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Evaluation of the B-D Urine Culture Kit

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Split samples of urine transported to the laboratory at 5°C and in a boric acid-glycerol-sodium formate preservative (B-D Urine Culture Kit; Becton, Dickinson & Co.) were cultured immediately and, in the case of preserved urine, after 24 and 48 h of storage at 25°C. Agreement between the results for cultures of specimens originally yielding $\geq 10^5$ colony-forming units (CFU) per ml and the results for urine preserved for 24 and 48 h was 85 and 71%, respectively. One-third of the specimens originally yielding 10^4 to 10^5 CFU per ml yielded $<10^4$ CFU per ml after 24 h of storage in preservative. Provided $\geq 10^4$ CFU per ml in specimens preserved for up to 24 h is regarded as equivalent to $\geq 10^5$ CFU per ml in original urine specimens, agreement of results was ≥90%.

It is recommended that unrefrigerated urine specimens received in the laboratory more than 2 h after collection be rejected for culturing and that another specimen be requested (4) because delays of greater than 2 h in inoculating cultures may produce spuriously positive results (6). Colony counts of refrigerated urine, however, have been shown to remain stable for as long as 24 h (3, 7) and, in some instances, for many days (8).

Prompt transport or, when delays are anticipated, refrigeration of urine specimens may not always be feasible; therefore, other alternatives, such as chemical preservation, have been evaluated for transport of urine. Porter and Brodie (9) reported close agreement between the results for cultures of natural urine and those for urine preserved for 3 days in 1.8% boric acid. Amies and Corpas (1, 2) reported similar results for urine preserved in a mixture of NaCl and polyvinyl pyrrolidone. Lauer et al. (7) evaluated a boric acid-glycerol-sodium formate preservative in a commercially prepared kit (B-D Urine Culture Kit; Becton, Dickinson & Co.) and found that 93.2% of 88 specimens which were positive ($>10^5$ colony-forming units [CFU] per ml) on initial culturing remained positive after 24 h of refrigeration or preservation in the kit. Further studies of the kit, however, seemed warranted because of the very small number of positive cultures in the study by Lauer et al. (7) with bacteria other than *Escherichia coli* and coagulase-negative staphylococci and because the transit time of mailed-in specimens would more likely be 48 h.

MATERIALS AND METHODS

The study was conducted in two phases. In both phases, clean-voided midstream or catheterized urine specimens were collected from patients with suspected bacteriuria by a urine collection team (10). Hexachlorophene (3 parts) diluted in water (1 part) was the antiseptic used to prepare the periurethral area before midstream urine collection (10). In the first phase, a sample of approximately 5 ml of the specimen was drawn into the B-D Urine Culture Kit for transport at room temperature, and a sample of the remaining specimen was stored and transported to the laboratory in a sterile tube at 5°C. The samples in both tubes were cultured identically by a 0.01-ml quantitative loop, streak plate method on blood agar and eosin-methylene blue agar (10). The B-D Kit (preserved urine) was stored at room temperature and recultured quantitatively after 24 and 48 h.

Because of the large number of cultures yielding *E. coli*, a second-phase study was performed. Urine was collected into a sterile tube as described above, refrigerated, and transported to the laboratory, where it was cultured quantitatively onto blood agar and eosin-methylene blue agar and then stored under refrigeration. Cultures were examined after incubation for 18 to 24 h. We selected urine specimens yielding $\geq 10^4$ CFU of bacteria other than *E. coli* per ml for study by drawing approximately 5 ml of the refrigerated specimen from a nonsterile paper cup into the B-D Kit. Quantitative cultures of both the original refrigerated specimen and that just drawn into the B-D Kit were then made within the hour. The B-D Kit (preserved urine) was then stored at room temperature and recultured quantitatively after 24 and 48 h.

To determine whether the preservative in the B-D Urine Culture Kit was sufficient to produce dilutional effects on the number of CFU per milliliter in the original urine, we aspirated adjusted inocula of $>10^5$, 10^4 to 10^5, and 10^3 to 10^4 CFU per ml in broth into the kit and immediately cultured the inocula quantitatively. To test the effects of sample size on the number of CFU per milliliter preserved in the kit, we aspirated 3- and 5-ml samples of 21 known positive urine specimens which yielded various organism groups into the

kit and cultured the samples quantitatively after 24 and 48 h of storage at room temperature.

RESULTS

The results are summarized in Table 1. There was at least 98% agreement between the results for initial cultures of original urine yielding $>10^5$ CFU per ml and results for preserved urine. Agreement between the results for initial cultures of original urine yielding 10^4 to 10^5 CFU per ml and those for preserved urine was 79.3% in phase one and 100% in phase two. The number of specimens yielding 10^4 to 10^5 CFU per ml was too small to allow us to determine whether the apparent difference in agreement between the results for the two phases of study was significant or simply due to random sampling error. Agreement between the results for initial cultures of original urine yielding $>10^5$ CFU per ml and the results for urine preserved for 24 h was 87.3 and 82.6% in phases one and two, respectively; however, the agreement between results for initial cultures of original urine yielding 10^4 to 10^5 CFU per ml and urine preserved for 24 h was only 55.2 and 45.8% in phases one and two, respectively. By 48 h, there were substantial discrepancies between the number of CFU per milliliter of the original specimen and the number of CFU per milliliter of the preserved specimen.

The numbers of isolates, arranged by organism group determined in each phase of the study, are listed in Table 2. The numbers of discrepancies listed in Table 2 represent the instances in which the number of CFU per milliliter changed by at least 1 $\log_{10}$.

No dilutional effect was noted after inoculation of known numbers of *E. coli* into the B-D Urine Culture Kit. The number of CFU per milliliter of different sample sizes (3 and 5 ml) containing known positive specimens agreed completely in 18 of 21 instances after 24 h of storage and in 15 of 21 instances after 48 h of storage in the kit. In all but two of the discrepanices, the number of CFU per milliliter in the smaller (3-ml) sample was 1 $\log_{10}$ lower.

DISCUSSION

Provided $\geq 10^4$ CFU per ml in urine preserved for 24 h was considered equivalent to $\geq 10^5$ CFU per ml in freshly collected or refrigerated urine specimens, agreement between results for cultures of the two samples was acceptable (≥90%); however, agreement was unfortunately unacceptable for results of cultures of samples preserved for 48 h. Moreover, approximately one-third of the samples originally yielding 10^4 to 10^5 CFU per ml had $<10^4$ CFU per ml after 24 h of storage in preservative. Since pure cultures of 10^4 to 10^5 CFU of bacteria per ml from properly collected and transported clean-voided midstream urine specimens are often clinically significant, the reduction of the numbers of bacteria in such samples to below 10^4 CFU per ml represents a major drawback to the use of the kit and limits its utility in follow-up cultures from patients receiving antimicrobial therapy. This reduction in numbers of bacteria could not be ascribed to dilution of the urine by the preservative and was not limited to any specific organism group.

Agreement between the results for the initial cultures of original urine samples yielding $\geq 10^5$ CFU per ml and the results for samples preserved for 24 h was at least 98%, a figure comparable to that reported by Lauer et al. (7); however, the agreement in the two phases of our study between the results for initial cultures of original urine samples yielding $\geq 10^5$ CFU per ml and the results for samples preserved for 24 h averaged 85%, which is somewhat less than that (93.2%) reported by Lauer et al. (7). Differences between our two studies related to the number of samples and distribution of species compared

TABLE 1. *Agreement between number of CFU per milliliter of an original urine specimen and number of CFU per milliliter of a sample stored in B-D Urine Culture Kit*

No. of positive specimens	Original urine (CFU/ml)	Agreement (%) at indicated preservation times of results for urine stored in B-D Culture Kit											
		Initial				24 h				48 h			
		$\geq 10^5$[a]	10^4-10^5	10^3-10^4	$\leq 10^3$	$\geq 10^5$	10^4-10^5	10^3-10^4	$\leq 10^3$	$\geq 10^5$	10^4-10^5	10^3-10^4	$\leq 10^3$
Phase 1													
71	10^5	98.6	1.4			87.3	7.1	4.2	1.4	80.3	12.7	2.8	4.2
29	10^4-10^5	13.8	79.3	6.9		13.8	55.2	31.0		10.3	48.3	34.5	6.9
Phase 2													
115	10^5	98	2			82.6	7.8	4.4	5.2	62.6	13.9	10.4	13.1
24	10^4-10^5		100			8.3	45.8	33.3	12.6	8.3	29.2	37.5	25

[a] Results are expressed in CFU per milliliter.

TABLE 2. *Number of isolates and discrepancies by organism group*

Organism	Study phase one			Study phase two		
	Isolates (no.)	Discrepancies (no.)[a] at:		Isolates (no.)	Discrepancies (no.)[a] at:	
		24 h	48 h		24 h	48 h
E. coli	46	4	9	30	16	21
Citrobacter diversus				4		1
C. freundii	1			3	1	2
Klebsiella oxytoca	3					
K. pneumoniae	7			25	3	6
Enterobacter aerogenes	1			3		
E. cloacae				4	2	2
Serratia marcescens	1			3	1	1
Proteus mirabilis	3			12		4
P. (Morganella) morganii	3	1	1	2	1	1
Acinetobacter calcoaceticus				4	1	3
Alcaligenes spp.				2	1	1
Pseudomonas aeruginosa	5	1	2	19		5
Staphylococcus aureus	1		1	2		1
S. epidermidis	3	1	2	3	1	1
Streptococcus						
viridans	1			1		
group B	1		1	3	1	1
group D	12	1	5	13	2	5
Yeast spp.	12	5	8	6	3	2

[a] Discrepancies represented differences of ≥ 1 $\log_{10}$ between CFU per milliliter in original samples and CFU per milliliter in preserved samples.

could account for the differences in results.

The B-D Urine Culture Kit is a convenient device for the transport and preservation for up to 24 h of urine submitted for microbiological examination. Since overgrowth by contaminants does not occur, specimens may be collected in clean, nonsterile containers before being drawn into the tube containing the preservative. As such, the kit could be used in a manner like that described by Ellner and Papachristos (5) for dip-slides in a general hospital, where urine specimens are delayed and unrefrigerated in transit to the laboratory. Provided the transit time in the kit does not exceed 24 h, and $\geq 10^4$ CFU per ml in preserved urine is regarded as a positive result, agreement between results for cultures of original urine specimens yielding $\geq 10^5$ CFU per ml and results for preserved urine specimens is within acceptable limits. Caution, however, would have to be taken in interpreting negative results, since approximately one-third of the specimens originally containing 10^4 to 10^5 CFU per ml yielded $<10^4$ CFU per ml in cultures of urine preserved for 24 h.

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