Expert Image Analysis by COPAN WASPLab™ to Evaluate Urine Cultures

Karen Timm[1], Karissa Culbreath[1,2]

[1]TriCore Reference Laboratories, Albuquerque NM, [2]Department of Pathology, University of New Mexico School of Medicine, Albuquerque NM

Abstract

With the implementation of automation in clinical microbiology, laboratories have digital images which present the opportunity for developing image analysis software for differentiation of growth and colony morphology. COPAN has developed an expert image analysis system that is able distinguish between positive and negative cultures and make presumptive determination of colony morphology to determine significant and non-significant cultures. In this study 5,033 urine specimens for bacterial culture were quantitatively plated on sheep blood and MacConkey agar bi-plates by WASPLab™ using a 1µL loop. Images were captured after 0 and 18 h incubation. The software quantitated and qualified each culture and reported as: No Growth if the bi-plate contained less than 10 colonies, Non-Significant Growth if growth was considered not pathogenic according to the guidelines inserted in the expert software, and finally Significant when the growth on each plate was to be considered pathogenic according to the expert rules. Results were then compared to manual interpretation as either significant or non-significant for pathogens based on laboratory urine culture policy. Discrepancies were blinded and reviewed by a microbiologist.
The initial analysis to determine Growth or No-Growth yielded sensitivity and specificity of 98.3% and 77.6% respectively. After discrepant resolution, the sensitivity, specificity, NPV and PPV were 99.9%, 80.0%, 99.9% and 66.8% respectively. Next, the images were analyzed for isolate categorization as pathogen or non-pathogen. Based on quantity and type of pathogen images were identified as No Growth, Significant or Non-significant. When evaluated for significance, the image analysis yielded an initial sensitivity and specificity of 93.1% and 80.9% respectively. Following resolution of the discrepancies, the expert image analysis system yielded sensitivity, specificity, NPV and PPV of 99.7%, 82.6%, 99.7% and 84.1%, respectively. These results indicate that the COPAN expert image analysis software is able to differentiate positive and negative urine cultures with a high sensitivity and negative predictive value. This allows for use as a screening tool to eliminate negative and normal flora and would be an effective mechanism to reduce unnecessary review of negative cultures. Future studies may include evaluating enhanced tools to increase the specificity of the image analysis tools for improved organism characterization.

Introduction

Automation of the microbiology laboratory using WASPLab™ has revolutionized plating and incubation of cultures. In addition to workflow efficiencies, the laboratory now has digital images of cultures. These digital images now present a novel opportunity for digital image interpretation through use of image analysis and deep learning algorithms. Studies have already demonstrated the ability of COPAN PhenoMatrix™ image analysis to accurately discriminate between positive and negative cultures on chromogenic media for MRSA(1) and VRE(2). These studies demonstrated that image analysis was able to yield a higher sensitivity for detection of positive chromogenic cultures compared to manual evaluation. Here, we evaluate the performance of the COPAN PhenoMatrix™ image analysis system for detection of positive urine cultures, the ability of the system to accurately classify organisms into common bacterial phenotypic groups, and implementation of user-defined rules to provide preliminary interpretation of the cultures.

Methods

Samples
All samples were plated and imaged using WASP™ and WASPLab™ with 1µl of urine struck for quantitation on a Blood Agar/MacConkey agar bi-plate. Cultures were incubated for 18 hours in non-CO_2 conditions. Routine urine cultures consisted of 5,053 consecutively collected urine cultures. All contrived samples were generated by spiking known organisms at known concentrations into sterile saline.

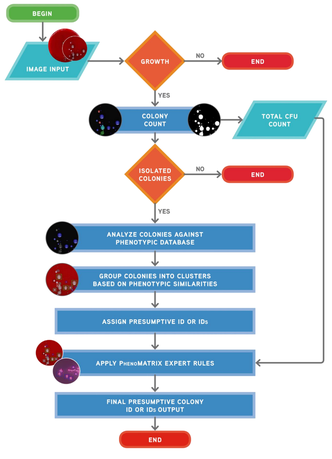
Interpretation
Images were taken at the 18 hour time point and evaluated in the WASPLab™ web application manually and using the PhenoMatrix™ system. Cultures were quantitated and identifications were generated using routine laboratory methods.

Urine Culture Interpretation Rules
Urine culture interpretation rules were generated based on the rules for the routine culture interpretation per standard laboratory protocols.

Statistical Analysis
Results generated by PhenoMatrix™ were compared to the manual interpretation. Discrepant results were reviewed for accurate organism classification and colony counts and resolved for final interpretation.

Sensitivity, specificity, positive and negative predictive values were obtained for growth and no growth segregation performance. The Cohen's kappa coefficient was used to determine the inter-rater performance of the PhenoMatrix™ compared to manual interpretation of urine cultures based on user define rules.

Figure 1. PhenoMatrix™ Algorithm



Results

Table 1: Initial Results for PhenoMatrix™ Urine Segregation Software. Identification of positive urine cultures defined as >10 CFU. Sensitivity 98.3%, Specificity 77.6%.

Software Interpretation	Laboratory		
		Negative	Positive
	Negative	1546	51
	Positive	447	3009

Table 2: Final Analysis for PhenoMatrix™ Urine Culture Segregation Software.
Discordant cultures were manually reviewed and colonies were counted to verify positive or negative interpretation. Following discordant resolution, identification of positive urine cultures defined as >10 CFU per plate improved to Sensitivity 99.9%, Specificity 80.0%.

Software Interpretation	Laboratory		
		Negative	Positive
	Negative	1595	2
	Positive	400	3056

Table 3: Limit of Detection. In monomicrobic suspensions of microorganism. *Enterococcus faecalis, E. coli,* and *K. pneumoniae* the limit of detection was 10^3 CFU/ml (1 colony per plate), for *S. aureus* the limit of detection was 10^4 CFU/ml (5-10 colony per plate). These are all within the routine limit of culture review for urine cultures of 10 CFU. In contrived polymicrobic suspensions, the PhenoMatriX™ system was able to detect mixtures of organisms >90% of the time when they were present at concentrations of at least 10^5 CFU/mL. At 10^4 and 10^5 CFU/mL when morphologies were not detected, they were in 2 and 3 organism mixtures 10-fold higher.

Polymicrobic Mixes	10^4			10^5			10^6		
	n	Detected	Percent	n	Detected	Percent	n	Detected	Percent
Enterococcus faecalis	11	9	82%	10	10	100%	10	10	100%
E. coli	11	8	73%	10	9	90%	10	10	100%
K. pnemoniae	11	9	82%	10	10	100%	10	10	100%
S. aureus	11	8	73%	10	9	90%	10	10	100%

Organism Classifications	n	Correct Classification	Percent	Unclassified	Percent	Misclassified	Percent	Correct Gram Classification	Percent
***Staphylococcus* species**	28	24	86%	4	14%	0	0%	28	100%
***Candida* species**	17	16	94%	0	0%	1	6%	17	100%
***Streptococcus* species**	37	24	65%	5	14%	8	22%	37	100%
Enterobacteriacae	69	62	90%	6	9%	1	1%	69	100%
Pseudomonas aeruginosa	10	7	70%	3	30%	0	0%	10	100%
***Enterococcus* species**	20	20	100%	0	0%	0	0%	20	100%

Table 4: PhenoMatrix™ Colony classification. Each isolated colony on the urine culture plate is evaluated against a phenotypic database and clustered according to phenotypic similarities and categorized into a classification group. The system was challenged with 181 known organisms. There was 100% agreement with gram classification and 65-100% correct classification of the colony into morphologic groupings. The most frequent incorrect classification occurred with Streptococci that were classified as Enterococcus species. Within the Staphylococcus species, 9/10 *S. aureus* were correctly sub-classified as "Possible *S. aureus*" the remaining culture was unclassified.

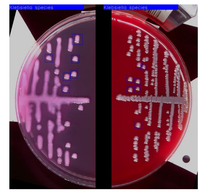


Figure 2. PhenoMatrix™ software interpretation view.

Observation	Interpretation
Organism count <10K	No growth
Growth of ≥3 organisms of the genital tract origin	Normal genitourinary flora/Contamination
One organism >10K Two organisms, each ≥10K Three or more organisms: <50K NGUF, >50K pathogen	Culture Review for ID/AST

Table 5. Laboratory defined protocol for PhenoMatrix™-Based culture interpretation. Abbreviations: NGUF; Normal genitourinary flora, ID/AST; Identification and Antimicrobial Susceptibility Testing

Software Interpretation		Manual Interpretation			Analysis	
		No Growth	Normal Flora/ Contaminated	Culture Review for ID/AST	% Agreement	Kappa Value
	No Growth	2044	5	4	99.6%	0.98 (0.98-0.99)
	NGUF/ Contaminated	7	360	8	96.0%	0.61 (0.59 -0.67)
	Culture Review for ID/AST	15	194	2414	92.0%	0.92 (0.90- 0.93)

Table 6. PhenoMatrix™ software interpretation based on user-defined protocols. Each culture was interpreted using PhenoMatrix™ software and compared to manual interpretation results. Agreement was >90% for all results with very strong kappa correlation for No Growth and Culture Review categorization and moderate kappa correlation for NGUF/contaminated cultures. Abbreviations NGUF; Normal genitourinary flora, ID/AST; Identification and Antimicrobial Susceptibility Testing

Conclusions

- COPAN PhenoMatrix™ accurately classified urine cultures on Blood Agar/MacConkey bi-plates as growth and no growth at a sensitivity of 99.9%
- The PhenoMatrix™ system is able to classify colonies into categorical groups with high accuracy and to a clinically significant limit of detection for urine cultures.
- The classifications generated by PhenoMatrix™ allow for the development of user-derived protocols for interpretation of cultures

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