Performance of the PREVI™ Color Gram automated staining system

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ABSTRACT

Gram stain is a critical stage of the microbiological diagnosis. Even though it is usually carried out manually, automation has become an alternative. Bacteriologists used to the manual method may, however, consider that automation allows to subtly adapt the decolorization step to the thickness of the smear.

Objectives: A study was performed to evaluate the performance of the PREVI™ Color Gram system (bioMérieux) on pure strains and biological samples in comparison with the manual staining method. This new system ensures an automatic Gram staining of slides disposed on a carrousel. Dyes are sprayed on slides during rotation.

Methods: Manual and automatic stainings were carried outin parallel. Manual Gram stain was performed according to the conventional procedure. The Gram staining on PREVI Color Gram system was performed using the Decolorizer 2 and 3 programs. Slides were then mixed and read by a trained bacteriologist not knowing the bacterial identity and the staining type carried out on each slide.

The first part of the study consisted of the staining of 40 reference strains. Results were considered to be concordant when morphology, arrangement and Gram reaction of bacteria were identical. The second part of the study included 117 clinical specimens from various origins. Smears were randomly attributed to the manual or the two automated protocols. Preparations were considered to be concordant when proportion of bacterial population , morphology, arrangement, Gram reaction were identical.

Results: 321 preparations were compared, 8 of them were declared non concordant, but not significant as real discrepancies. These discrepancies involved 4 samples and were due to a) insufficient decolorization by the manual technique, b) washing out of the smear during manual staining, c) difference in thickness of the smears in the case of a very mucoid expectoration, and d) staining problem which could not be allocated to one or the other of the techniques. The two automated protocols gave the same results.The PREVI Color Gram using the Decolorizer 3 and 2 provided 100% and99% agreement with the manual method for reference strains and clinical samples respectively. Discrepancies analysis was never in discredit of the automatic technique.

Conclusions: Even using a manual standardized protocol, significant staining differences may persist between operators. The PREVI Color Gram system provides distinct and reproducible results, thus contributing to stain standardization, besides saving dyes and technician time.

INTRODUCTION

The PREVI™ Color Gram system ensures an automatic Gram staining of slides disposed on a carrousel. Dyes are sprayed on slides during rotation.

This study was performed to evaluate the performance of the PREVI Color Gram system on pure strains and biological samples in comparison with the manual staining method.

MATERIAL AND METHODS

Manual and automatic stainings were carried out in parallel. Manual Gram stain was performed according to the conventional procedure (K.C. Chapin and T.L. Lauderdale, Reagents, stains and media : bacteriology in Man Clin Microbiol, 9th éd., ASM Press, p.335), using Color Gram 2 reagents (bioMérieux, France) and home-made acetone-alcohol.

The Gram staining on PREVI Color Gram system was performed using the Decolorizer 2 and 3 programs. Slides were then mixed and read by a trained bacteriologist not knowing the bacterial identity and the staining type carried out on each slide.

The first part of the study consisted of the staining of 40 reference strains: 18 Gram-positive, 20 Gram-negative and 2 yeasts. Results were considered to be concordant when morphology, arran-gement and Gram reaction of bacteria were identical. The second part of the study included 117 clinical specimens from various origins (31 urines, 10 blood cultures including 6 with charcoal, 20 faeces, 20 genital swabs, 5 bronchoalveolar lavage fluids, 10 CSF and 21 sputum specimens).

Smears were randomly attributed to the manual or to one of the two automated protocols. Preparations were considered to be concordant when the main bacterial populations were found in equivalent proportions.

PREVI Color Gram system

RESULTS

Reference strains:

When working on colonies from reference strains, the PREVI Color Gram system provided 100% agreement [confidence interval CI= 90,91-100 %] with the manual technique. The automatic discolouration of Gram negative bacteria proved to be more regular than with the manual process.

Clinical specimens:

The 117 specimens were compared using the manual (M), automatic # 2 (A2) or automatic #3 (A3) protocols. Comparing M to A2, 4/117 specimens were declared non concordant. When results were analyzed in term of superiority, only one of the discordant preparations could not be allocated to one or the techniques. This discrepancy being excluded, 115/116 smears were equally or best stained with the A2 protocol (table 1). In protocol 3 versus manual technique comparison (table 2), 3 non concordant specimens were registered and discrepancies analysis was never in discredit of the automatic technique. Between the two automated protocols, no significant difference could be noticed (table 3).

Thus, the PREVI Color Gram using either Decolorizer 2 or 3 automatic programs provided total agreement with the manual method for reference strains and 99% for clinical samples.

Table 1: Sample repartition according to equivalence or superiority between manual (M) and program 2 of automatic staining (A2),

Method comparison	Number	%	CI 95%
A2 < M	1	0.85	[0.15%; 4.81%]
A2 ≥M	115	99.14	[95.15%; 99.85%]

Table 2: Sample repartition according to equivalence between manual and program 3 automatic staining

Equivalence	Number	%	CI 95%
No	3	2.56	[0.86%; 7.41%]
Yes	114	97.44	[92.59%; 99.14%]

Table 3: Sample repartition according to equivalence between programme 2 and program 3 automatic staining

Equivalence	Number	%	CI 95%
No	1	0.85	[0.15%; 4.81%]
Yes	116	99.15	[95.19%; 99.85%]

CONCLUSION

Gram stain is a critical stage of the microbiological diagnosis.

The bacteriologists accustomed to the manual technique may consider that it makes it possible to adapt the decolorization step to the thickness of the smear. Actually, even using a manual standardized protocol, significant staining differences may persist between operators.

The PREVI Color Gram system was shown to provide distinct and reproducible results, thus contributing to stain standardization, besides saving dyes and technician time.