

Urine Culture Incubation Time: One vs Two Days!

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ABSTRACT:

Background: Shorter incubation time for non-invasive urine culture has been a matter of controversy, since striking a balance between potential of losing slower growing organisms weighs heavily over shorter incubation time. Our study at Sutter Health Shared Laboratory (SHSL) indicates if routine urine cultures are incubated in CO₂ atmosphere and the no-growth plates are examined using a benchtop magnifier there are no statistically significant loss of uropathogenic organism after one day of incubation compared to two days. Incubating no growth urine cultures for one day can significantly improve the performance efficiency, workflow, incubation space, and the labor especially for laboratories with high volume of urine cultures.

Methods: Only routinely collected urine cultures has been included in this study and invasive collection such as nephrostomy, straight or diagnostic catheter collection were excluded. SHSL urine culture procedure the cut off for workup of uropathogenic organisms in a routine culture is 10,000 CFU/mL. Total of 2,709 urine specimens were processed using WASP automated plating system. 1µL sterile loop were used to inoculate TSA-II Blood Agar and MacConkey II bi-plates. Plates were incubated in 5% CO₂ at 35°C for at least 18 hours and maximum 24 hours for the first read. All media that shown no bacterial growth were examined with a regular bench top magnifier/light for evidence of growth. All No-Growth plates were incubated for an extra overnight incubation (18-24 hour) and examined for growth. Organism identifications performed using Vitek MS instrument.

Results: Total of 501 out of 2,709 samples were determined No-Growth on the first day exam, and after 2nd day of incubation 435 stayed as No-Growth (86.8%), 66 samples (13.2%) indicate growth of normal Uro-Genital (UG) microbiota, and no uropathogenic organisms detected. Among those with growth 54 (10.8%) samples grew <10K, 10 samples (2.0%) grew 10-50K, and 2 samples (0.4%) grew >50K CFU/mL of normal UG microbiota.

Conclusion: Although small percentage with low level urogenital microbiota was missed on the first day of incubation, there were no uro-pathogenic organisms missed. Therefore the one day incubation of routine urine culture plates in CO₂, and careful examination of the plates appeared to have same efficiency of 2 days incubation in pathogen detection. One day incubation can result in significant saving in labor and incubation space for large volume laboratories, and fully automated microbiology testing systems.

Introduction:

• With recent focus on increasing laboratory operation efficiency, the old question becomes relevant: how long we should incubate the urine cultures without losing the chance of detecting pathogenic organisms? Is one day of incubation enough? While many current laboratory practice references stating 16 – 24 hours is sufficient for detection of uropathogenic organisms^{1,2}, we were not able to find any concrete data in the literature who backs it up. Our study was design to determine the loss of pathogenic organism detection level when incubation time is one day (18-24 hours) compare to 2 days, when plates are incubated in CO₂^{3,4}, and examined carefully using visual aids.

Methods:

- All plates incubated in CO₂ at 35°C and were incubated for at least 18 hours before examination by testing personnel.
- All negative plates were examined carefully by a bench top magnifier (10X) light combo [Figures 1 and 2].
- Statistical calculations for confidence interval performed using Wald method for normal approximation.

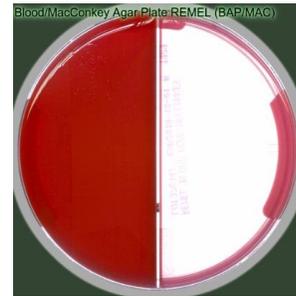


Figure 1: No visible growth after 18 hours incubation of plates,

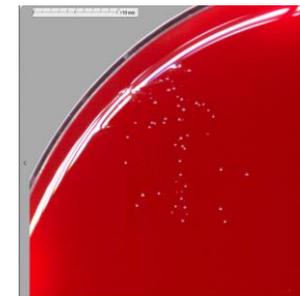


Figure 2: Same Plate, Visible colonies using magnification

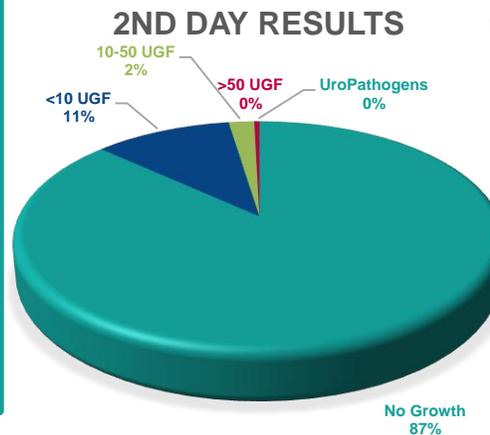


Table 1: details of growth after 2nd days of incubation of all plates that were no growth 1st day

2 Days incubation	#	Ratio	95% CI	
No Growth	435	86.8%	83.6	89.5
<10 CFU/mL	54	10.8%	9.2	12.2
10-50 CFU/mL	10	2.0%	1.2	2.7
>50 CFU/ML	2	0.4%	0.1	1.5
Uropathogens	0	0%	--	--

Conclusions:

- Our study confirmed 2 days of incubation will not increase the rate of recovery of uropathogenic organism, provided that extra care is taken to exam the plates and incubation is performed in CO₂.
- Effectiveness of such procedure for invasively collected urine, such as suprapubic, nephrostomy, and diagnostic catheter has not been studied and results only applies to clean catch, voided, routine specimens.
- The emergence of microbiology total-automation systems such as WASP-Lab® and Kiestra® provides automated imaging capability and artificial intelligence screening of urine culture plates. In many instances higher resolution, magnifying capability, and automated segregating software make this systems performing in higher accuracy than the manual screening of plates, and improve performance of one-day incubation results. Interestingly there will be significant amount of saving that one-day incubation will bring to total lab automation projects by reducing the required incubators capacity up to 50% for incubating urine culture plates.
- Presence of uro-genital normal microbiota such as Lactobacilli may not assist with detection of UTI, however in cases that they grow in large numbers, it can serve as an indicator for improper collection or contaminated sample. Although two-days incubation may promote better growth of the slow-growing urogenital flora, CO₂ incubation will enhance their growth and make them detectable sooner. Our data shows one-day incubation may miss some normal urogenital microbiota growth, however, it very rarely (0.4%) misses heavy growth of them. This acceptable growth of urogenital normal microbiota rate after one day incubation make it possible to effectively use it as a contamination indicator of urine specimens.
- One day incubation in CO₂, paired with proper plate examination clearly has no negative impact in recovery and detection of uropathogenic organisms with fast or moderate rate of growth.

References:

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