**UW Medicine - Pathology**

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Brown and Brenn for Gram Positive and Gram Negative Bacteria Procedure

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PURPOSE

To identify the method for performing the special stain of Brown and Brenn method for demonstration of Gram Positive and Gram Negative Bacteria procedure.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Paraffin sections cut at 4 microns.

*NOTE:* Use Type II deionized water for all solution preparation.

**Solutions:**

**Crystal Violet**

Richard Allan Scientific. Ready made.

**Gram’s Iodine**

Richard Allan Scientific. Ready made.

**Stock Basic Fuchsin**

Basic fuchsin 0.50 gm

Distilled water 100.0 ml

*Working:*

**Basic Fuchsin**

Stock Basic Fuchsin 20.0 ml

Distilled water 50.0 ml

**Decolorizing Solution**

Richard Allan Scientific. Ready made.

**Tartrazine Yellow**

Richard Allan Scientific. Ready made.

**Procedure:**

Use control slide.

1. Deparaffinize and hydrate to distilled water.
2. Place slides on a staining rack. Flood slides with working crystal violet for 1 minute.
3. Rinse in tap water.
4. Flood slides with Gram’s iodine for 5 minutes.
5. Rinse in tap water
6. Decolorize with decolorizer until no more color runs off.
7. Rinse in running water for 3 minutes.
8. Working basic fuchsin for 1:30 minutes.
9. Wash in tap water.
10. Flood slides with Tartrazine Yellow for 20 seconds.
11. Dehydrate quickly, clear and mount.

**Results:**

Gram positive bacteria blue-black

Gram negative bacteria red

Filaments of Norcardia and actinomyces blue

Nuclei red

Other tissue elements yellow

**Comments:**

This method is invaluable in the demonstration of the filaments of *Nocardia* and *Actinomyces*. It must be realized, however, that these filaments are not completely or strongly Gram positive. It is possible to obtain either Gram positive or Gram negative results depending on the degree of differentiation. It is suggested, therefore, that at least two slides be run with varying degrees of differentiations.

Basically, the procedure involves the application of crystal violet solutions, followed by an iodine mordant to form a dye lake. Both Gram positive and Gram negative cells are colored blue-black after these two steps.

Decolorization is the third major step, and its purpose is to render the Gram negative cells colorless while leaving the blue-black dye lake in the Gram positive cells. If sections are exposed too long to the action of the decolorizing agent, even Gram positive cells will loose the dye lake and become colorless.

The final major step is counterstaining with basic fuchsin and the Gram negative cells are dyed pink-red.

There numerous theories that attempt to explain the Gram reaction. Although the dye lake is most likely distributed throughout the entire cell, the cell wall of the bacteria is believed to play a role in keeping the dye lake within the Gram positive cells. Studies have shown that Gram positive cells have walls 3-4 layers thick; Gram negative cells consist of 2 layers. This would explain differential decolorization on a somewhat physical basis as the Gram negative cell wall would not be thick enough to resist decolorization relative to the Gram positive cell wall.

Another factor is that Gram positive cell walls have a greater proportion of lipoprotein and polysaccharide components within them; hence, they are relatively more impermeable cell walls compared to the Gram negative cell walls. this lesser permeability would aid in dye lake retention once the lake had entered the Gram positive cell.

A chemical factor may also be involved in Gram staining as experiments have shown that Gram positive cells contain an acidic substance, (magnesium ribonucleate), within the cell wall. This substance is known to form insoluble complexes with crystal violet and iodine and these complexes do not decolorize with conventional decolorizing agents. Gram negative cells lack this substance.

REFERENCES

Luna, Lee G.: *Manual of Histologic Staining Methods of the AFIP*, McGraw-Hill Book co., 1968, pg. 222-223. Modified by Histopathology Laboratory, Harborview Medical Center, Seattle, WA.

Sheehan, D.C. and Hrapchak, B.B.: *Theory and Practice of Histotechnology,* The C. V. Mosby Co., 1980, pg.234.

Written By: Director Approval:

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