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Comparison of clinical performance of commercial urine growth stabilization products☆

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ABSTRACT

Introduction: Urine specimens for quantitative culture for the diagnosis of urinary tract infection may be unreliable due to bacterial overgrowth within 4 h after collection, at room temperature. Because specimen transportation may take longer than 4 h, urine preservatives may reduce overgrowth. Further evidence is needed to support a recommendation for use of preservative and to compare preservative products.
Methods: Consecutive midstream urine specimens submitted for culture were quantitatively cultured on receipt and then inoculated into 3 storage conditions [BD Urine Vacutainer (BD), Copan UriSwab (US), and refrigeration, with a room temperature control] for 72 h, with quantitative culture performed every 24 h. Odds ratio for significant growth interpretation was reported.
Results: Ninety-five of 501 (19.0%) urine specimens demonstrated significant growth. Within 24 h of storage, unpreserved urine at room temperature demonstrated a significantly increased odds ratio for significant growth as compared to preserved urine, and urine in refrigeration demonstrated similar odds ratio for significant growth as compared to preserved. There was no significant difference between the performance of US and BD. Over 48 and 72 h of storage, odds ratio for significant growth further increased.
Conclusions: Preservation performed similarly to refrigeration. Preserved urine demonstrated a doubling in odds ratio for significant growth after 24 h. This increase may negatively impact antibiotic treatment decisions.

1. Introduction

Urine is the most commonly submitted specimen to the clinical bacteriology laboratory, and urine culture results influence antibiotic treatment significantly (Burd and Kehl, 2011). Reduction of urine culture contamination through preanalytical quality initiatives may significantly impact antimicrobial stewardship. Urine forms a suitable growth medium for bacteria during transportation to the laboratory. Therefore, bacterial counts present at receipt may be greater than counts present on collection, providing falsely positive culture results and encouraging inappropriate antibiotic therapy (Gupta et al., 2011). Within 2 h of collection, bacterial populations in fresh urine held at room temperature may increase by 1 $\log_{10}$ (Hindman et al., 1976), with a bacterial doubling time in urine approximating the doubling time in growth media (Lewinson, 2008). After 4 h at room temperature, interpretation of urine culture is unreliable (Jefferson et al., 1975; Wheldon and Slack, 1977).

With increasing centralization of bacteriology laboratories, transportation time for urine specimens is increasing. Commercial products are available which stabilize bacterial growth in urine during transportation since refrigeration during the entire transportation period is not generally feasible.

A meta-analysis of preanalytical practices for urine culture concluded that boric acid and refrigeration both preserved urine adequately over 24 h, although the strength of this evidence was considered low (LaRocco et al., 2016). The authors did not make a recommendation on the use of preservative or refrigeration and requested that more systematic studies be performed.

Two new products have been marketed since initial studies were reported. The Becton Dickenson Vacutainer® Plus C + S preservative tube (BD, Becton Dickenson, USA) contains lyophilized boric acid, sodium formate, and sodium borate. It was evaluated using 79 clinical urine specimens (30 with growth), funded by the company, and found to preserve equally to refrigeration over 48 h at room temperature (Eisinger et al., 2011). The Copan UriSwab® (US, Copan Diagnostics, USA) contains boric acid and sodium formate impregnated on a sponge. It was evaluated in a comparison with BD using 293 clinical urine specimens (51 with growth) kept at room temperature over 48 h but without a refrigeration control. No statistical analysis was performed

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(Bourbeau and Swartz, 2007). One author worked for Copan. Based on these very limited unpublished data, both companies claim that stabilization products can maintain bacterial populations for up to 48 h without refrigeration. However, users are uncertain how controlled laboratory studies generalize to real-world application of these products.

One comparative performance study of BD and US has been published (Rennie et al., 2016). Two hundred outpatient urines were collected into both products and cultured after 1–24 h of transportation. The study did not compare equal durations of transportation between products.

We previously evaluated US using 816 consecutive clinical urine specimens (165 with growth) as compared to refrigeration using clinical interpretation categories for significance of growth. We found a statistically significant difference in percent positives within 24 h of incubation [odds ratio (OR) for significant growth in refrigeration compared to room temperature 1.03 (95% confidence interval {CI}= 0.96–1.10), OR for significant growth in US compared to room temperature 1.54 (95% CI=1.35–1.77), $P < 0.001$]. This suggested that US was less effective than refrigeration in clinical application (Stokes et al., 2012). The study was funded by Copan.

Previous evaluations of commercial urine transport products have demonstrated design weaknesses. These studies were not reported according to STARD guidelines (Bossuyt et al., 2003) and often examined selected specimens instead of consecutive, did not describe the population that specimens were collected from, did not use blinding among laboratory staff, did not report statistical significance of differences, and did not report the role of the funding source. Studies must report changes in clinical interpretation categories (from "no significant growth" to reported growth) to consider the actual impact of overgrowth during transportation on treatment decisions. Studies reporting control strains inoculated into urine do not represent clinical conditions.

The widespread use of commercial urine preservative products may not be adequately supported by the existent data, and we felt that a large prospective, effectiveness comparison study between the 2 products would be justified. The hypothesis was that commercial urine preservatives are not as effective as refrigeration in the maintenance of bacterial populations over time.

2. Materials and methods

2.1. Specimens

Consecutive urine specimens received over 10 days in July 2012 at a tertiary care hospital microbiology laboratory in St. John's, Canada, were considered. Specimens not collected via midstream method, that contained less than 20 mL, or mislabeled were excluded. St. John's has a population of 219,000 people, and 1 microbiology laboratory services the city, reporting approximately 30 positive urine cultures per day. Seventy-five percent of urines are received from outpatients. Although the age of urine specimens at receipt is not measured, most are received within 24 h of collection.

2.2. Reference standard testing

Urine specimens were inoculated on receipt onto blood agar and MacKonkey agar using automated inoculation (Copan WASP®) with a calibrated 1-μL loop. Specimens with low volume (20–35 mL) were inoculated manually. Plates were incubated at 35 °C in ambient air for 18–24 h. Uropathogens (Gram-negative bacilli, *Enterococcus*, beta-hemolytic *Streptococcus*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *lugdunensis*, *Corynebacterium urealyticum*, *Aerococcus urinae*, and *sanguinicola*) were identified according to laboratory protocol by a single untrained investigator (YG) without blinding. Interpretation of growth followed laboratory protocol (Table 1).

Table 1
Growth interpretation.

Types	Count (CFU/mL)	Interpretation
1	$<10^4$	No significant growth
1	$>10^4$	Significant growth
2	Both $>10^5$	Significant growth
≥2	One $>10^5$, Others $<10^5$	Significant growth
≥2	All $<10^5$	No significant growth
Any	All $<10^4$	No significant growth
≥3	All $>10^5$	Mixed growth (no significant growth)

2.3. Urine preservative testing

At the time of receipt, 3 US and 3 BD containers were charged with urine according to manufacturer's recommendations and stored at room temperature. One sterile container (SC) of residual urine was incubated at 4 °C, and 1 control SC was incubated at room temperature. US, BD, and SC were inoculated onto blood and MacKonkey agars at 24, 48 and 72 h. Preservative containers were only entered once to perform inoculation of culture, but sterile containers were reentered with each inoculation of culture.

2.4. Ethics

Specimens were anonymized before inclusion. Permission from the local ethics committee and patient consent were not required.

2.5. Statistical methods

Sample size was not precalculated. The outcome was proportion of urines with significant growth at each time point as compared to results at the time of receipt. Logistic regression (generalized estimation equation model) was performed, and reported as an OR with 95% CI. Subgroup analysis by organism type was also performed.

3. Results

A total of 1155 specimens were received during the study period. Six hundred fifty-four of 1155 (56.6%) were rejected due to collection from catheters or surgical procedures, inadequate volume, missing data, or mislabeling. Five hundred one specimens were included in the study, and 501 results were available in each storage condition arm.

At time zero, 95/501 (19.0%) specimens demonstrated significant growth. After 24 h, significant growth was observed in 205/501 (40.9%) of specimens in BD, 191/501 (38.9%) of specimens in US, 181/501 (36.1%) of specimens in the refrigerated SC, and 355/501 (70.9%) in the room temperature SC. After 48 h, significant growth was observed in 255/501 (50.9%) of specimens in BD, 253/501 (50.3%) of specimens in US, 218/501 (40.5%) of specimens in the refrigerated SC, and 395/501 (78.8%) in the room temperature SC. After 72 h, significant growth was observed in 302/501 (60.3%) of specimens in BD, 272/501 (54.3%) of specimens in US, 253/501 (50.5%) of specimens in the refrigerated SC, and 403/501 (80.4%) in the room temperature SC.

Table 2 reports the results of logistic regression analysis. Within 24 h of storage, urine in sterile container at room temperature demonstrated significantly increased OR for significant growth as compared to US and BD. Urine in US and BD at room temperature for 24 h demonstrated an increased OR of significant growth (US 2.72, BD 2.96), although these were (nonsignificantly) higher ORs for significant growth compared to refrigeration. There was no significant difference between the performance of US and BD. Over 48 and 72 h of storage, OR for significant growth further increased.

Fig. 1 describes the specific organisms detected during storage, including a control rate of nonsignificant growth (mixed growth). Gram-negative bacilli, streptococci, and enterococci increased during storage at a similar slope, while staphylococci overgrew at a slower

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