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Urine Culture Transport Tubes: Effect of Sample Volume on Bacterial Toxicity of the Preservative

KIM K. NICKANDER, CAROL J. SHANHOLTZER, AND LANCE R. PETERSON*

Microbiology Section, Laboratory Medicine Service, Veterans Administration Medical Center, Minneapolis, Minnesota 55417

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Stable bacterial counts in urine specimens before culture are necessary to assure the accurate diagnosis of urinary tract infections. Preservative-containing tubes are commercially available for urine transport. As these tube containers are not always filled to the manufacturer's specifications, we studied the effects of stabilizer formulas with low urine volumes. The Sage Urine Culture Tube and the Becton-Dickinson Urine Culture Kit were evaluated by using 30 cultures diluted in urine to 10^5 colony-forming units per ml. Both tube types were injected with 1, 2, 3, and 4 to 5 ml (tube capacity) of urine containing each culture. Specimens were held at 22°C and cultured at 0, 4, and 24 h. Colony counts were corrected for the dilution due to the preservative. The Becton-Dickinson Urine Culture Kits were toxic to *Escherichia coli* and *Klebsiella pneumoniae* in specimens containing up to 2 ml of urine, and the minimum usable amount of urine for reliable results was 3 ml. The Sage Urine Culture Tube maintained the number of bacteria in 1 to 4.5 ml of urine in 83% of the specimens. However, the Sage tube was toxic to *E. coli* when held for 24 h. Quantitative counts of enterococci tended to significantly increase in specimens that contained 2 ml or more of urine with either system. The limitations of preservative-containing tubes for urine transport need to be recognized in order to avoid false-positive and false-negative results.

The time involved in transporting urine specimens from collection to culturing is often a problem in hospitals. Specimens may become overgrown with organisms if they are not received in the laboratory within 1 h or if they are not refrigerated until cultured (1). Two manufacturers have produced urine transport tubes, the Sage Urine Culture Tube (Sage Products, Inc., Cary, Ill.) and the Becton-Dickinson (B-D) Urine Culture Kit (Becton, Dickinson & Co., Rutherford, N.J.), which have a preservative to maintain constant colony-forming unit counts for up to 24 h at room temperature (22°C). The B-D instructions caution that "failure to add greater than 4 ml could result in reduction of organisms in 24 h" and recommend a fill volume of 5 ml. The Sage instructions imply that a minimum of 1.5 ml of urine is acceptable and recommend a fill volume of 4 to 4.5 ml. The purpose of this report is to determine the minimum amount of urine necessary to obtain accurate results with each system.

MATERIALS AND METHODS

Urine transport tubes. The Sage Urine Culture Tube contains 1 ml of 5.5% boric acid solution and is evacuated to draw 4 to 4.5 ml of urine. The B-D tube contains 0.5 ml of a solution made with 250 g of glycerol, 20 g of boric acid, and 10 g of sodium formate per liter and is evacuated to draw approximately 5 ml of urine. One commercial lot of each system was used in the study.

Bacterial strains and inoculum. We collected five isolates each of *Escherichia coli*, *Enterococcus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Morganella morganii* from fresh clinical specimens. Each organism was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 1.5 h and then diluted with sterile water to match the turbidity of a 0.5 McFarland standard. We then added 0.3 ml of this suspension to 30 ml of pooled human urine, known to be sterile by prior culture, to give a final bacterial concentration of 10^5 colony-forming units per ml. Each seeded urine specimen was injected into both tubes in volumes of 1, 2, and 3 ml and the full capacity of the tube, providing 240 specimens.

Cultures. All 240 specimens were plated with a 0.001-ml calibrated loop on sheep blood agar plates in duplicate at 0, 4, and 24 h. The original urine dilution was also plated in duplicate at 0 h. All B-D and Sage tube specimens were held at 22°C (room temperature) between samplings. Sheep blood agar plates were incubated for 18 to 24 h at 37°C. The colonies were counted with a Biotran II automated colony counter (New Brunswick Scientific Co., New Brunswick, N.J.).

Calculations. The number of colonies on the duplicate plates was averaged and corrected for the dilution caused by the volume of preservative fluid in each tube. Without correction for preservative volume in

TABLE 1. Comparison of the effect of urine volume on colony counts of experimentally infected urine preserved in B-D and Sage urine transport tubes

Sample	Colony-forming units (log_{10}) per ml[a]																	
	E. coli			*Enterococcus*			*S. epidermidis*			*P. aeruginosa*			*K. pneumoniae*			*M. morganii*		
	0[b]	4	24	0	4	24	0	4	24	0	4	24	0	4	24	0	4	24
Starting urine	5.135			5.141			4.970			5.056			5.089			5.217		
B-D, 1 ml	4.817[c]	4.165[c]	3.276[c]	5.298[c]	5.316[c]	5.168	5.058	5.065	4.771[c]	5.185	5.091	4.413[c]	4.792[c]	3.839[c]	3.606[c]	5.260	5.251	4.837[c]
B-D, 2 ml	5.153	4.927[c]	4.910[c]	5.245	5.302[c]	5.336[c]	5.038	5.044	4.937	5.142	5.080	5.014	5.154	4.804[c]	4.711[c]	5.285	5.191	5.234
B-D, 3 ml	5.068	5.044	5.046	5.221	5.261	5.351[c]	5.047	5.059	4.914	5.140	5.156	5.081	5.122	5.041	4.985	5.257	5.256	5.252
B-D, full	5.129	5.083	5.127	5.164	5.262	5.355[c]	5.034	5.028	4.944	5.114	5.099	5.078	5.104	5.117	5.104	5.273	5.239	5.234
Sage, 1 ml	5.217	5.169	5.135	5.308[c]	5.281[c]	5.220	5.095	5.014	4.421[c]	5.232[c]	5.219[c]	4.933	5.123	5.094	4.965	5.399[c]	5.344	5.182
Sage, 2 ml	5.169	5.111	5.130	5.219	5.301[c]	5.349[c]	5.040	5.071	4.803[c]	5.191[c]	5.149	4.942	5.143	5.075	5.062	5.369[c]	5.325	5.236
Sage, 3 ml	5.164	5.117	4.955[c]	5.215	5.263	5.405[c]	4.999	5.068	4.961	5.124	5.170	5.017	5.096	5.127	5.036	5.292	5.268	5.221
Sage, full	5.087	5.063	4.941[c]	5.227	5.320[c]	5.503[c]	5.004	5.116[c]	4.945	5.155	5.131	5.017	5.114	5.121	4.981	5.308	5.268	5.220

[a] Each value is the mean of five isolates.
[b] Duration of storage (hours) at 22°C (room temperature).
[c] Significant change greater than ± 0.129 log_{10} from starting concentration.

the B-D tube system, the colony counts would have been artificially lowered by 33% for the 1-ml urine volume, 20% for the 2-ml volume, 14% for the 3-ml volume, and 9% for the full tube. The absence of a preservative volume correction in the Sage tube would have resulted in an estimate low by 50% in the 1-ml urine sample, 33% in the 2-ml sample, 25% in the 3-ml sample, and 18 to 20% in the full tube. A reduction of 25.7% or more would have been considered a significant decrease in bacterial counts, using three standard deviations as the criteria for a significant change in this study.

The geometric mean was determined for each group of five isolates. The change in bacterial count was determined by subtracting the geometric mean of each group of five isolates at each incubation time from the counts of the initial sample dilution. The reproducibility of the counting method was determined by performing 20 replicate samplings from one full B-D tube containing *E. coli*. The geometric mean and standard deviation were determined from the counts on the 20 determinations.

RESULTS

The 20 replicate determinations from a single tube showed the mean and standard deviation counts to be 5.135 ± 0.043 log_{10}. We subsequently deemed any change in bacterial counts greater than three standard deviations, or ± 0.129 log_{10} (confidence interval, 99.7%), to be a significant change.

Tube incubations of 0 to 4 h. The results of the 0- to 4-h tube incubations are shown in Table 1. Counts of *K. pneumoniae* and *E. coli* were significantly reduced immediately in the B-D tubes containing 1 ml of urine, indicating the toxicity of a high concentration of the B-D preservative on some bacteria. At 4 h, all B-D tubes containing 3 ml or more of urine had remained within ± 0.129 log_{10} of the starting specimen. Half of the B-D tubes containing 2 ml of urine differed by more than ± 0.129 log_{10} from the starting specimen. Although the Sage tubes were nontoxic to all specimens between 0 and 4 h, enterococcus-containing specimens increased by more than 0.129 log_{10} over the starting inoculum.

Tube incubations of 24 h. Specimens containing five of six types of microorganisms in B-D tubes with 1 ml of urine had significantly reduced colony counts by 24 h. *E. coli*, enterococcus, and *K. pneumoniae* counts had also changed by more than ± 0.129 log_{10} in B-D tubes containing 2 ml of urine. In B-D tubes containing 3 ml of urine or more, only enterococcus specimens had changed significantly; all others had remained within ± 0.129 log_{10}. This is in agreement with a previous study on the effectiveness of the B-D tube for gram-negative bacilli (2). Lauer et al. (2) did not find increased colony counts with enterococci at 24 h, but they tested only two cultures of this bacterial species. After

24 h, colony counts of *S. epidermidis* had been significantly reduced in Sage tubes containing 1 and 2 ml of urine. *E. coli* also was significantly reduced in Sage tubes containing 3 ml of urine or more. Enterococcus counts significantly increased in tubes containing 2 or 3 ml or the full amounts of urine in both B-D and Sage tubes.

DISCUSSION

The B-D Urine Culture Kit and the Sage Urine Culture Tube are comparable, although the B-D system appears to be superior when more than 4 h is expected to elapse between specimen collection and actual plating of the urine culture and when the specimen volume is greater than 3 ml. The Sage tube will maintain the number of bacteria in any amount of urine for up to 4 h. When transporting specimens takes longer, one must be aware when using Sage tubes that some bacterial counts, especially *E. coli*, may be significantly reduced, even when the tubes are filled to the manufacturer's specification. B-D tubes are toxic to many bacteria in tubes containing 1 or 2 ml of urine, yet will maintain bacterial counts for up to 24 h in tubes containing 3 ml or more. Neither system, however, prevents growth of enterococci.

Hamilton and Gross reported that B-D tubes gave only 0.6% false-positive results at 24 h; however, there were 10% discrepancies as compared with immediate culture in which the preservative system was used (J. R. Hamilton and C. Gross, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C255, p. 305). However, this clinical study did not comment on specific bacteria or urine volumes in their specimens. Guenther and Washington, in another clinical study, also found deterioration in colony counts in specimens stored in B-D tubes for 24 h. This study also did not comment on specific bacteria or urine specimen volumes (K. L. Guenther and J. A. Washington, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C253, p. 304). Hubbard and Elsasser noted a 40% decrease in positive urine cultures at 24 h when the B-D tube was used in conjunction with automated urine culture screening. They had difficulties primarily with *E. coli* and *S. epidermidis* and did not comment on the urine culture volumes received for culture (W. A. Hubbard and P. J. Elsasser, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C254, p. 305). We did not observe diminished bacterial counts at 24 h for these or other bacteria when 3 ml or more of urine was aspirated into the B-D tube kit.

Landau et al. evaluated the Sage collection tube in a clinical study. They found good agreement between the preservative-containing tube and conventional methods, but did not comment on the organisms studied or the numbers of negative and positive specimens (W. Landau, J. E. Barrett, and R. L. Kaplan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C259, p. 305).

We performed our study with spiked, pooled normal urine with known numbers of bacteria for a determination of the ability of the two commercial systems to maintain stable bacterial counts. This may remove individual patient variation, but it should provide an adequate assessment of the ability of the preservative-containing tubes to function as designed. We found that the B-D system performed well for all bacteria up to 4 h after collection if properly filled, but was toxic to many gram-negative bacilli if 2 ml or less of urine was aspirated into the tube. At 24 h, the B-D system had allowed enterococci to significantly increase in colony counts. Filled Sage tubes were toxic to *E. coli* by 24 h and allowed enterococci to significantly increase in numbers even by 4 h at 22°C.

Finally, correction of counts altered by the preservative volume needs to be considered, as the addition of 3 ml or less to Sage Urine Culture Tubes could result in false-negative cultures unless the dilution factor is considered when reporting colony counts of the specimen. With the B-D system, the dilution factor is not likely to be significant unless 2 ml or less of urine is added, and at that point the preservative will likely be toxic to bacteria in the specimen. Graduations on the side of the tube would aid in an estimation of the volume of urine, allowing for correction of dilution and of possible preservative toxicity.

The commercially available urine transport tubes can be useful when a prolonged time between the collection of a specimen and its receipt in the laboratory is anticipated. However, care must be taken to follow the recommendation for the specimen volume to avoid potential toxicity against bacteria in the specimen. Laboratory personnel also need to be aware of problems of interpretation caused by dilution of the specimen with preservative, especially in the Sage system, and the fact that enterococci may multiply in either tube system with prolonged (≥4 h) storage.

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