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Abstract: Preserving Bacterial Viability in Human Urine: Phosphate Buffer as an Alternative to refrigeration and Boric Acid.

Accurate diagnosis of urinary tract infections (UTIs) depends on preserving bacterial viability in urine samples before culture. In many clinical and field settings, delays in processing can lead to false-negative results if bacteria die off or overgrow during storage. For this project, I personally collected and analyzed 80 human urine samples stored at 4°C, monitoring bacterial recovery at 24, 48, and 72 hours. A sample was considered positive for bacterial growth if colony-forming units (CFU) exceeded 10,000 CFU/mL, the clinical threshold for UTI diagnosis. My preliminary data showed that *E. coli* and *Proteus vulgaris* (Gram-negative rods) increased in 25% of samples after 48 hours but declined below detection in another 25% by 72 hours, suggesting instability during storage. Gram-positive cocci like *S. pyogenes* and *Aerococcus urinae* also showed variable recovery, with several dropping below the diagnostic threshold after extended refrigeration.Overall, about 40% of samples showed reduced or absent bacterial growth by 72 hours, raising concerns about relying on refrigeration alone. Although boric acid is often used as a preservative, it can suppress bacterial growth too strongly, especially in low-load or sensitive samples. As an alternative, I propose using a phosphate buffer, which maintains urine pH in the physiological range of 5–6. This range helps prevent ammonia-driven alkalinization and supports bacterial viability without promoting overgrowth. Phosphate buffer's gentle, stabilizing chemistry makes it a promising method for improving urine sample preservation and diagnostic accuracy when immediate processing is not possible.