

BROWN AND HOPPS GRAM STAIN

Purpose The demonstration of gram-negative and gram-positive bacteria.

Principle Crystal violet is applied first and then followed by an iodine mordant, forming a dye lake. At this point both gram-positive and gram-negative organisms are stained. Both types of bacteria's walls are composed of peptidoglycan but gram-positive walls are thicker. Therefore, decolorizing will differentiate them from gram-negative organisms.

Fixative 10% neutral buffered formalin.

Reagents

1% Crystal Violet

Crystal violet ... 5.0 g

Distilled water ... 500.0 ml

Gram's Iodine

Iodine ... 3.0 g

Potassium iodide ... 6.0 g

Distilled water ... 900.0 ml

Place the iodine and potassium iodide in approximately 150 ml of water. Stir or vortex until dissolved. Then add remaining water.

Stock Basic Fuchsin Solution

Basic fuchsin ... 0.25 g

Distilled water ... 100.0 ml

Working Basic Fuchsin Solution

Stock basic fuchsin solution ... 2.0 ml

Distilled water ... 50.0 ml

Gallego's Solution

Distilled water ... 50.0 ml

Formalin, 37-40% ... 1.0 ml

Glacial acetic acid ... 0.5 ml

Picric Acid-Acetone Solution

Picric acid ... 0.5 g

Acetone ... 1,000.0 ml

Quality Control

Use a control slide with known gram-negative and gram-positive tissue.

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Procedure

1. Deparaffinize and hydrate sections to water.
2. Stain in crystal violet for 2 minutes.
3. Rinse in water.
4. Stain in Gram's Iodine for 5 minutes.
5. Rinse in water.
6. Blot one slide at a time with slightly damp filter paper and decolorize quickly in 100% acetone.
7. Rinse quickly in water.
8. Stain with basic fuchsin working solution for 5 minutes.
9. Rinse in water.
10. Differentiate in Gallego's solution for 5 minutes with periodic agitation.
11. Rinse in water. Blot but do not dry.
12. Dip quickly 3 times in 100% acetone.
13. Dip quickly 3 times in picric acid-acetone solution.
14. Dip quick 3 times.
15. Pass through acetone/xylene to clear in xylene.
16. Mount in synthetic resin.

Results

Gram-positive bacteria ... blue
Gram negative bacteria ... red
Background ... yellow
Nuclei ... light red

References

Histotechnology, A Self-Instructional Text, Freida L. Carson, p. 194.