickinson & Co.) is a ready-to-use, disposable culture kit that utilizes the dip-inoculum technique (4) with a plastic media-containing "paddle." This report presents data on 1,000 clinical urine specimens processed by both the pour plate and the new dip method.

It is hoped that adoption of this or similar devices will enable the physician in practice to diagnose more readily and follow patients with urinary tract infections. Direct inoculation in hospital wards may also prove useful in decreasing the problem of bacterial multiplication prior to receipt by the laboratory.

MATERIALS AND METHODS

Bacteriuria screening test unit. The functional unit consists of a clear plastic dual-chambered "paddle" containing both a general purpose and a differential medium, Trypticase soy agar (TSA) and Levine eosin méthylene blue (EMB) agar, respectively. It is housed in a clear plastic screw-cap vial. The media beds are parallel and face the same direction; each has a surface size of about 310 mm^2. The outer wall of the media wells is constructed to prevent medium dislodgement during handling and shipment and the trapping of urine between the wall and media (Fig. 1).

To inoculate the unit, the media portion is momentarily dipped into the urine to wet the entire agar surfaces. It is then returned to the vial and incubated in an upright position overnight at 37 C.

The number of colonies per milliliter of urine is estimated by comparing the density of growth with a standard chart obtained from two- or fivefold dilutions of known cultures. The functional unit and the standard dilution series are depicted in Fig. 1.

Individual colonies can be picked from the media for subculture and further identification. Preliminary identification of lactose-fermenting bacteria (primarily *Escherichia coli*) can be made by the charac-

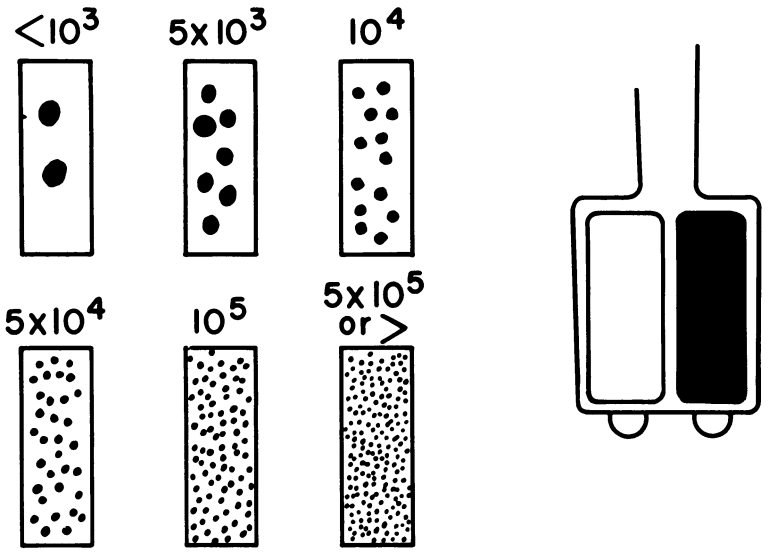

FIG. 1. *"Paddle" quantitative urine culture device. Media, EMB agar (blackened area) and Trypticase soy agar (clear area), are layered side by side on a plastic surface. Only one face is used. The test is read by pattern as shown in the figure. The entire device is packaged in a sterile, clean container. The handle of the device is much larger than shown to permit easy manipulation.*

teristic purple sheen on EMB agar. Gram-positive organisms will grow well on the TSA and be inhibited on EMB.

Clinical material. One thousand urine specimens were obtained from three hospitals in Madison, Wisc. over a period of 6 weeks (753 from University Hospitals, 222 from Veterans Administration Hospital, and 25 from Madison General Hospital). These specimens were clean-catch, midstream, or catheterized urines that had been submitted to the hospital diagnostic bacteriology laboratory and stored at 4 C until tested. Each urine sample was cultured simultaneously by the serial dilution pour plate method in TSA pour plates (dilutions of 10^{-1}, 10^{-3}, and 10^{-5}) and by the new dip method.

The same technician performed each test by both methods, but two additional individuals (one of whom had no previous bacteriology training) independently compared the density growth on the "paddle" with the standard dilution series.

RESULTS

Comparative bacterial counts of the 1,000 urine specimens by pour plate and the new dip method, for TSA medium, are shown in Table 1. All three readers of the dip cultures reported the same results. Identical results between pour plate and the dip method were obtained in 829 specimens (82.9%). In 127 other specimens (12.7%), the difference was no greater than a factor of five. Of the remaining 44 specimens, 36 (3.6%) differed by a single 10-fold dilution, and only 8 (0.8%) varied by a factor of 50 or more. For gram-negative bacteria, there was slightly better correlation between the two methods using the growth density on the Levine EMB agar.

For gram-positive organisms, 6 of 15 cultures of *Staphylococcus aureus* failed to grow on EMB but were detected on TSA medium. This series included only three strains of streptococci, but these were recovered on both media. With these differences in growth on the two media for gram-positive and gram-negative organisms, data obtained using both media differed from those shown in Table 1 for TSA medium alone and gave better overall correlation with the pour plate method.

The dip method detected counts of 100,000/ml or greater on both media in 226 of 258 specimens (87.5%) with similar counts by pour plate. An additional nine specimens (3.5%) with gram-negative organisms had counts of 100,000/ml or greater only on Levine EMB agar, and four specimens (1.6%) with gram-positive organisms had comparable counts only on TSA. This gives a correlation with the pour plate of 92.6% when both media are used. The great majority of the remaining 19 samples contained 50,000 organisms per ml by the dip "paddle" method. Good correlation was obtained with low as well as high counts.

A growth density of 10,000 or 50,000 on one medium with similar or less growth density on the other medium was obtained in 104 specimens by the dip method. Nineteen of these specimens (18.3%) had colony counts of 100,000/ml or greater by pour plate. No false-

negative results were obtained with a growth density less than 10,000 by the dip "paddle" method. False-positive cultures by the dip method occurred in three instances (two *S. epidermidis* and one *Proteus*). There was no growth on the Levine EMB agar in the two specimens with *Staphylococcus*.

The bacterial species recovered on subculture from pour plates containing 50,000 or more colonies per ml are presented in Table 2. All but one of the 277 samples with high colony counts contained a single organism. In each instance, the same organism was also isolated from the dip method. For the one mixed culture only the predominant organism (*Pseudomonas*) was isolated from the paddle. Quantitative differences were limited to one-half to one $\log_{10}$ lower bacterial counts with the "paddle." In five instances (two *E. coli*, two *Klebsiella-Enterobacter*, and one *Proteus*) there were major differences between the two methods.

DISCUSSION

The new dip method using a plastic "paddle"-like device is similar to the dip slide in being compact, simple to use, inexpensive to manufacture, capable of immediate inoculation, and able to give reliable quantitative information. The "paddle" has the advantage in that the media face the same direction and can be viewed and compared simultaneously. The smaller surface size aids in the inoculation of complete surfaces when only small volumes of urine are available.

This unit should provide the physician in

TABLE 1. *Comparison of bacterial colony counts (per milliliter) between pour plates and "paddle" culture methods in 1,000 urine specimens using Trypticase soy agar*[a]

"Paddle" method	Pour plate[b]						
	$<10^3$	10^3	5×10^3	10^4	5×10^4	10^5	5×10^5 or >
$<10^3$	477	0	0	0	0	0	0
10^3	21	50	25	3	1	0	0
5×10^3	1	6	48	22	5	0 (1)[c]	0
10^4	0	2	5	30	12	4 (1)	2
5×10^4	0	2	3	8	14	6 (2)	16
10^5	0	0	1	1	0	5	18
5×10^5 or >	0	1	0	0	0	2	205

[a] Double underline indicates identical results; single underline indicates deviation by one-half $\log_{10}$ interval.

[b] Pour plate counts are rounded off to the one-half $\log_{10}$.

[c] Parentheses indicate specimens with colony counts of 80,000 to 90,000 by pour plate. These four specimens are not included in the total of 258 with counts of 10^5 or greater by pour plate.

TABLE 2. *Relation of colony counts to bacterial species isolated from Trypticase soy agar pour plates containing 50,000 or more colonies and plastic "paddle" cultures*[a]

Organism isolated	Same count		Plate higher						Plate lower		Total no. studied
			0.5×10^1		10^1		5×10^1–10×10^1		0.5×10^1		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Escherichia coli	130	79.8	13	8.0	14	8.6	2	1.2	4	2.4	163
Klebsiella-Enterobacter	44	84.6	3	5.8	2	3.8	2	3.8	1	1.9	52
Pseudomonas	21	91.3	2	8.6	0	0	0	0	0	0	23
Proteus	18	85.6	1	4.8	1	4.8	1	4.8	0	0	21
Staphylococcus	11	73.3	2	13.3	2	13.3	0	0	0	0	15
Streptococcus	2	66.7	1	33.3	0	0	0	0	0	0	3
Total	226	81.7	22	7.9	19	6.8	5	1.8	5	1.8	277

[a] For comparison, Levine EMB agar for gram-negative and Trypticase soy agar for gram-positive organisms were used.

office or clinic practice with another convenient method for both diagnosis and follow-up of patients with urinary tract infections without great expense to the patient. The simplicity of the procedure should allow patients to culture their own urine and even read the results themselves. The method is also potentially useful for mass screening programs since the units can be inoculated in the field immediately and later returned to the laboratory for evaluation. It must be emphasized, however, that proper collection of a clean-voided urine specimen, followed by prompt culture is essential for the reliability of any quantitative urine culture method.

The dip method must be considered as a screening device which closely correlates with the more definitive pour plate method. Accordingly, it is essential that growth densities of 10,000 or 50,000 per ml be considered an indication for repeat culture before excluding the possibility that significant bacteriuria is present. Final identification of species and antimicrobial sensitivity testing require appropriate subculture and utilization of standard bacteriological methods.

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