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Evaluation of Preservative Fluid for Urine Collected for Culture

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Immediate culture or refrigeration of urine is recommended, but not always practical. Therefore, we evaluated the Becton-Dickinson Urine Culture Kit containing a boric acid-glycerol-sodium formate preservative in a study of 1,000 clinical urine specimens. Each specimen was cultured a total of four times by the surface streak technique with a 0.001-ml calibrated loop. After an initial reference culture (culture 1), a portion of urine was poured into a clean nonsterile paper cup, aspirated into a urine transport tube, and recultured immediately (culture 2). The original specimen cup was refrigerated for 18 to 24 h (culture 3), and the urine transport tube was held at room temperature for 18 to 24 h (culture 4) before repeat cultures. Eighty-eight of the initial reference cultures were positive (pure growth of $>10^5$ bacteria per ml). Eighty-two (93.2%) of the 88 specimens positive on the reference culture were also positive after refrigeration or holding at room temperature in the transport tube for 24 h. There was one false-positive culture from refrigerated urine but none from the transport tube. Mixing of urine in a nonsterile container did not introduce detectable contamination. We conclude that the Becton-Dickinson Urine Culture Kit maintains a stable bacterial population in urine for up to 24 h as reliably as refrigeration. Urine for culture may be collected in a nonsterile container if it is immediately aspirated into the transport tube so that contaminants are not allowed to multiply.

Voided urine is often contaminated with urethral, skin, genital, or fecal flora despite efforts to obtain clean-catch, midstream specimens. Contaminating bacteria are usually present in low numbers ($<10^4$ colony-forming units [CFU] per ml), but at temperatures above 15°C they may multiply rapidly in urine and necessitate prompt inoculation of media or refrigeration (6, 9). Because accurate quantitative cultures are essential to the laboratory diagnosis of urinary tract infection, it is recommended that urine for culture be refrigerated within 1 or 2 h of collection (2, 6). Unfortunately, transportation of specimens to the bacteriology laboratory frequently is delayed by many hours, and specimens often are not refrigerated before their arrival (5). Consequently, the culture results may be uninterpretable or frankly misleading (3, 5, 10, 12). Chemical preservation of urine would simplify transport and assure the quality of specimens, but previously suggested compounds have been only partially successful.

Sterile containers are also recommended for collection of urine (2); however, they are substantially more expensive than nonsterile containers and add to the cost of culturing urine. If contaminants introduced from nonsterile cups were few and their growth during transport was prevented, the use of nonsterile cups would be possible.

The objectives of this study were to evaluate the boric acid-glycerol-sodium formate preservative in the Becton-Dickinson (BD) Urine Culture Kit (patent pending) and to evaluate the use of ordinary paper cups for collection of urine.

MATERIALS AND METHODS

We examined 1,000 urine specimens submitted to the Clinical Microbiology Laboratory at Colorado General Hospital. The specimens were from children and adults with symptoms suggesting urinary tract infection and from pregnant women being screened for asymptomatic bacteriuria. Urine samples were gathered by nurses and house staff and transported to the laboratory in sterile plastic cups. Specimens were included in the study unless the volume of urine was insufficient (<6 ml).

Upon arrival in the laboratory specimens were refrigerated immediately until they could be plated. Each specimen was cultured four times on 5% sheep blood agar (Trypticase soy base) and MacConkey agar biplates by the surface streak technique with a calibrated 0.001-ml platinum loop at the intervals de-

scribed below (4). Well-mixed urine plated directly from the sterile container according to routine laboratory procedures served as the initial reference culture (plate 1) for all subsequent comparisons. A portion of urine then was poured into a nonsterile, plastic-lined paper cup (8.5-ounce [ca. 0.25-liter] Lily cup no. 8 RL6V; Owens-Illinois, Toledo, Ohio), drawn into a BD Urine Culture Kit, mixed briefly in a Vortex mixer, and recultured (plate 2). The BD Urine Culture Kit consists of a rubber-stoppered glass tube containing boric acid, glycerol, and sodium formate and a disposable plastic transfer device that draws 4 to 6 ml of urine by vacuum into the tube (J. Mehl and J. S. Desai, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C176, p. 339). The original specimen was refrigerated (4 to 10°C) for 18 to 24 h and the urine transport tube was left on the laboratory bench at room temperature (25 ± 2°C) before repeat cultures (plates 3 and 4, respectively). Agar plates were incubated for 18 to 24 h at 35°C in a 5% CO_2 atmosphere. Colony counts were read from the blood agar side of the biplate.

For purposes of data analysis, urine specimens that grew $>10^5$ CFU of a potential urinary tract pathogen per ml in pure culture were considered positive. Isolates from positive urines were identified by standard biochemical tests at least to the genus level. Cultures growing $<10^5$ CFU/ml or a mixture of organisms were considered negative. Isolates from negative cultures were identified and reported only by colonial appearance, Gram stain, and pattern of growth on sheep blood agar and MacConkey agar (e.g., gram-negative rods, gram-positive cocci, and mixed gram-negative rods and gram-positive cocci). Colony counts were recorded as $>10^5$, 10^4–10^5, 10^3–10^4, or no growth. A single colony was classified as no growth.

RESULTS

Urine usually was collected by the clean-catch, midstream method (78.5%), but some specimens were obtained by single catheterization (8.2%) or from indwelling catheters (7.7%). Only a few were unspecified collections (5.6%).

TABLE 1. *Comparison of quantitative urine cultures after refrigeration or chemical preservation at room temperature for 18 to 24 h with results of reference cultures*

Plate 1: Reference culture[a] (initial plating)	No. of cultures	No. of cultures in agreement with reference (%)		
		Plate 2[b]	Plate 3[c]	Plate 4[d]
Positive ($>10^5$ CFU/ml)	88	84 (95.4)	82 (93.2)	82 (93.2)
Negative ($<10^5$ CFU/ml or mixed flora)	912	911 (99.9)	911 (99.9)	912 (100)

[a] Sterile container.
[b] Urine transport tube; plated immediately after passing through clean, nonsterile paper cup.
[c] Sterile container after refrigeration at 4°C for 24 h.
[d] Urine transport tube after passing through clean, nonsterile paper cup and holding at 25°C for 24 h.

The results are summarized in Table 1. Eighty-eight reference cultures were positive, i.e., grew $>10^5$ CFU of a single potential urinary tract pathogen per ml. *Escherichia coli* was identified most commonly in positive cultures (Table 2). Culture results were comparable after refrigeration or chemical preservation for 24 h; both methods detected 82 of the 88 positive reference cultures (93.2%). The discrepancies mostly consisted of growth in addition to the pathogen of a few gram-positive urogenital tract contaminants on comparison plates but not on the reference cultures (Table 3).

A total of 912 reference cultures were negative, including 349 plates that showed no growth. Of the 912 specimens only 1 was false-positive after 24 h of refrigeration, and none was false-positive after chemical preservation. Bacterial counts of the predominant microorganisms in the urine specimens preserved by either refrigeration or the boric acid-glycerol-sodium formate solution agreed within 1 $\log_{10}$ CFU/ml with the reference culture 98% of the time.

Urine drawn into the transport tube from a clean, nonsterile paper cup and plated immediately gave quantitative results similar to the reference urine cultures plated at the same time from sterile containers (Tables 1 and 3). Thus, no misleading contamination was introduced by passing the urine through ordinary paper cups.

DISCUSSION

Urine is an excellent culture medium for many bacteria. Contaminants initially present in low numbers (10^2 to 10^4 CFU/ml) may multiply in urine to 10^5 to 10^8 CFU/ml (3, 5, 12). The minimum time required for contaminants to reach

TABLE 2. *Identification of bacteria isolated from 88 positive urine cultures*[a]

Bacteria	No. of cultures (%)
Escherichia coli	55 (62.5)
Coagulase-negative staphylococci	13 (14.8)
Pseudomonas aeruginosa	4 (4.5)
Klebsiella pneumoniae	4 (4.5)
Proteus mirabilis	3 (3.4)
Citrobacter sp.	2 (2.3)
Enterococci	2 (2.3)
Staphylococcus aureus	2 (2.3)
Enterobacter sp.	1 (1.1)
Serratia marcescens	1 (1.1)
Beta-hemolytic streptococci (not group A or D)	1 (1.1)

[a] $>10^5$ CFU/ml in pure growth of a urinary tract pathogen specifically excluding *Corynebacteria* sp., *Haemophilus vaginalis*, *Lactobacillus* sp., and non-enterococcal, alpha- and nonhemolytic streptococci.

TABLE 3. *Discrepancies between results of quantitative reference urine cultures and urine cultures after refrigeration or chemical preservation*[a]

Reference culture[b] (initial plating)	Urine transport tube[c] (initial plating)	Refrigeration[b] (4°C for 24 h)	Urine transport tube[c] (25°C for 24 h)
>10^5 *E. coli*	10^4-10^5 *E. coli*	10^4-10^5 *E. coli* 10^3-10^4 mixed+	10^3-10^4 *E. coli* 10^3-10^4 mixed+
>10^5 *E. coli*	I	I & 10^3 mixed+	I & 10^3 mixed+
>10^5 *E. coli*	I	I & 10^3 mixed+	I
>10^5 *E. coli*	I	I	10^4-10^5 *E. coli* & 10^4-10^5 lactobacilli
>10^5 β-streptococcus (not group A or D)	I & 10^4-10^5 nonhemolytic streptococci	I	I
>10^5 *S. aureus*	I	I & 10^4-10^5 LF	I
>10^5 *Klebsiella*	10^4-10^5 *Klebsiella*	I	10^4-10^5 *Klebsiella*
>10^5 *E. coli*	I	I	I & 10^3-10^4 *S. epidermidis*
>10^5 *S. epidermidis*	I	10^4-10^5 *E. coli* & 10^4-10^5 mixed+	I
>10^5 *E. coli*	I	10^4-10^5 *E. coli* & 10^4-10^5 mixed+	I & 10^4-10^5 mixed+
>10^5 *E. coli*	I & 10^3-10^4 *S. epidermidis*	I	I

[a] Culture results expressed as CFU per milliliter of urine. I, Identical result to reference culture; mixed+, mixed gram-positive flora; LF, lactose fermenter.
[b] Sterile container.
[c] Clean, nonsterile paper cup.

concentrations indicative of significant bacteriuria (>10^5 CFU/ml) is variable and depends upon the growth rate of the organism, the initial degree of contamination, and the ambient temperature; however, most studies set the maximum safe delay in plating at 2 to 4 h (3, 5, 6, 12). The usual recommendation that urine be plated within 1 to 2 h or be refrigerated is, therefore, sensible, although sometimes unrealistic (2, 6).

Chemical preservatives have been tried in an attempt to eliminate the need for refrigeration of specimens. Two preservatives have been recommended: NaCl-polyvinylpyrrolidone (PVP) and boric acid. The activity of NaCl-PVP depends on the bacteriostatic activity of NaCl; PVP serves as a protective colloid and aids in dispersal of bacteria. NaCl-PVP was reported to be effective in experiments with simulated infected urine at final concentrations in urine of 9% NaCl and 1% PVP (1). In a clinical trial NaCl-PVP also proved effective, but 6 of 69 specimens showed a slight decline in bacterial counts over 6 to 8 h (1).

Boric acid has been used to preserve blood and milk samples for serological or bacteriological examination and urine for chemical analyses (8). When used to preserve urine for culture, boric acid (final concentration of 1.8%) was effective for up to 4 days (8). No *E. coli* were inhibited, but the viable counts of some strains of *Proteus* and *Klebsiella* were reduced.

A comparative study of 1.8% boric acid, NaCl-PVP, and refrigeration for preservation of simulated infected urine showed that boric acid was toxic for *Pseudomonas* sp. in less than 24 h and toxic for *E. coli* and *Proteus* sp. after 24 h. Moreover, boric acid powder was difficult to dissolve and required prolonged mixing. NaCl-PVP was toxic for *E. coli* and appeared not to retard the growth of *Micrococcus* subgroup 3. Refrigeration stabilized the viable count for up to 3 days (11).

The BD Urine Culture Kit uses boric acid and sodium formate dissolved in glycerol. Because the boric acid and sodium formate are already dissolved before the addition of urine to the tube, prolonged mixing is not required. In this study the boric acid-glycerol-sodium formate solution did not appear to be toxic for *E. coli*, *Proteus* sp., or *Pseudomonas* sp. Most importantly, the BD Urine Culture Kit was clearly as effective as refrigeration in maintaining urine reliably for at least 24 h before quantitative cultures.

An important finding was that nonsterile specimen cups did not contaminate specimens sufficiently to influence the interpretation of colony counts. This confirms the conclusions of a previous study that used Dixie cups in a screening program for asymptomatic bacteriuria in children (7). The cost of a disposable, nonsterile cup is about $0.01 compared with about $0.10 to $0.25 for sterile plastic urine containers. The continued use of sterile containers does not seem to be necessary as long as urine is drawn immediately into the transport tube.

Several questions about the urine transport tube remain to be answered. First is whether or

not the boric acid-glycerol-sodium formate preservative will ultimately prove to be toxic for particular species or strains of bacteria not encountered in this study. Second, the duration of the preservative's effect beyond 24 h needs to be determined, because specimens that must be sent through the mail to a distant laboratory may not arrive for several days. Third, the preservative's effect on leukocytes, erythrocytes, casts, Gram stain, and tests for glucose, protein, hemoglobin, and specific gravity should be studied, since physicians often request these tests when they submit urine for culture. Porter and Brodie found that cellular elements, albumin, and glucose were well preserved by 1.8% boric acid at room temperature, but cellular elements deteriorated in urine at room temperature without preservative as the urine became alkaline (8).

In summary, the BD Urine Culture Kit was as effective as refrigeration for up to 24 h in maintaining the quality of urine for culture. The chemical preservative stabilized the bacterial counts of urinary tract pathogens and the usual contaminants. No significant contamination resulted from the use of nonsterile containers before filling the transport tube. The BD Urine Culture Kit is a reliable and convenient alternative to refrigeration and should be particularly useful in those settings where prompt refrigeration or plating of urine cannot be assured.

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