Improved Recovery of Beta-Hemolytic Streptococcus Species from Group A **Streptococcus** Cultures Using WASP® Instrumentation in Streak Only Mode

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Abstract

Objective: Compare recovery of beta-hemolytic Streptococcus species from culture using manual inoculation and WASP® (Copan Diagnostics Inc.) instrumentation in "streak only mode" to conventional

processing.

Methods: A total of 504 throat specimens were collected and transported to the laboratory using traditional swab transport devices (Amies Agar Gel) and inoculated onto Remel™ Strep Selective II Agar (Thermo Fisher Scientific) in duplicate. One plate from each specimen was manually struck for isolation using a 4 quadrant streak pattern. The second plate was loaded onto the WASP® for plate streaking using a single streak pattern. Plates were incubated at 35-37°C in 5-7% CO₂. Cultures were read for the presence of beta-hemolytic colonies after 18-24 hours of incubation. If negative for Streptococcus pyogenes, the cultures were incubated for an additional 18-24 hours and read again for the presence of beta-hemolytic colonies. Beta-hemolytic colony types were identified using a combination of Remel™ PathoDx™ Strep Grouping Kit (Thermo Fisher Scientific), catalase reagent, and Gram stain morphology. Comparison of the recovery of beta-hemolytic Streptococcus species Results:

		Manual Method				
		S. pyogenes	Streptococcus, not group A	Negative		
WASP*	S. pyogenes	17 (3.4%)	0	2 (0.4%)		
	Streptococcus, not group A	0	56 (11.1%)	18 (3.6%)		
	Negative	0	6 (1.2%)	405 (80.4%)		

Conclusions: 478 of 504 (95%) specimens tested were in agreement. Seventeen (17) of 19 (89.5%) specimens positive for S. pyogenes were in agreement between the two methods. Two (2) specimens were positive for S. pyogenes on the plates struck by the WASP® only. There were 18 WASP® struck specimens that yielded non-group A beta-hemolytic streptococci (14 group B; 3 group C; 1 group F) that were not detected on the manually struck plates. There were 6 manually struck specimens that yielded beta-hemolytic streptococci (2 group C; 2 group F; 1 group B; 1 beta-hemolytic Streptococcus species, not group A or B, that was not further characterized) that were not detected on the plates struck by the WASP®. Use of the WASP® instrumentation in "streak only mode" yielded more beta-hemolytic streptococcal isolates (n=93) (Sensitivity: 94%; 95% Confidence Interval: 87-98%) than the manually struck plates (n=79) (Sensitivity: 80%; 95% Confidence Interval: 70-87%). Additionally, the WASP® struck

I. Introduction

plates were easy to read due to the consistent streak pattern.

WASP® (Walk Away Specimen Processor, Copan Diagnostics Inc.) instruments are currently used to plate and streak urine culture samples in our laboratory. Traditional swab transports are not compatible for use with the WASP® instrumentation. However, the WASP® instruments are capable of using a "streak only mode" to streak plates from cultures submitted with traditional swab transports that have been manually inoculated. The WASP® instrumentation has options for multiple streak patterns and varying number of loop sterilizations. This study evaluated recovery of beta-hemolytic Streptococcus species from culture using conventional processing as compared to manual inoculation and the use of the WASP® instrumentation in "streak only mode".

II. Methods

A total of 504 throat specimens were collected and transported to the laboratory using traditional swab transport devices (Amies Agar Gel) and were inoculated onto Remel™ Strep Selective II Agar (Thermo Fisher Scientific Inc.) in duplicate. One plate from each specimen was manually struck for isolation using a conventional 4 quadrant streak pattern. The second inoculated plate was loaded onto a WASP® instrument for plate streaking using the SST6 single directional streak pattern (Figure 1). The SST6 pattern was chosen for optimal distribution of growth and decreased instrument processing time as compared to a 4 quadrant streak pattern. Plates were incubated at 35-37°C in 5-7% CO₂. Cultures were read for the presence of beta-hemolytic colonies after 18-24 hours of incubation. If negative for Streptococcus pyogenes, the cultures were incubated for an additional 18-24 hours and read again for the presence of beta-hemolytic colonies. Beta-hemolytic colony types were identified using a combination of Remel™ PathoDx™ Strep Grouping Kit (Thermo Fisher Scientific), catalase reagent, and Gram stain morphology.

III. Results

Of the 504 specimens plated in duplicate using the two different plate streak patterns and methods (Table 1), 478 (95%) were in agreement. Seventeen (17) of the 19 (89.5%) specimens positive for S. pyogenes were in agreement. S. pyogenes was recovered from two specimen on the plates struck by

the WASP® and not isolated from the manually struck plates. Fifty-six (56) specimens had non-group A beta-hemolytic streptococci recovered using both methods. Eighteen (18) WASP® struck specimens yielded non-group A beta-hemolytic streptococci (14 group B; 3 group C; 1 group F) that were not detected on the manually struck plates. On six (6) manually struck specimens beta-hemolytic streptococci (2 group C; 2 group F; 1 group B; 1 beta-hemolytic Streptococcus species, not group A or B, that was not further characterized) were isolated that were not detected on the plates struck by the

Table 1: Comparison of the recovery of beta-hemolytic Streptococcus species

		Manual Method		
		S. pyogenes	Streptococcus, not group A	Negative
	S. pyogenes	17 (3.4%)	0	2 (0.4%)
WASP®	Streptococcus, not group A	0	56 (11.1%)	18 (3.6%)
	Negative	0	6 (1.2%)	405 (80.4%

Use of the WASP® instrumentation in "streak only mode" yielded a total of 93 beta-hemolytic streptococcal isolates (Sensitivity: 94%; 95% Confidence Interval: 87-98%) while the manually struck plates yielded a total of 79 beta-hemolytic streptococcal isolates (Sensitivity: 80%; 95% Confidence Interval: 70-87%).

The WASP® struck plates were consistently well struck with good colony separation as compared to the manually struck plates. This is consistent with a previous urine culture study that suggested that the streak pattern, specifically the number and length of the lines, is critical for optimizing the number of single colonies recovered (1).

It has previously been shown that improved efficiency in plate reading can be achieved with better colony separation (2). During this evaluation, the WASP® stuck plates were well received by the techs reading the cultures, however, the reading efficiency was not specifically measured. Additionally, the WASP® instrumentation has previously been shown to have decreased plate contamination occurring during the streaking process (unpublished data).

IV. Conclusion

Use of the WASP® instrumentation in "streak only mode" yielded significantly more beta-hemolytic streptococcal isolates (93) (Sensitivity: 94%; 95% Confidence Interval: 87-98%) than the manually struck plates (79) (Sensitivity: 80%; 95% Confidence Interval: 70-87%). Additionally, the WASP® struck plates were easy to read due to the consistent streak pattern. This study suggests that the WASP® can be utilized in "streak only mode" using a single streak pattern for cultures plated to selective

V. References

1. Quiblier C, et al. 2016. Performance of Copan WASP for routine urine microbiology. J Clin Microbiol 54:585-592. 2. Croxatto A, et al. 2015. Comparison of inoculation with the InoqulA and WASP automated systems with manual inoculation. J Clin Microbiol 53:2298-2307.

Figure 1: Beta-hemolytic colonies on SSA struck using WASP®





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