Gram Smear Preparation In a Total Automated Microbiology Laboratory



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Introduction

The gram stain remains one of the oldest, trusted, simplest and most reliable testing techniques in the microbiology laboratory. The gram stain is the first test performed on specimens and often leads to clinical decision making and antibiotic treatment. There are, however, inherent sources of error in the gram stain test such as inconsistency in sample inoculum, slide preparation, staining technique and interpretation by the Medical Laboratory Technologist. The objective of this study is to utilize total laboratory automation such as WASP[™] for sample inoculum and slide preparation and automated gram staining to provide consistent reliable gram stained slides.



Figure 1: Blood culture gram stain



Figure 2: Eswab vaginal gram stain

This study used WASP[™] to process 210 specimens collected with ESwab[®] kits as well as 40 body fluids. Gram slides were prepared by the WASP[™] slide preparation module. Various loop sizes were used to determine the optimal inoculum volume. Slides were methanol fixed and stained with an automated Previstainer. Gram stains were read and screened for the presence of pus cells and bacteria. Results were compared to manual inoculation and staining processes.





Figure 3: Gram stain interpretation

Materials and Methods

Figure 4: WASP[™] slide prep module

Results

Of the 210 specimens processed, 192 were wound swabs, 11 eye swabs and 7 vaginal. 10 swabs were processed using a 30 ul loop. These specimens tended to wash off during the staining process. 50 slides were prepared using a 1 ul loop and stained with the Previstainer. 3 of these slides showed discordance from manual methods with the WASP[™] slides showing more bacteria and pus cells. 150 slides were prepared using a 10 ul loop with 25 slides showing discordance with the WASP[™] slides showing more bacteria and pus cells. 40 body fluids were processed with a 10 ul loop and stained with the automated stainer. The gram stain results were consistent with manual methods.

Conclusions

WASP[™] slide preparation and automated staining resulted in equal or better quality gram stains compared to manual preparation and staining methods. ESwab[®] kit allows the entire specimen to elute from the flocked swab creating a homogeneous suspension for a more precise and even smear. Smears were more consistent in both thickness and staining. Using total laboratory automation for gram slide preparation and also frees up time for the technologist and allows for the opportunity to implement skill mix by using laboratory assistants to process and stain specimens.

