Boric acid-free UriSponge™: comparison with commercially available boric acid transport system

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INTRODUCTION

Urinary tract infections (UTI) are one of the most common causes of bacterial infection worldwide, thus it is not surprising that the highest percentage of samples normally managed and analyzed by microbiology labs are urine.

HOW URINE CULTURE IS CARRIED OUT

Urine culture is the standard technique for the diagnosis of UTI.

One microliter of urine is usually seeded onto an agar plate. According to common guidelines, blood agar (non-selective medium) and MacConkey agar (selective and differential for Gram-negative rods) are probably the most commonly recommended and used media for routine urine cultures. As an alternative, chromogenic agar has been proposed as standard media for urine culture Sabouraud agar should be added, in addition to the usual bacterial media, to culture the urine of patients in particular care units or if yeasts have been seen by microscopic examination.

After 18-24 hours of incubation at 35-37 °C the number of colonies on the surface of each medium are counted. Each colony growing on the agar plate represents one colony forming unit (cfu)/µL (according to the size of the loop), which is equal to 1000 cfu/mL.

In freshly voided urine, a value ≥ 100 cfu per bacterial type (corresponding to ≥ 10^5 cfu/mL) has usually been regarded as a cutoff for UTI. If 10–100 colonies are counted (the number of bacteria is between 10^4 and 10^5 cfu/mL), the result should be evaluated according to the clinical status. On the other hand, the probability of UTI is low if the number of colonies is <10 (the number of bacteria is < 10^4 cfu/mL). For cultures containing colonies from two pathogens, UTI is diagnosed if ≥ 100 colonies are counted for at least one of the two pathogens. Sub-cultures, for further identification and antimicrobial susceptibility testing, should be performed for each type counting ≥ 100 colonies, but it is also recommended to request a new sample. If both types have < 100 colonies, for each one, UTI is not likely and the sample is often contaminated. If there are more than two types of colonies, the sample is often contaminated. A new sample should be requested.

Is clear how is critical good sample collection, transport, and preservation, as sample degradation may dramatically impact the final outcomes.

SAMPLE FOR URINE CULTURE

The standard sample for UTI culture is mid-stream urine. Patients shall avoid collecting the first void urine (the first 10-20 mL) as normally the sample contamination is higher by the commensal flora normally present in the urinary tract. The urine sample should be delivered to the lab and analyzed within two hours.

As it is really unlikely that the sample can respect this very short time interval, urine must be refrigerated or preserved with additives in order to preserve the vitality of uropathogens and to avoid the overgrowth of the commensal flora and of the high-fitness pathogenic bacteria.

Assuring the cold chain during sample storage before testing is extremely difficult and expensive, especially for a such high-volume sample like urine for culture.

Thus, different preservatives based on boric acid-based were formulated over time. Several different boric acid formulations were used, including boric acid alone, glycerol-boric acid-sodium formate, and sorbitol-boric acid-sodium formate. Boric acid is included in the Candidate List of substances of very high concern for Authorisation published in accordance with Article 59(10) of the Registration, Evaluation, Authorisation and restriction of chemicals (REACH) Regulation due to its toxicity for reproduction during industrial processes .

This inclusion poses a health concern, especially for long-term exposure in manufacturing sites, as well as apprehension for its future availability on the market of 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 NEW BORIC ACID-FREE URISPONGE™

Copan developed a new boric acid-free preservation formula, which is proved to be extremely efficient to preserve urine samples for culture application.

Apart from the excellent performances, the UriSponge™ is characterized by an increased user experience as:

- It doesn't need any ancillary device (straw, needles, special cups) for collecting the sample.
- The urine is collected by passive adsorption of the sponges, so there is no problem of vacuum leaks that affect the suction capacity and subsequent respect of the preservative/urine ratio.

COMPARISON OF BORIC ACID-FREE URISPONGE™ VERSUS THREE COMMERCIALLY AVAILABLE BORIC ACID BASED URINE TRANSPORT SYSTEM

MATERIAL AND METHODS

The recovery and preservation performance of three commercially available boric acid-based urine collection devices were compared vs performances of the new boric acid-free UriSponge™.

Device 1 and 2 are vacuum tubes with boric acid salts based as preservatives, while Device 3 is a syringe-based device.

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

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 collection was performed for all the 4 devices according their Instruction for Use.

UriSponge™ was verified to preserve viability after incubation 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).

Data are results from three replicates from the same inoculum in three different tubes, for each device type.

RESULTS

	UriSponge	Device 1	Device 2	Device 3
GRAM -				
E. coli	-0,36	0,17	0,49	0,04
E. cloacae	-0,16	-0,23	1,29	-0,26
P. mirabilis	-0,11	0,23	0,62	-0,14
C. freundii	-0,15	-0,07	1,24	0,00
K. pneumoniae	-0,22	0,58	1,45	0,60
P. aeruginosa	-0,24	-0,23	-0,10	-0,15
GRAM +				
E. faecalis	0,07	0,27	0,62	0,02
S. saprophyticus	-0,02	0,04	0,59	-0,01
YEAST				
C. glabrata	-0,09	-1,72	-0,50	-0,34
C. albicans	0,08	-0,14	0,28	-0,08
CLINICAL ISOLATED STRAINS				
E. faecalis	0,14	0,45	1,17	0,01
E. coli	-0,19	0,31	0,37	0,00
P. rettgeri	-0,14	0,08	1,53	-0,09
M. morganii	-0,15	-0,25	0,15	-0,15

Table 1: *CFU/mL logarithmic difference at 24 hours vs T0. Inoculated devices were kept at RT (20 – 25 °C). Values in red are out of the acceptance criteria for sample preservation, accordingly to the CLSI M40-A2 guideline.*

On gram-negative bacteria after 24 hours of incubation, UriSponge™, Device 1 and 3 demonstrated good performances, even if *K. pneumoniae* start to worryingly grow. Devices 2 seems already to not be effective in preserving the urine with *E. cloacae*, *C. freundii* and *K. pneumoniae* outside the acceptance criteria with an overgrowth of 10 times versus the time 0.

Gram-positive bacteria on the other hand are well preserved by all four devices, even if Device 2 showed a significant grow with respect to time zero.

Yeasts are well preserved by UriSponge™, Device 2, and by Device 3. Device 1 seems to already allow to uncontrolled death of *C. glabrata* well outside the M40-A2 parameter.

Analyzing the data of the clinically isolated strains, Device 2 showed an unacceptable preservation performance on *E. faecalis* and *P. rettgeri.* UriSponge™, Device 1 and 3 on the other hands, showed a good preservation performance over the 24 hour.

Overall, after 24 hours of incubation at room temperature, UriSponge™ and Device 3 demonstrated very good preservation performances. Device 1 demonstrated to lack preservation capability on *C. glabrata* and a worrying growth rate of *K. pneumoniae.* Device 2 is unable to preserve the sample already after 24 hours of incubation.

	UriSponge	Device 1	Device 2	Device 3
GRAM -				
E. coli	-0,15	0,28	1,51	0,25
E. cloacae	-0,18	0,38	1,59	-0,19
P. mirabilis	-0,25	0,46	1,49	-0,37
C. freundii	-0,26	0,12	1,57	-0,03
K. pneumoniae	-0,28	1,29	1,55	0,95
P. aeruginosa	-0,76	-0,46	-0,40	-0,45
GRAM +				
E. faecalis	0,12	0,41	1,43	0,06
S. saprophyticus	-0,19	0,02	1,48	0,01
YEAST				
C. glabrata	0,02	-2,48	-0,18	-1,03
C. albicans	0,35	-0,30	0,56	-0,10
CLINICAL				
E. faecalis	0,00	1,02	1,61	0,05
E. coli	-0,31	0,54	1,39	-0,16
P. rettgeri	-0,30	0,35	1,59	-0,25
M. morganii	0,02	0,14	0,48	0,07

Table 2: *CFU/mL logarithmic difference at 48 hours vs T0. Inoculated devices were kept at RT (20 – 25 °C). Values in red are out of the acceptance criteria for sample preservation, accordingly to the CLSI M40-A2 guideline.*

After 48 hours of incubation at room temperature, the only urine collection and preservation device which proof to effectively preserve the sample is the UriSponge™.

On gram-negative bacteria, the UriSponge™ demonstrated extremely low deviance from T0, apart from *P. aeruginosa* which decrease its concentration of 0.76 cycles, still well into the the acceptance criteria.

Device 1 showed its inability to preserve *K. pneumoniae* that overgrowth of around 20 times with respect of T0. Device 2 is characterized by a general overgrowth of all the tested microorganisms, apart from *P. aeruginosa*. Device 3 has all the microorganisms in the reference range, even if *K. pneumoniae* is extremely close to the acceptance range.

On gram-positive UriSponge™, Device 1 and 3 showed a good preservation performance. Device 2 let all the reference strains test overgrowth above the acceptance level.

Yeasts are perfectly preserved by UriSponge™ and Device 2. Device 1 confirmed the inability to preserve the vitality of *C. glabrata*. Also, Device 3 is unable to preserve *C. glabrata*.

On the clinically isolated strains, UriSponge™ was able to perfectly preserve all the strains used in this study. Device 3 showed also comparable results. Device 2 also in this case seems to be completely inefficient to avoid microbial overgrowth in urine sample.

Finally, Device 1 seems unable to avoid the overgrowth of the clinically isolated *E. faecalis* even if it gave acceptable results on the *E. faecalis* ATCC strain.

Results reported in table 1 and 2 are the average of three replicates from the same inoculum in three different tubes, for each device type.

To further show the preservation uniformity intra-device, we report here below the plates that generates the results.

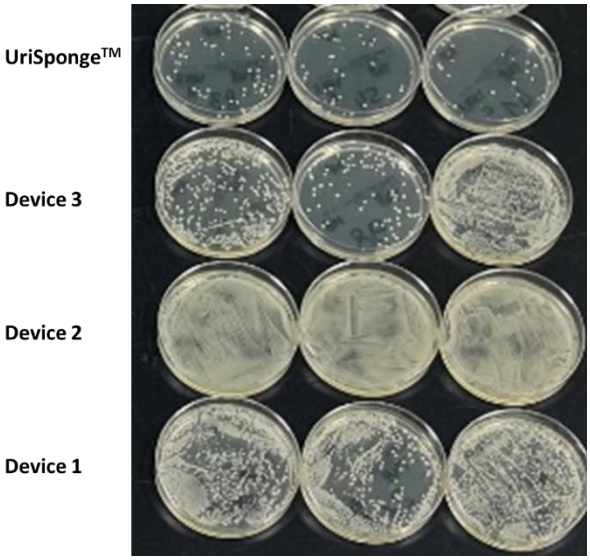

Fig 1: *growing plates of three replicates of K. pneumoniae - ATCC® 700-603 after incubation in UriSponge™, Device 1, Device 2 and, Device 3 at 20 – 25 °C for 48 hours*

In Figure 1 we reported the growing plates of three replicates of *K. pneumoniae* - ATCC® 700-

603 after incubation in UriSponge™, Device 1, Device 2, and, Device 3 at 20 – 25 °C for 48 hours.

As evident, UriSponge™ is the urine preservation device that allows the highest degree of uniformity. Devices 1 and 2 were not able to properly preserve the sample as let *K. pneumoniae* overgrow over 1 logarithmic cycle, and this is also pretty clear from the images.

Device 3, which conforms to the M40-A2 parameter with a 0.95 cycle overgrow (max allowed ± 1 logarithmic cycle VS T0), generated two plates out of the limit, while the third is well into the limit. Overall, the final result is compliant but with a very dispersed dataset. This has a huge impact on the everyday clinical life of these kinds of devices, as results are generated in single, and not in replicate. Thus, the probability to deliver a false result is not negligible.

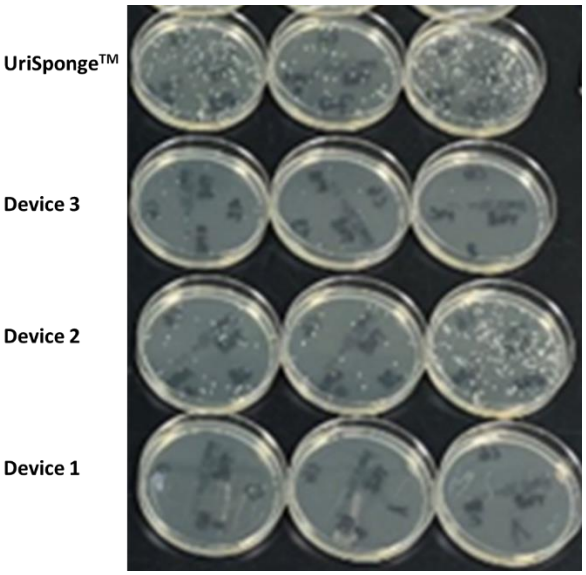

Fig 2: *growing plates of three replicates of C. glabrata* - ATCC® 15126 *after incubation in UriSponge™, Device 1, Device 2 and, Device 3 at 20 – 25 °C for 48 hours*

On the other hand, *C. glabrata* - ATCC® 15126 was poorly preserved by Device 1 and 3, and this is extremely evident in figure 2. Device 2 is compliant to M40-A2, but with an extremely ununiform results between replicates. UriSponge™ is the only device that proved to preserve the vitality of *C. glabrata* with a very limited results variability between the replicates.

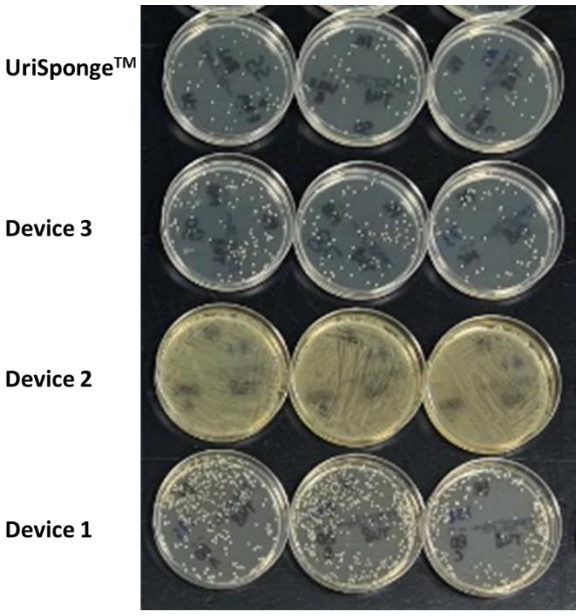

Fig 3: *growing plates of three replicates of P. rettgeri clinical strain after incubation in UriSponge™, Device 1, Device 2 and, Device 3 at 20 – 25 °C for 48 hours*

In Figure 3, the results for *P. rettgeri*. For this microorganism, only Device 2 was not compliant with the M40-A2. But, even if both Device 1 and 2 were compliant, it is evident, especially for Device 1, the high variability between devices. UriSponge™ demonstrated to generate results with the highest level of repeatability.

CONCLUSIONS

In conclusion, only the UriSponge™ proved to be efficient to preserve the vitality and avoiding the overgrowth of the most common UTI's etiological agents for up to 48 hours.

Device 3 behaved fairly comparably to the UriSponge™, apart from *C. glabrata* which decreased its concentration below the acceptance value, and *K. pneumoniae*, which even if still at the acceptance level, increased its concentration fairly close to the limit.

Device 1 showed a problem in preserving the vitality of *C. glabrata* already at 24 hours, a problem that becomes extremely serious at 48 hours with over 400 folds decrease in vitality. Device 1 also showed unacceptable results at 48 hours in vitality preservation of *K. pneumoniae* and overgrowth problem for the clinically isolated *E. faecalis.*

Device 2 seems not to be a suitable transport device for urine for culture application, as it seems to not be able to generally preserve microorganisms for both overgrowth and vitality preservation problems.

Finally, a very important outcome of this evaluation is the very high repeatability of the replicated generated by the UriSponge™ compared with the results generated by the three other devices, that are extremely scattered.

This is a fundamental point. Even if the average data seems to prove a good preservation quality, in the real life where results are produced from one device only, the possibility to generate false results is extremely high. Risk that is mitigate by the use of the UriSponge™.

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