

# Preserving Bacterial Viability in Human Urine

Phosphate Buffer as an Alternative to Refrigeration and Boric Acid

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## INTRODUCTION

Urinary tract infections (UTIs) are common, especially in women, and diagnosis relies on accurate bacterial culture of urine samples. However, delays in sample processing due to transport or limited lab access can lead to false negatives or positives. Refrigeration (up to 24 hours) and boric acid (up to 48 hours) are current preservation methods, but both have limitations. My research investigates the viability of bacterial cultures in refrigerated urine samples stored up to 72 hours and proposes phosphate buffer as a future alternative preservative.

Urine culture for GBS test; Picture retrieved from:  
<https://www.groupbstrepinternational.org/gbs-testing.html>



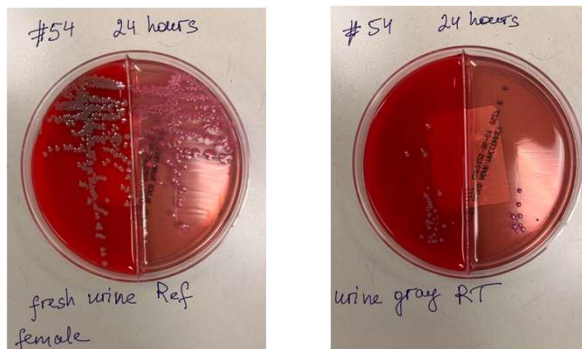
**Figure 1.** *E. coli* Growth on Differential Media (24 Hours Post-Collection)  
This image shows a urine sample plated 24 hours after collection on agar, containing Blood Agar (left) and MacConkey Agar (right). The fresh, refrigerated urine was streaked using a 10  $\mu$ L calibrated loop. (Picture is taken by Hanna Powell)

## METHODS AND MATERIALS

- 80 fresh human urine samples were collected using sterile technique.
- Samples were stored at 4C (refrigeration) in sterile containers with no preservatives.
- Plated every 24, 48, and 72 hours on Blood and MacConkey agar using a 10  $\mu$ L inoculating loop (double plate technique).
- Bacterial growth was recorded based on colony presence, morphology, and CFU estimation ( $\geq 10,000$  CFU/mL considered clinically positive).
- Neither phosphate buffer nor boric acid has been used during this phase.

## OBJECTIVE

To evaluate bacterial viability in refrigerated human urine samples over a 72-hour period, and to explore the potential use of phosphate buffer as a future alternative to refrigeration and boric acid for urine sample preservation.



**Figure 2.** Comparison of Gram-negative bacterial growth on MacConkey agar after 24 hours incubation. Left plate: fresh urine sample (female) stored under refrigeration (Ref) shows numerous pink to purple lactose-fermenting Gram-negative colonies. Right plate: urine sample from the same patient stored at room temperature (RT) in a gray-top tube shows fewer colonies with similar lactose-fermenting Gram-negative morphology. (Pictures are taken by Hanna Powell)

## FUTURE DIRECTIONS

- Test phosphate buffer (pH 5-6) as a preservative in urine samples.
- Monitor stability and bacterial survival for up to 96 hours. Compare phosphate buffer vs boric acid vs refrigeration.
- Use MALDI-TOF for species-level identification of cultured organisms.
- Evaluate safety, cost-effectiveness, and field applicability of phosphate buffer in clinical settings.

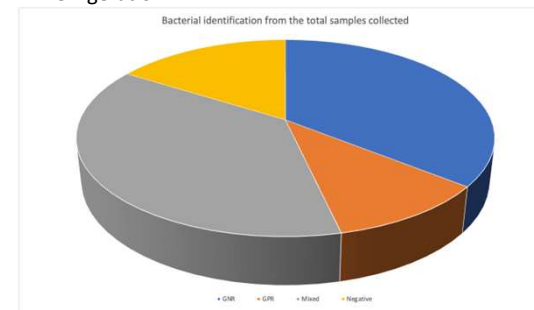
## REFERENCES



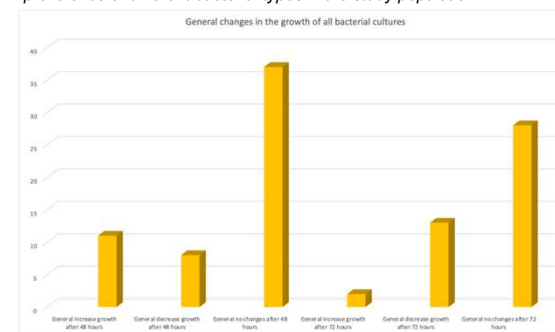
Fresh Urine Data have been collected in the LabCorp Microbiology Dept. under supervision of Dr. Naccache, the technical director of the microbiology department of the LabCorp, MLS, PhD in microbiology

## RESULTS

- Gram-negative rods (e.g., *E. coli*, *Proteus*): 25% increased growth at 48h; 25% fell below detection by 72h.
- Gram-positive cocci (e.g., *Streptococcus*, *Aerococcus*): showed instability with 12.5% dropping below detection by 48h.
- Mixed flora and variability were observed across samples. 12 samples showed no growth throughout the test window.
- Overall, 40% of samples showed loss or reduction in bacterial growth by 72 hours, raising concerns about relying solely on refrigeration.



**Figure 3.** Distribution of bacterial groups recovered from urine samples cultured on Blood and MacConkey agar. The pie chart represents the total number of samples categorized by type of bacterial growth: Gram-negative rods (GNR), Gram-positive cocci (GPC), mixed urogenital flora, and no bacterial growth. Percentages reflect the proportion of each group among all cultured samples, illustrating the relative prevalence of different bacterial types in the study population.



**Figure 4.** General changes in bacterial group recovery from urine samples plated on Blood and MacConkey agar at 24 (fresh), 48, and 72 hours after collection under refrigeration (4°C). The graph displays trends in the number of samples showing growth of Gram-negative rods (GNR), Gram-positive cocci (GPC), mixed urogenital flora, and no bacterial growth over time. Results illustrate the impact of prolonged cold storage on the viability and detectability of different bacterial groups.