SONIC HEALTHCARE UK

The effect of container, time, and temperature on microbiological urine culture results

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Introduction

Quantitative urine culture is a key method in the detection of urinary tract infection (UTI) which is one of the most common infections in the UK[1]. Culture interpretation is based on bacterial colony count and number of isolates. It is therefore essential that sample integrity is preserved during production, transit, processing and storage[2]. This study investigated whether increased temperature during sample transport would influence the clinical interpretation of routine urine cultures, and determined the optimal timeframe, container, and conditions for storage of urine samples in a hub and spoke model modern diagnostic laboratory.

Methods

Experiment 1

To determine optimal storage timeframe, conditions, and sample container:

- 100 urine samples growing a single reportable predominant organism in a growth of 10^4-10^5 CFU/mL were identified.
- These were split into 3 containers and incubated at 2-8°C, room temperature (25-28°C), and at 35-37°C.
- Samples were re-cultured, incubated, and interpreted daily.

Experiment 2

To determine the effect of sample transport during the hottest days of the year in London,

- 100 urine samples were cultured then stored at 35-37°C for 8 hours and cultured every 2 hours during this time.
- Cultures were then read in sequence and changes in interpretation recorded.

Culture was performed using Oxoid Brilliance UTI Clarity Agar ready-poured plates (Figure 1) and incubated for 18 hours at 35-37°C as per manufacturer guidelines.

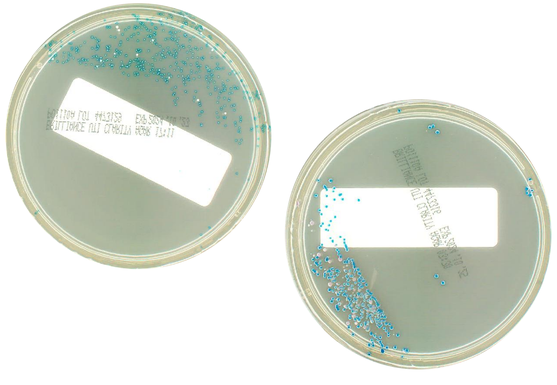

Figure 1. Routine urine cultures growing single reportable predominant organism in 10^4-10^5 CFU/mL growth on UTI media

Results: Experiment 1

There was a visible increase in significantly changed culture results for boric acid compared to plain container samples for all storage conditions (Figure 2.) however, this difference was not statistically significant (p=0.364).

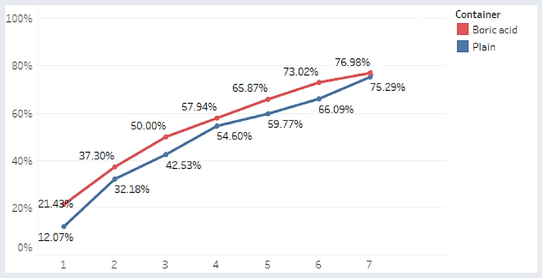

Figure 2. Percentage of boric acid vs plain container samples with a clinically significant result change each day.

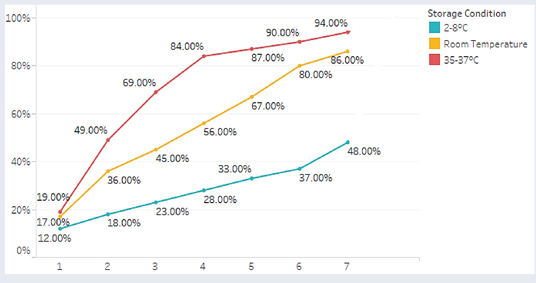

Figure 3. Percentage of samples with a clinically significant culture result change each day for each temperature condition.

All storage conditions (Figure 3.) were significantly different when compared to each other (p<0.05) with refrigerated samples demonstrating the slowest rate of culture result change.

Results: Experiment 2

Boric acid containers showed evidence of improvement (Figure 4) over plain containers (p=0.07).

There was a significant change in culture result between hours 2 and 6 (p=0.046).

No samples changed from no growth to positive growth.

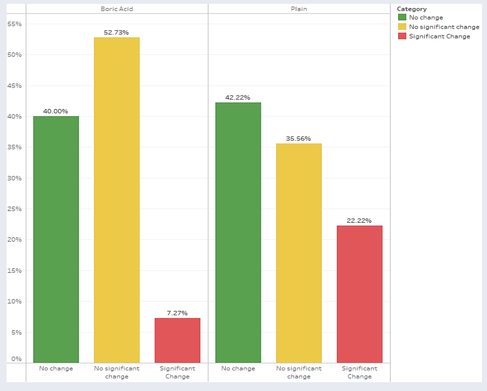

Figure 4. Proportion of samples changing result over 8 hours of incubation at 35-37°C

Other observations

The most commonly isolated organism during Experiment 1 was *E. coli* and the second was *E. faecalis.* There was a significantly higher percentage of predominant *E. faecalis* isolates from plain containers compared to boric acid (p=0.009).

Discussion

All samples used in this study were true laboratory samples, therefore are reflective of those received in diagnostic microbiology laboratories.

As expected, the optimal condition for long term storage for all urine samples was 2-8°C, confirming that current practice is correct.

Whilst boric acid did not preserve samples more effectively than plain containers in long-term storage, it was more effective during the 8 hours of simulated transport, therefore its use should continue to be encouraged. This is reflected in recommendation 21 by EFLM. [3]

Boric acid suppressed *E. faecalis* growth which is often considered to be a contaminant. [4]

The significant differences between the 2 and 6-hour incubation in Experiment 2 suggests that 6 hours is the maximum time samples should be stored/ transported un-refrigerated prior to culture.

Recommendations

1. Boric acid sample containers should be used for routine urine culture to preserve samples during transport and to prevent the growth of contaminants.
2. Urine samples should be refrigerated as soon as possible following culture. Controlled transport should be investigated for urine samples with a transit time exceeding 6 hours, and samples should also be refrigerated pre-transit and prior to culture if there is a delay.
3. Smaller collection tubes, such as 10mL boric acid containers, could be used across all sites to streamline filing and to offset additional space requirement raised by recommendation 2.
4. Re-culture of urine samples should be discouraged and not offered as a laboratory service following 24 hours of storage.
5. Clearer guidelines for clinicians and patients on sample collection and the effects of sample handling on results and ultimately, patient treatment.

References

1. UK Standards for Microbiology Investigations; Issued by the Standards Unit, Public Health England; Bacteriology; B 41; Issue no: 8.7; Issue date: 11.01.19
2. Silver SA, Baillie L, Simor AE. Positive urine cultures: A major cause of inappropriate antimicrobial use in hospitals? Can J Infect Dis Med Microbiol. 2009 Winter;20(4):107-11. doi: 10.1155/2009/702545. PMID: 21119801; PMCID: PMC2807252.
3. The EFLM European Urinalysis Guideline 2023; Kouri T, Hofmann W, Falbo R, Oyaert M, Schubert S, Gertsen J, Merens A, Pestel-Caron M, on behalf of the Task and Finish Group for Urinalysis (TFG-U), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM); Clin Chem Lab Med 2024; https://doi.org/10.1515/cclm-2024-0070
4. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med. 2013 Nov 14;369(20):1883-91. doi: 10.1056/NEJMoa1302186. PMID: 24224622; PMCID: PMC4041367.