Evaluation of Growth Segregation Software for Automatic Interpretation of Urine Bi-Plate Cultures Comprised of Blood Agar and CHROMagar™ Orientation using WASPLab™

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ABSTRACT

Full laboratory automation is a relatively new concept for microbiology laboratories. One exciting aspect is use of digital imaging of culture plates combined with segregation software (SSW) to separate negative plates from positive plates. We evaluated the COPAN WASPLab™ and SSW with digital analysis of urine specimens inoculated to a bi-plate with blood agar and CHROMagar™ Orientation. SSW was applied with image analysis at 0 hr and 20 hr incubation and was compared to manual Clinical Laboratory Scientist (CLS) read image analysis of the culture plates. Discrepancies between SSW and CLS visual image analysis were resolved after review by a director. A total of 5183 routine urine plates, processed by the WASPLab system according to standard laboratory procedures with a 1 µL loop were evaluated after 20 hr incubation at 35-37°C according to the following criteria: No growth (<1,000 CFU/mL=NG), <10,000 CFU/mL (<10IG, and considered negative for SSW), ≥10,000 CFU/mL (≥10 and considered positive), and multiple organisms present (MOP and if >10 considered positive). There were 2032 urines considered negative (NG and <10IG) and 1680 considered positive by both CLS reading and SSW. SSW called an additional 1471 positive while the CLS reading was considered negative (NG or <10IG) or insignificant (MOP). Of these 1471 samples, 417 were visually read as <10IG, 55 were read as NG, and 999 were considered MOP by the CLS. The plate images were further reviewed with >75% of the 417 <10IG and >50% of the 55 NG deemed accurate by the SSW. Breakdown of the 2032 SSW negatives included CLS results of 1295 true NG, 726 <10IG, and 10 MOP (but <10IG). There was only 1 culture out of 5183 called negative by SSW (false negative) that was considered positive by visual reading. Use of SSW to remove negative and insignificant growth urine cultures from the routine workflow should result in better laboratory efficiency and use of staff resources. The Copan SSW is a powerful tool and can be applied with WASPLab™ for bi-plate urine cultures with blood and CHROMagar™ Orientation agars.

INTRODUCTION

Urine cultures make up the bulk of most microbiology laboratories workload. Many of these cultures are either no growth or have insignificant quantity of colonies for work-up, yet require technical time for plate examination. Laboratory automation with digital imaging of culture plates along with programmable software can be used to detect the presence or absence of colony growth as well as actual quantitation capabilities that fit many urine culture guidelines. The ability to directly remove and report (with technologist confirmation) cultures with no growth or insignificant quantity of colonies can help laboratory workflows, especially for urine cultures. The Copan Diagnostics WASPLab™ includes dedicated incubators with digital imaging capabilities of culture plates that allow for image analysis by technologists to determine whether the culture is negative (no growth or insignificant growth) or non-negative and would require visual examination by the technologist who would determine if further work-up is necessary. Copan has developed software that has the ability to segregate plates that are negative from those considered non-negative and would require further examination and processing. This Segregation Software (SSW) has been reported to work with chromogenic media for MRSA and for VRE[1,2].

The Southern California Permanente Medical Group (Kaiser Permanente) laboratory at Chino Hills has 4 WASP® instruments with 3 WASPLab™ automated lines and 7 dedicated incubators. SSW algorithms have been developed specifically for the Kaiser model with a urine bi-plate of blood agar/CHROMagar™ Orientation. In this study, we evaluated the SSW developed by Copan for our urine bi-plates

METHODS

1. A total of 5,183 routine urine cultures were processed on one WASP line with incubation at 35-37°C in either of two WASPLab incubators.
   a. All urines were plated by the WASP with a 1 µL loop to a bi-plate of blood agar and CHROMagar™ Orientation.
   b. All plates had images taken at time 0 and after 20 hr incubation.
2. Automated digital analysis by the SSW was established with the following criteria
   a. Both blood and CHROMagar™ Orientation agars were analyzed with 2 different lighting patterns, one each to display the characteristic colony morphologies on each medium.
   b. Negative urines included
      i. No growth (<1,000 CFU/mL)
      ii. <10,000 CFU/mL (Insignificant growth)
   c. Positive urines: ≥10,000 CFU/mL (non-negative)
3. All cultures were analyzed by a technologist (CLS) prior to the SSW implementation and image review of discrepancies was performed by a manager/director.
4. Discrepancies of SSW positive, CLS negative were not considered significant. CLS would be directed by SSW to manually examine plate for further workup.

Results

1. 5,183 total urine cultures were evaluated by a CLS and by the SSW. See Table 1 and 2.
2. There were 2032 (39.7%) urine cultures considered negative by the SSW.
   a. 1295 were negative by both CLS read and SSW.
   b. 726 were <10,000 CFU/mL by both the CLS and SSW.
   c. 10 were <10,000 by SSW and considered contaminated (≥3 colony types, MOP) by the CLS, but total colony counts were <10,000 CFU/mL
   d. Only 1 false negative occurred. Visual plate review indicated 20,000 CFU/mL of tiny alpha hemolytic colonies not detected by SSW. Sensitivity >99.9%.
3. There were 3151 (60.8%) urine cultures considered positive by SSW.
   a. 1680 were positive (>10,000 CFU/mL) by both CLS read and SSW.
   b. 1471 were reported as positive by SSW by initial CLS examination indicated no growth, <10,000 CFU/mL, or MOP.
      i. 999 were >10,000 CFU/mL but reported by CLS as contaminated, MOP
      ii. 317 of 417 (>75%) reported as <10,000 CFU/mL by CLS read were correctly called >10,000 CFU/mL by SSW with manager review. The remainder were mostly 2 or more colony types each with <10,000 CFU/mL and not significant to CLS read and report.
      iii. 29 of 55 (>50%) reported as no growth by CLS but positive by SSW were apparently due to imperfections in the media or to indentations produced by the loop. No growth was observed by visual examination.

TABLE 1. Overall results of Segregation Software (prior to manager review)

		LAB READ	
		Negative	Positive
SSW	Negative	2031	1
	Positive	1518	1633

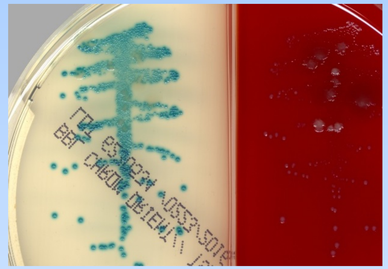
TABLE 2. Data with <10,000 and MOP.

		LAB READ			
		Negative	<10,000	MOP	Positive
SSW	Negative	1295	726	10*	1
	Positive	55	417	999	1680

* All were <10,000 CFU/mL

Conclusion

1. Evaluation of 5183 urine samples by Copan Segregation Software resulted in a sensitivity of >99.9%.
   a. The one error has been corrected in the software algorithm.
2. Specificity was not determined since all software positive plates would be subject to further CLS evaluation.
3. The SSW is a powerful tool to automatically remove negatives as defined by our laboratory, thus allowing better workflow. System was implemented 4/4/2017.



References

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2. Faron, M.L., et al. Automated digital analysis of chromogenic media for vancomycin-resistant-Enterococcus species using COPAN WASPLab. J Clin Microbiol 54:2464-2469, 2016.

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