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ANALYSIS

Evaluation of a new method for Gram staining of bacteria

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ABSTRACT

Gram staining is a very important and useful technique for bacterial identification, often helping in selection of empirical antibiotic therapy. Some of the reagents for Gram stain can be replaced by those which can be useful in other stains also. We here evaluate one such method.

Keywords: Gram stain, new method.

Abbreviations: *M. leprae*: *Mycobacterium leprae.*

1. INTRODUCTION

Gram stain is a rapid and important method for bacterial differentiation (Beveridge TJ, 2001). Named after Hans Christian Gram who developed the method in 1884, the Gram stain helps to distinguish between Gram-positive and Gram-negative bacteria on the basis of differential staining with crystal violet-iodine complex and a safranine counterstain(Coico R, 2005). Acid alcohol (1%) is useful in Acid -fast staining for *M.leprae*(Acharya T). So if this 1% acid-alcohol mixture is useful as a decoloriser in Gram stain, it can replace acetone which is conventionally used for this, and consequently save cost. Hence this study was planned to compare the efficacy of a mixture of 1% acid in alcohol (70% ethanol) as decoloriser with that of acetone.

2. MATERIALS AND METHODS

This was a laboratory-based observational study, carried out in the Department of Microbiology of the institute, from mid-June 2015 to End of July, 2015 (one and half months.).Ten clinical isolates each of *Staphylococcus aureus*, *Bacillus* spp. and *Escherichia coli* were selected randomly

for the study. Each time on a fresh clean glass slide, 2 smears were prepared, 1 from *Staphylococcus aureus* or *Bacillus* spp. (alternatingly), and 1 from *Eschericha coli*.

In the conventional method, smear was prepared, air-dried and heat fixed, and Crystal violet was poured on it and kept for 1 minute. Then Gram's iodine was added to it and kept for 1 minute, followed by decolorisation with Acetone for 10-15 seconds and counterstaining with 0.5% Safranine.

In the new method, smear was prepared, air-dried and heat fixed, and and Crystal violet was poured on it and kept for 1 minute. After that, Gram's iodine was added to it and kept for 1 minute, followed by decolorisation with 1% acid-alcohol for 4-5 seconds (99 ml of 70% ethanol +1 ml of conc. sulfuric acid). Counterstaining was done by 0.5% Safranine.

The methods were repeated three times for 1 particular isolate. Slides were dried, blotted on tissue paper and observed under oil immersion lens (100X objective) for results.

3. RESULTS

The new decoloriser was equally effective and non-inferior to acetone. Using the new method, *S. aureus* and *Bacillus* spp. were Gram positive and E. coli was Gram negative. Thus there was 100% concordance between the two methods.

4. DISCUSSION

Gram staining is a very old staining technique for medically important bacteria, with innumerable modifications (Gram stain Protocol). It helps in instituting effective empirical treatment to the patients, and is very important since treatment will vary considerably depending on Gram staining pattern(Perez-Jorge and Burdette). Keeping these things in mind, our new staining method, especially new method of decolourisation is very useful. Acetone usage can be associated with potential toxic effects like headache, dizziness when inhaled accidentally in large amounts(Overview.What Is Acetone Poisoning).Acid alcohol can replace acetone in Gram stain, as found in our study. Further studies are required regarding this.

5. CONCLUSION

Acid-alcohol in the said percentage is a new decoloriser in Gram staining that can replace acetone, being safer and useful in other stains also.

SUMMARY OF RESEARCH

This is a new method of decolorisation in Gram stain and further such studies are needed and awaited.

FUTURE ISSUES

In our opinion researchers will foind this method of decolorisation easy , safe and effective in the long run and it can replace acetone as a component of Gram stain.

DISCLOSURE STATEMENT

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