

Comparison of PREVI™ Color Gram Automated Gram Stain to Manual Gram Stain

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ABSTRACT

Background: Gram stains provide valuable information about specimens submitted for culture. Although considerable expertise is required, specimen quality, information regarding presence or absence of infection and a preview to the cause of infection can be obtained very quickly. Unlike cultures, gram stains can be performed STAT and therefore used to guide empiric therapy decisions. However, traditional manual gram staining provides several challenges. Inconsistent results due to tech variability, stricter regulations regarding proper disposal of waste stain reagents and more emphasis on productivity in the Microbiology department led us to evaluate the automated PREVI™ Color Gram (bioMérieux, SA). **Methods:** 1000 specimens were included in the evaluation. Specimen types included urine, positive blood cultures, wounds, stools, genital swabs, bronchial lavage and sputum. Two slides were prepared for each. One slide was stained using the existing manual procedure and the other using the automated PREVI Color Gram. Both slides were read by the same technologist and results recorded along with comments regarding the quality of each slide. To optimize the study, these assessments were performed without knowledge of the method used for each slide set. In addition, measurements were taken of the quantity of reagents used, quantity of waste produced and technologist time required when doing manual and automated staining. **Results:** The stains prepared using the PREVI Color Gram were of more consistent high quality and overall easier to read than those prepared using the manual method. The PREVI Color Gram took 7 minutes and 32 seconds to produce a batch of 12 stained and dried slides, produced 105ml of waste and used 20ml of stain reagents at an estimated cost of \$0.95. The manual method took 14 minutes and 23 seconds to produce the same number of stained and dried slides, produced 4470ml of waste and used 90ml of reagent at an estimated cost of \$1.04. **Conclusions:** The PREVI Color Gram is an excellent cost effective alternative to manual gram staining. The system saved tech time, reagents and waste production while improving the consistency and quality of the gram stains.

INTRODUCTION

Gram stain continues to be a rapid and comprehensive method for obtaining preliminary results for which to make diagnostic and therapeutic decisions. Although much more sensitive and specific tests have been developed, the speed, low cost and wide range of information that can be obtained from this one test prevents its replacement by these newer technologies. Hands on time, hazardous waste production, and variability in technique, however, are definite challenges of this procedure. We compared the manual gram stain to the automated PREVI Color Gram for turnaround time, hands on time, waste production, reagent use and slide quality to see if automating this procedure would reduce these challenges.

bioMérieux PREVI Color Gram



METHODS

Quality Study

Two slides were prepared from each of 1000 specimens. 150 urines, 150 aerobic blood cultures bottles, 150 anaerobic blood culture bottles, 150 wound swabs, 50 stools, 100 genital swabs, 50 bronchial lavages, 100 sputum, and 100 culture isolates were included. One slide of each pair was labeled as "A" and one was labeled "B". The slides labeled "A" were stained using the manual gram stain following the department's written procedure (1 minute crystal violet, 1 minute iodine, 2-10 seconds decolorizer and 1 minute safranin). The slides labeled "B" were stained with the PREVI Color Gram. One technologist performed all of the staining. A separate technologist read all of the slides without knowledge of which stain procedures were used. Results were documented and one of each pair was selected as the best quality. Discrepant results between slides were resolved by slide review and culture result review.

Waste Study

To measure the waste produced by the automated PREVI Color Gram, 12 slides were run using the 12 slide carousel and the waste was poured from the waste container and measured in mls. This was repeated for the 30 slide run using the 30 slide carousel. To measure the waste produced for the manual method, an empty waste container was placed in the sink to catch the waste. After 12 slides were processed, this waste was measured. This was repeated staining 30 slides. The cost of waste was then calculated based on an estimated \$4.75/gallon and our facilities average of 2500 gram stains per month (30000/year)

Time Study

In order to obtain the total turnaround time for the manual gram stain, the measurement began as slides were placed onto the slide warmer and ended when slides were dry enough to read. The 12 slide run was performed in two batches because only 8 slides fit comfortably on our manual staining rack. The 30 slide run was performed in four batches. To obtain the PREVI Color Gram's time, measurement began with loading the slides onto the carousel and ended with unloading the slides following completion of the stain program. Measurements were taken for a load of 12 slides and repeated for a load of 30 slides.

Reagent Use

Reagent use was calculated for the manual staining method by flooding a slide with stain and aspirating it using a needle and syringe to obtain a measurement. This was repeated three times to obtain an average. Reagent use for the PREVI Color Gram was obtained from the Operators Manual since this is a programmed amount.¹

Reagent Cost Study

Reagent costs were calculated using our current reagent pricing and reagent use as obtained above. After converting pricing from cost/ordering unit to cost per ml, a cost per slide and cost per batch of 12 slides were obtained.



Manual Staining Station

PREVI Color Gram Staining Station

RESULTS

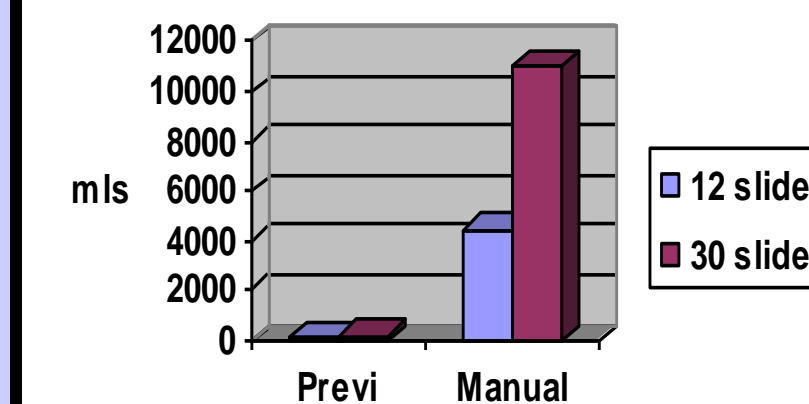
Of the 1000 pair of slides evaluated, 8 produced discrepant results. After review of both slides and culture results, it was determined that the PREVI slide results were correct in 6 of the 8 pair for a 0.2% overall error rate. The manually stained slide was correct in 2 of the 8 pair resulting in a 0.6% overall error rate. For overall quality of the slides, the PREVI slide was preferred in 943 of the 1000 pair examined (94.3%).

The time involved in staining was significantly less with the PREVI Color Gram which took approximately 50% less time to stain batches of 12 or 30 than did the manual staining method. The system produced a stained batch of 12 slides in 7 minutes and 32 seconds compared to the 14 minutes and 23 seconds required of the manual method. For the batch of 30 slides, the PREVI took 10 minutes and 7 seconds and the manual stain took 22 minutes and 10 seconds. Additionally, the tech hands on time for the PREVI was much less at only 2 minutes and 24 seconds for 12 slides and 4 minutes and 4 seconds for 30 stained slides compared to the manual stain which took 9 minutes and 16 seconds and 16 minutes and 53 seconds respectively.

There was also less reagent use with the PREVI Color Gram. It used slightly less than 20ml (19.4ml) of reagent to stain 12 slides. The manual method averaged 2.5ml of each reagent per slide for an average of 90ml for 12 slides. However, there was no significant difference in reagent cost per slide.

The most dramatic difference found between the PREVI Color Gram and manual stain method was in waste production. Using the PREVI, staining of 12 slides using the 12 slide carousel and 30 slides using the 30 slide carousel produced only 105ml and 220ml respectively but the manual staining method produced 4470ml and 10980ml for those same size runs. This equates to an average of 8ml of waste/slide using the PREVI Color Gram and 369ml of waste/slide with manual staining which is a 97% reduction in waste with the PREVI Color Gram. For facilities with similar volume as ours (average of 30000 gram stains per year) who must pay to have gram stain waste disposed of, this could equate to a savings of \$13,590 per year.

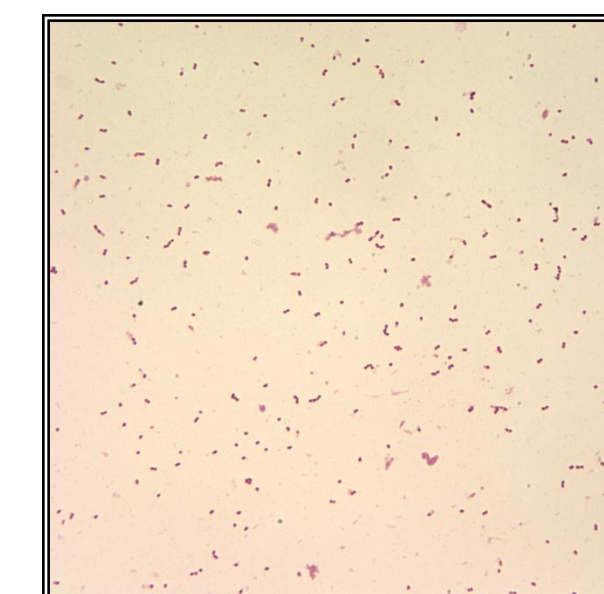
Waste Study



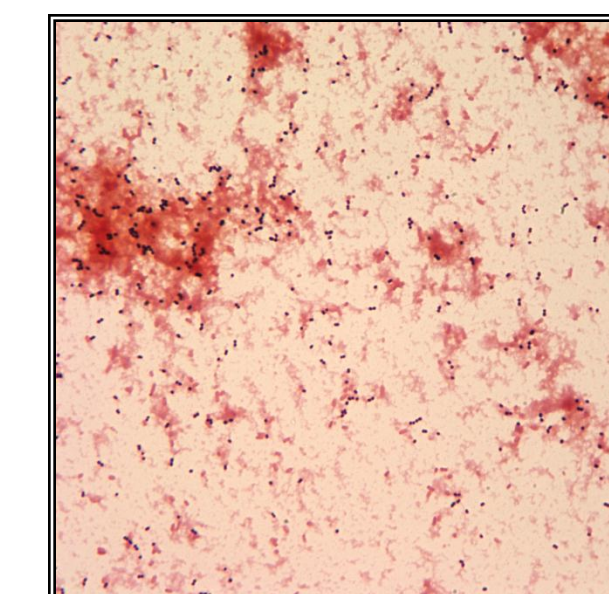
Waste produced from staining 12 slides

QUALITY STUDY

(Blood culture containing *Klebsiella pneumoniae* and *Enterococcus faecalis*)



Manual Stain



PREVI Color Gram Stain

TIME STUDY

Method	12 slide run Total time	30 slide run Total time	12 slide run Hands on time	30 slide run Hands on time
Previ™ Color Gram	7 min 32 sec	2 min 24 sec	4 min 4 sec	10 min 7sec
Manual	14 min 23 sec	9 min 16 sec	16 min 53 sec	22 min 10 sec

CONCLUSIONS

The PREVI Color Gram is an excellent cost effective alternative to manual gram staining. The system saves tech time, reagents and waste production while improving the consistency and quality of the gram stains. It provided a cleaner more lean work area and has been implemented in our laboratory as standard procedure for gram stains. As a side note, bioMérieux also has available a cytopspin rotor that may be used with the PREVI Color Gram. Using a cytopspin method vs. a 15 minute centrifugation and slide drying significantly decreased CSF gram stain turn around time in our experience.

REFERENCES

¹ PREVI Color Gram Operators Manual.

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