Instructions For Use

BBS-IFU

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Gram Stain Kit

(Modified Brown & Brenn)

Description and Principle

The Gram Stain Kit (Modified Brown & Brenn) is intended for the demonstration and differentiation of Gram-positive and Gram-negative bacteria.

Gentian Violet along with Lugol's Iodine forms a dye lake staining both gram-positive and gram-negative organisms. Gram-positive and gram-negative bacteria are differentiated due to differences in cell wall composition. Gentian violet-iodine complex is removed from gram-negative bacteria while gram-positive bacteria retain the stain. Safranin O is then applied as a counterstain for the differentiated gram-negative bacteria.

Expected Results

Gram Positive Bacteria:	Blue
Gram Negative Bacteria:	Red
Background:	Yellow
Nuclei:	Red

Kit Contents

Kit Contents	Storage
1. Gentian Violet Solution	18-25°C
2. Lugol's Iodine Solution	18-25°C
3. Gram's Decolorizer Solution	18-25°C
4. Safranin O Solution (For Gram Staining)	18-25°C
5. Picric Acid - Acetone Solution (0.1%)	18-25°C.

Suggested Controls *(not provided)*

Tissue or cell smear containing both gram-positive and gram-negative organisms

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure

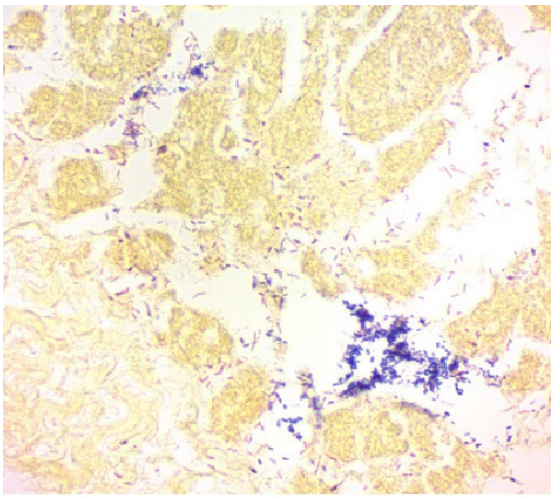
1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Apply Gentian Violet Solution to tissue section for 2 minutes.

3. Rinse slide in distilled water to remove excess stain.

4. Apply Lugol's Iodine Solution to tissue section for 1 minute.

5. Rinse slide in distilled water to remove excess solution.



Gram stain on Avian Liver demonstrating gram-positive and gram-negative bacteria. Magnification 400X

6. Carefully apply Gram's Decolorizer dropwise until color no longer bleeds off section. Note: Application of this decolorizer for longer than 5 seconds may remove stain from gram positive bacteria.

7. Rinse slide quickly in distilled water.

8. Apply Safranin O Solution to tissue section for 4 minutes.

9. Rinse slide in distilled water to remove excess stain.

Note: Alcohol and Picric Acid-Acetone (steps 11-12) are required to remove red stain from background, but excess incubation with these solutions can also remove stain from bacteria.

10. Dip slide once in absolute alcohol and then remove excess alcohol from slide by blotting.

11. Carefully apply a few drops of Picric Acid - Acetone Solution (0.1%) while gently agitating for 2-10 seconds, then immediately and briefly rinse slide in absolute alcohol. If tissue is still strongly red, repeat step 12 until it is mostly yellow – a tinge of red may remain due to presence of nuclei or large amounts of gram(-) bacteria.

12. Allow slide to air dry.

13. Clear, and mount in synthetic resin.

References

1. Isenberg, H.H. Clinical Microbiology Procedures Handbook. American Society for Microbiology, 1992.
2. Sheehan, DC., Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 235.
3. Brown, J.H., Brenn, L. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. Bulletin John Hopkins Hospital, 1931, Volume 48, pages 69-73.
4. Gram, C. Fosrtchr. Med., Volume 2, page 185, 1884.

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