Boric acid-free UriSponge™: performance and benefits

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Introduction

Urinary Tract Infections (UTIs) are among the most common bacterial infections affecting millions of people worldwide every year. Characterized by discomfort and frequent urination, UTIs can range from mild to severe and affect any part of the urinary system, including the kidneys, ureters, bladder, and urethra.

Most UTIs are caused by bacterial pathogens, and the most common by far is ***Escherichia coli***. *E. coli* is a type of bacteria that naturally resides in the gastrointestinal tract, but certain strains can cause infections when they enter and proliferate in the urinary tract. It is responsible for approximately **80-85%** of all UTIs.

Apart from *E. coli*, several other bacteria and pathogens can also cause UTIs, though they are less frequent. Some of the other common pathogens associated with UTIs include:

Staphylococcus saprophyticus: it is the second most common cause of UTIs, especially in young sexually active women.

Klebsiella pneumoniae: it is a common cause of UTIs in healthcare settings and can be associated with severe infections, especially in individuals with compromised immune systems.

Proteus mirabilis: it is known for its ability to produce urease, an enzyme that contributes to the formation of kidney stones, worsening the severity of the infection.

Enterococcus species: Enterococci can cause both uncomplicated and complicated UTIs, and they are particularly significant due to their **increasing antibiotic resistance**.

Pseudomonas aeruginosa: Usually seen in individuals with catheters or other urinary tract abnormalities, *Pseudomonas* infections can be challenging to treat due to their inherent resistance to many antibiotics.

Group B Streptococcus (*Streptococcus agalactiae*): A common cause of UTIs of concern in pregnant women and individuals with underlying health conditions.

It's important to note that the specific prevalence of these pathogens may vary depending on factors such as age, gender, geographical location, and other underlying health conditions. Additionally, advances in diagnostic techniques and research might reveal changes in the distribution of pathogens over time.

Urine culture preanalytics for

Urine culture is a laboratory test used to identify and determine the type of bacteria or other pathogens that are causing a UTI. It is an essential technique to identify the specific pathogen and its susceptibility to antibiotics, to provide patients with the appropriate antibiotic treatment.

According to Eisinger et al. (*Am J Clin Pathol*. 2013 Sep;140(3):306-13): "Usually, contaminant organisms are present in low numbers, i.e., less than 10^4 colony-forming units (CFU)/mL, whereas uropathogens are present in significantly higher numbers, usually greater than 10^5 CFU/mL. However, over time, even contaminant microorganisms can grow to significantly high

numbers when specimens are left at room temperature (>15°C). The effects of delayed urine culture and the impact of various transport and storage conditions have been described. The current guidelines for urine collection, transport, and culture emphasize the need to use either transport tubes containing preservatives or not exceeding the 2-hour interval from collection to processing. If preservative tubes are not used and a transport time of less than 2 hours may not be achievable, refrigeration of the urine specimen has been shown to be an alternative to limit the overgrowth of organisms; however, it is unrealistic to expect that no urine specimen will spend more than two cumulative hours unrefrigerated in most settings. In the 2005 College of American Pathologists (CAP) Q-Probes study on urine culture contamination, the investigators found that only a few microbiology laboratories enforce the 2-hour cutoff rule for limiting the transport time of urine specimens."

BORIC ACID FOR URINE PRESERVATION

Boric acid is a widely used preservative substance for urine for culture application due to its ability to maintain the viability of the main pathogens causing UTIs and avoid the overgrowth of commensal flora.

Boric acid is included in the Candidate List of substances of very high concern for Authorization published in accordance with Article 59(10) of the REACH Regulation due to its toxicity for reproduction.

This inclusion poses a health concern, especially for long-term exposure, as well as apprehension for its future availability on the market for all commercial devices for urine preservation based on this chemical.

Nonetheless, common organisms causing UTIs, such as ***Escherichia coli***, ***Enterococcus fecalis***, and ***Klebsiella pneumoniae***, have been noted **to be inhibited** when boric acid is used as a storage medium.

COPAN URISPONGE™

Copan UriSponge™ is a sponge-based device intended to transport and preserve urine specimens, transferred from their initial container from the collection site to the testing laboratory. In the laboratory, UriSponge™ specimens are processed using standard clinical laboratory operating procedures for uropathogenic bacteria and yeast culture. The urine adsorption is given by passive adsorption onto the sponge, guaranteeing that only the correct amount of urine is collected and assuring that the ratio between urine and the preservative solution is always respected.

The new UriSponge™ formulation is based on sodium propionate and potassium sorbate, and it is characterized to be completely toxin-free.

UriSponge™ is CE-IVDR and M40-A2 compliant.

VALIDATION OF BORIC ACID-FREE URISPONGE™

MATERIAL AND METHODS

Tests were conducted using the organisms recommended by the Clinical and Laboratory Standards Institute (CLSI) M40-A2 and other ATCC® strains.

UriSponge was validated using the most common etiological agents that cause UTIs.

The following microorganism and strains were used for the validation:

GRAM -
M. morganii - ATCC®25829
E. coli - ATCC®25922
E. cloacae - ATCC®13047
P. mirabilis - ATCC® 7002
C. freundii - ATCC® 8090
K. pneumoniae - ATCC® 700-603
P. aeruginosa - ATCC® 27853

GRAM +
E. faecalis - ATCC® 29212
S. saprophyticus - ATCC® 15305

YEAST
C. glabrata – ATCC® 15126
C. albicans – ATCC® 24433

To further explore the performance of the new formulation free of boric acid, we also include the following clinically isolated strains:

E. faecalis - clinical strain
E. coli - clinical strain
P. rettgeri clinical strain
M. morganii - clinical strain

A suspension of approximately $1.5x10^4$ CFU/mL from fresh microorganism culture was used to spike artificial urine to reach a concentration of $1.5x10^3$ CFU/mL useful to inoculate the device.

Urine was collected by dipping the UriSponge™ into the cup for 5 seconds.

UriSponge™ was verified to preserve viability after storage at 2-8°C (refrigeration) and at 20-25°C (room temperature) for up to 48 hours.

From every time-point/tube combination, 100 µL were plated in triplicate on the appropriate culture medium. Plates were incubated at 35°C for 20/24 hours to count CFU and delta log from baseline (Time 0).

Urine was released from the sponges by either centrifugation or by hand "shaking."

RESULTS

The new boric acid-free formulation has been shown to be highly effective in preserving the viability of the most common pathogens that cause UTIs.

Transport at 20-25 °C

Organism	24h	48h
GRAM -		
M. morganii	-0.15	0.02
E. coli	-0.36	-0.15
E. cloacae	-0.16	-0.18
P. mirabilis	-0.11	-0.25
C. freundii	-0.15	-0.26
K. pneumoniae	-0.22	-0.28
P. aeruginosa	-0.24	-0.76
GRAM +		
E. faecalis	0.07	0.12
S. saprophyticus	-0.02	-0.19
YEAST		
C. glabrata	-0.09	0.02
C. albicans	0.08	0.35

Table 1: *CFU/mL logarithmic difference at 24 and 48 hours vs T0. Inoculated UriSponge was kept at RT (20 – 25 °C)*

As shown in Table 1, the new boric-acid-free formulation demonstrated an excellent capacity to preserve the viability of UTIs pathogens, as well as to avoid overgrowths.

On Gram-negative bacteria, at 24 hours, most of the pathogens decrease their concentration less than 0.25 log cycle. Only *E. coli* decreases its concentration to 0.36 cycles, but it seems to recover vitality at 48 hours.

At 48 hours, only *P. aeruginosa* shows a decrease in vitality at 0.76 cycles. This decrease is, in any case, below the 1 log cycle, which is set as a limit by the CLSI M40-A2. Thus, this decrease in viability is not of concern.

Gram-positive bacteria show perfect vitality preservation at booth 24 and 48 hours.

Among yeast, *C. glabrata* remains perfectly viable without any overgrowth, whereas *C. albicans* slightly increases its concentration during the 48-hour time frame.

Transport at 2-6 °C		
Organism	**24h**	**48h**
GRAM -		
E. cloacae	-0.09	-0.08
M. morganii	-0.20	-0.14
E. coli	-0.18	-0.15
P. aeruginosa	-0.02	-0.15
P. mirabilis	-0.07	-0.32
K. pneumoniae	-0.31	-0.41
C. freundii	-0.67	-0.88
GRAM +		
S. saprophyticus	0.01	-0.15
E. faecalis	-0.31	-0.31
YEAST		
C. albicans	-0.08	-0.17
C. glabrata	-0.21	-0.32

Table 2: *CFU/mL logarithmic difference at 24 hours and 48 hours vs T0. Inoculated UriSponge was kept refrigerated (2-6 °C)*

If UriSponge™ is kept refrigerated, no major performance earn is measured compared to the same samples kept at room temperature.

Among gram-negative bacteria, only *Citrobacter freundii* showed a significative decrease in vitality, mostly probably due to a metabolic delay caused by the low temperature. On the other hand, *P. aeruginosa* looks to improve its fitness if preserved at 2-6 °C vs 20-25 °C.

Among gram-positive bacteria and yeast, the preservation performances at 2-6 °C are comparable to the ones at room temperature.

Recovery tests were also carried out on clinically isolated samples.

Transport at 20-25 °C		
Organism	**24h**	**48h**
Clinical isolated samples		
E. faecalis	0.14	0.00
E. coli	-0.19	-0.31
P. rettgeri	-0.14	-0.30
M. morganii	-0.15	0.02

Table 2: *CFU/mL logarithmic difference at 24 hours and 48 hours vs T0. Inoculated UriSponge was keep at RT (20 – 25 °C)*

As reported in Table 3, clinically isolated samples were also preserved with minimal modification of the final recovery after 48 hours of preservation.

Finally, as urine can be released from the sponges by either centrifugation or manual "shaking," the differences in sample release were tested with specific strains.

Organism	Release by centrifugation	Manual release
C. albicans	*0.00*	*0.11*
E. coli	*0.21*	*0.16*
S. saprophyticus	*-0.06*	*-0.03*

Table 4: *Logarithmic reduction (-) or increase (+) in inoculated urine with different release method*

No significant differences were measured between the two release methods.

CONCLUSIONS

The new boric acid-free preservative solution used by the UriSponge™ has proved extremely effective in preserving the most common microorganisms that cause UTIs.
The preservation performances assure a good recovery for all reference strains of gram-positive, gram-negative, and yeast tested. Also, clinically isolated strains were preserved with minimal differences in the final recovery, even at 48 hours.

The new formulation, free of boric acid, looks extremely efficient at either room (20 – 25 °C) or refrigerated temperature (2-6 °C).

Both release methods – by centrifugation and manually – efficiently recovered the samples from the sponges.

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