

Comparison of the quality of Gram stain prepared using different swab transport systems.

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Abstract

Objectives: Gram staining is one of the most useful tests performed in the microbiology laboratory. This valuable, rapid and inexpensive staining technique is very often chosen to confirm presumptive identification, contribute to the selection of culture media, especially with mixed flora, and provides guidance in the selection of further identification. The aim of the present study was to compare the Gram stain exam results of smears prepared from five different swab transport systems (Medical Wire & Equipment Sigma-swab, Sigma-Transwab, charcoal swab and Copan Diagnostic eSwab and Healthlink Amies transport system) using control strains of gram positive and gram negative organisms.

Method: Swabs in triplicate were inoculated with a suspension of control strains of gram positive and gram negative rods and cocci with different arrangements based on their planes of division and placed back into the transport device. Three sets of slides were prepared for each swab system. Slides were stained using the standard Gram staining procedure. The microscopic examination results were compared and noted.

Results: Microscopic examination of Gram stain slides from the five different transport systems showed that the quality of slide prepared from dry Sigma-swab was superior to those obtained using gel or liquid media. There was a heavy background of molecules resembling gram-negative cocci observed with eSwab, which can lead to negative and misleading interpretation of results.

Conclusion: Gram staining is the most productive first line of defence procedure in the clinical laboratory to detect bacteria as well as TB, fungi and parasites. This study showed that the Gram stain prepared using dry Sigma-swab demonstrated the highest quality in comparison to gel and liquid transport systems. The quality of the transport swabs used to prepare the stain is very important. The presence of background and non-viable microorganisms in the transport medium can cause misleading results.

Introduction

Gram's stain is a widely used method of staining bacteria as a aid to their identification and it can be a critical test for the rapid presumptive diagnosis of infectious agents. A good specimen collection system is very important especially if the Gram stain is prepared directly at the time of collection. Interpretation of Gram-stained smears involves consideration of staining characteristics and cell size, shape and arrangements. These characteristics may be influence by a number of variables, including media, culture age, incubation atmosphere, staining methods and the presence of inhibitory substances. Similar considerations apply to the interpretation of smears from clinical specimens and additional factors include different host cell types and possible phagocytosis. The aim of the present study was to compare the Gram Stain exam results of smears prepared from five different swab transport systems (Medical Wire & Equipment Sigma-swab, Sigma-Transwab, charcoal swab and Copan Diagnostic eSwab and Healthlink Amies transport system) using control strains of gram positive and gram negative organisms.

Methods

Swabs in triplicate were inoculated with control strains of *S.aureus*, *E.coli* and their mixtures and then placed back immediately into the transport tube. The eSwab and Sigma-Transwab tubes were vortexed , swabs were removed and 50µl of liquid medium was used to prepare slides by spreading onto the slide surface with the help of a second slide. Slides from gel transystems were prepared directly by rolling and smearing the swab on the slide. Slides from Sigma dry swab were prepared by dipping the swab into the drop of distilled water placed onto the slide surface and spreading it over the surface. All slides were air dried and stained using standard Gram staining procedure.

Results

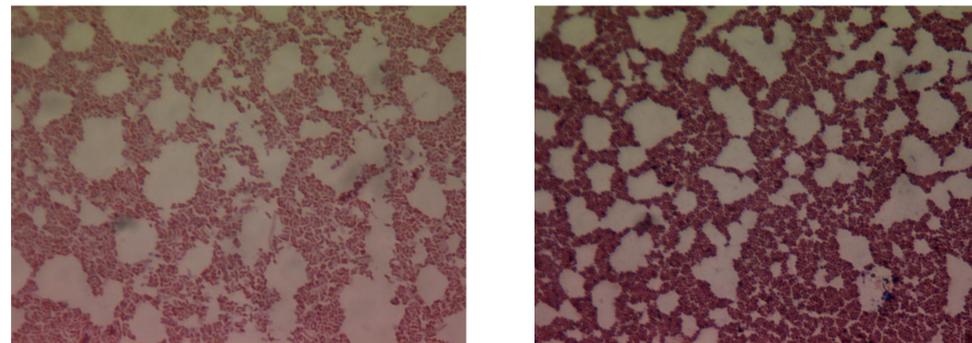


Figure 1. Gram Stain from Sigma swab – *E.coli* (left picture), *S.aureus* (right picture). There were no background in any of microscopic fields.

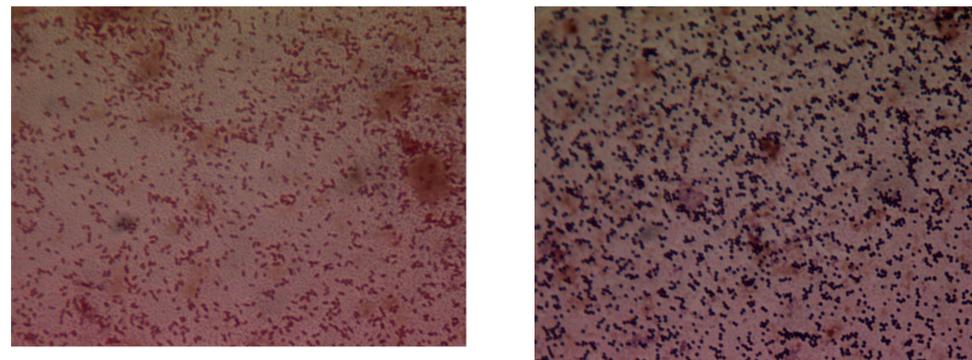


Figure2. Gram Stain from eSwab – *E.coli* (left picture), *S.aureus* (right picture). Heavy background of gram-negative molecules in every microscopic field.

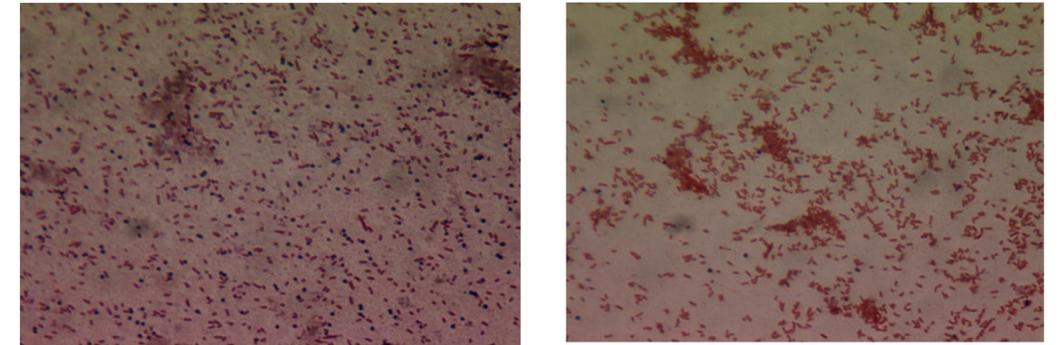


Figure 3. Gram Stain from eSwab – mixture of *E.coli* and *S.aureus* (left picture), plain eSwab inoculated with *E.coli* (right picture). There is a background in every microscopic field even with plain eSwab inoculated with bacteria.

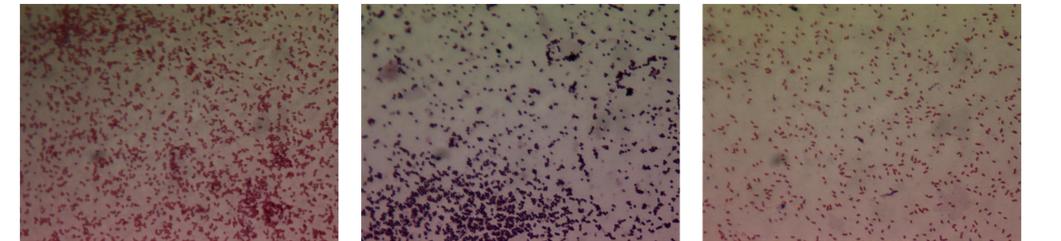


Figure 3. Gram stain from Sigma Transwab – *E.coli* (left picture), Gram stain from Charcoal swab – *S.aureus* (middle picture). Gram stain from HealthLink swab (right picture). Very light background in few microscopic fields with every type of swab.

Discussion/ Conclusion

Examination of Gram Stain slides from the five different transport systems showed that the quality of slide prepared from dry Sigma Swab was superior to those obtained using gel or liquid media. There was a heavy background of gram-negative molecules covering every microscopic field even with a slide prepared using dry eSwab without the medium. Gram stain slides prepared from Sigma Transwab, Charcoal Swab and HealthLink Amies swab showed very light background in few microscopic fields. The quality of the transport swabs used to prepare the stain is very important. The presence of background and non-viable microorganisms in the transport medium can cause misleading results.

References

1. Quality Control of Microbiological Transport Systems: Approved Standard. NCCLS document M40-A. 2003.
2. Fontana, C., Favaro, M., Limongi, D., Pivancova, J., Favalli, C. Comparison of the eSwab collection and transportation system to an amies gel transystem for Gram stain of clinical specimens. *BMC Research Notes* 2009, 2:244.

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