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Automated Digital Quantitation of Urine Cultures using the WASPLab

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Introduction

Advances in automation have reduced the time for specimen processing by using robotic systems to inoculate, label, track and incubate plates for culture. However, culture analysis is still a time intensive and costly procedure for the laboratory as technologists have to interpret colony counts to differentiate pathogens from normal flora for several hundred plates a day. Automation that can count and differentiate colony types on blood plates could help to reduce cost of urine cultures by sorting plates based on colony growth. Recently, software was developed for the WASPLab (Copan, Brescia, IT) that reads digital images and provides quantitation of colony forming units (CFU) from blood agar plates (BAP). In this study, we compare the accuracy of this software to manual analysis.

Method

Urine specimens submitted for bacterial culture were enrolled into the study and plated on BAP following standard of care testing. Specimens enrolled were processed by the WASPLab with a 1µL loop and digital images were taken at 0 and 24 hours post inoculation. The software quantitated each plate for colony counts, recorded as colony forming units per plate (CFU/plate), and results were compared to manual quantitation. Manual quantitation was performed by a technologist blinded to the results and colonies were counted using the same digital image viewed on a HD monitor. Specimens that contained >1000 CFU/plate were reported manually as 1000 CFU/plate. Results are reported in the following categories: 0 CFU, 1-10 CFU, 11-100 CFU and >100 CFU.

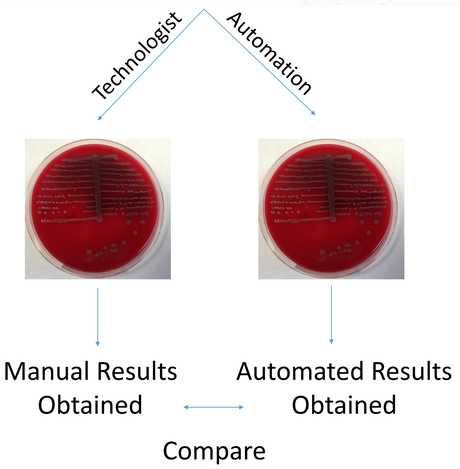


Figure 1. Digital analysis to differentiate colonies

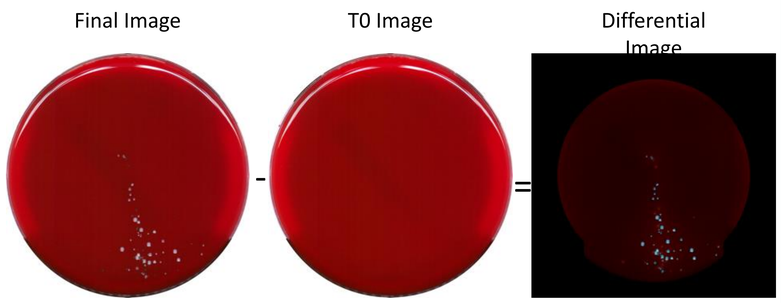
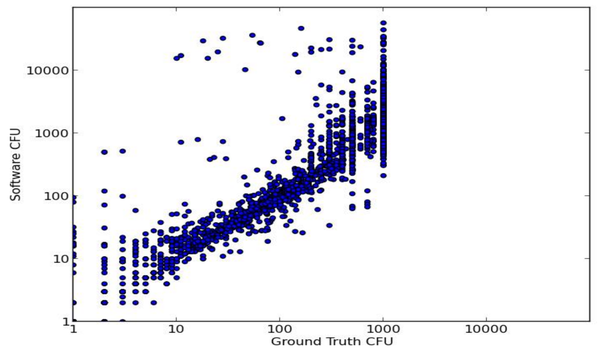


Figure 1. Detection of colonies by the software is performed by detecting differences between the final image and the initial time point 0 image. From this differential image, the software's algorithm separates colony growth for quantitation.

Figure 2. Scatter plot demonstrating correlation between automation and manual reading.



A cutoff of 100 CFU was used for manual plating. This was set to reflect reporting of urine cultures as specimens containing greater than 100 colonies are reported as > 100,000 CFU/mL

Table 2. Discrepant analysis of MN/AP specimens N = 10,348

Colony Count (CFU/mL)	Concordant	Automation overcall	Automation under called	Total	Concordance (%)
0	283	50[a]	NA	333	85.0
1-10	254	70[b]	0	324	65.1
11-100	450	35[c]	2	487	92.4
>100	882	N/A	23	905	97.4
Total	**1869**	**155**	**25[c]**	**2049**	**91.2**

[a] 18 plates were ≥ 2 logarithmic difference
[b] 4 plates were ≥ 2 logarithmic difference
[c] All plates were < 2 logarithmic difference

Table 1. Agreement of automation bacterial counting with manual reading

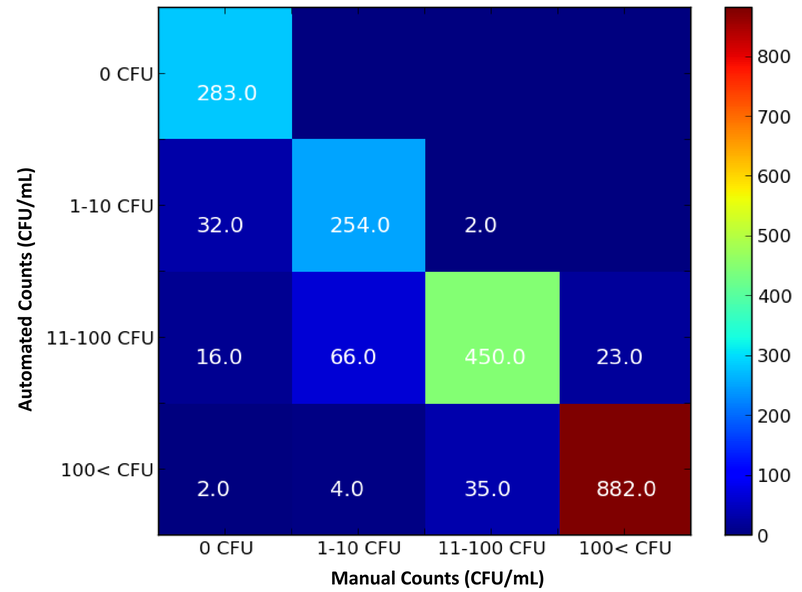


Table 1. A total of 2049 specimens were enrolled and tested. Plates were categorized as containing either 1-10, 11-100, or greater than 100 CFU. The counts for automation were compared together and graphed according to category. A heat map was created demonstrating the amount of specimens that fall within the comparison.

Conclusions

- The quantitation software was accurate at detecting growth on BAP with no false negative plates (automation 0, manual > 0 colonies) observed.
- The software was accurate with an overall concordance of 91.2% with manual plate counting.
- Discordant specimens were more often resulted in higher colony counts than manual reading with 7.5% of all specimens compared to 1.2% of specimen automatically reported as fewer than manual.
- The majority of discordant results were < 2 logs difference when compared to manual counting and only 22/180 (12.2%) of discordant plates had bacterial count differences of ≥ 2 log.
- Reliability of detecting colonies could allow workflow to batch view negative plates to increase productivity and reduce turnaround time (TAT).
- Currently, technologists are still required to interpret results of plates with growth as the software cannot differentiate between colony morphology.