

# Beaumont

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## Histology Special Stain - Twort's Gram Stain - Royal Oak

Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for differentially demonstrating gram positive and gram-negative bacteria.

### II. PRINCIPLE:

The cell walls of gram-positive bacteria, when compared with the wall of gram negative, are thicker, have more layers, have different lipoproteins and polysaccharides, and have a higher amount of an acidic substance. When both types of bacteria are stained, first with crystal violet and then with iodine mordant, a dye lake is formed. Both types of bacteria are stained blue. When the acetone is applied, the thicker cell walls of the gram-positive bacteria, with its more impermeable lipoproteins, will resist decolorization longer than the gram-negative bacteria. Also, the high acidic substance in the cell walls of the gram-positive bacteria form a complex with the crystal violet and iodine, which helps in resisting decolorization. The neutral red stains the gram-negative bacteria. Fast Green counterstains the background.

### III. SPECIMEN COLLECTION AND HANDLING:

- A. Fixation
  - 1. Any well-fixed tissue.
- B. Processing
  - 1. Standard, overnight processing.
- C. Section Thickness

1. Routine specimens-5 $\mu$ .
- D. Slide Drying
1. 60 minutes at 60°C.
- E. Type of Slide
1. Plain slides.

## IV. REAGENTS:

A. **Crystal Violet Solution**

Use pre-made solution

B. **Gram Iodine Solution**

Use pre-made solution

C. **0.2% Neutral Red In Ethanol**

<b>Neutral Red</b>	<b>0.2 gm</b>
<b>100% Ethanol</b>	<b>100.0 mL</b>

Mix well, store in brown bottle at room temperature for 6 months

D. **0.2% Fast Green In Ethanol**

<b>Fast Green</b>	<b>0.2 gm</b>
<b>100% Ethanol</b>	<b>100.0 mL</b>

Mix well, store in brown bottle at room temperature for 6 months

E. **Twort's Counterstain**

<b>0.2% Neutral Red in Ethanol</b>	<b>9.0 mL</b>
<b>0.2% Fast Green in Ethanol</b>	<b>1.0 mL</b>
<b>Distilled Water</b>	<b>30.0 mL</b>

Mix immediately before use

## V. EQUIPMENT:

- A. Balance
- B. Magnetic stirrer

## VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Coplin jars

## VII. QUALITY CONTROL (QC):

Tissue containing gram positive and gram-negative bacteria.

## VIII. SPECIAL SAFETY PRECAUTIONS:

### A. Crystal Violet

1. Is toxic.
2. Is a carcinogen.
3. May cause serious eye damage.

### B. Gram Iodine

1. May be fatal if swallowed.
2. Irritant to eyes and skin.

### C. Acetone

1. Is an extremely flammable liquid and vapor.
2. Vapor may cause flash fire.

### D. Neutral Red

1. Is an irritant.

### E. Fast Green

1. Is an irritant

## IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Stain slides with crystal violet.	1 minute	Steps 2, 4 and 6 may be done by placing the slides flat on a staining rack and flooding slides with solution.
3	Rinse slides running tap water.	5-10 seconds	
4	Stain slides with Gram iodine.	1 minute	
5	Rinse slides in running tap water.	5-10 seconds	
6	Decolorize slides quickly with acetone until background is clear.	1-2 seconds	
7	Rinse slides in distilled water.	30 seconds	
8	Counterstain in Twort's.	10	Must be made fresh before each use.

		minutes	
9	Rinse slides in running water.	30 seconds	
10	Dehydrate through alcohols and clear in xylene.		
11	Coverslip.		

## X. LIMITATIONS:

- A. The following may influence the validity of test results:
1. Keep the time of decolorization to a minimum, as gram positive bacteria can be over-decolorized, and will appear as gram negative.
  2. Old or dead gram-positive bacteria will stain red instead of violet blue.

## XI. RESULTS:

- A. Gram positive bacteria - **violet blue**  
 B. Gram negative bacteria - **red**  
 C. Background - **green**

## XII. REFERENCES:

- A. Preston, Morrell. J. Path. Bact. (1962), 84:241-5.  
 B. Twort. J State medicine (1924), 32:351.  
 C. Cherukian; Schenk. J. Histotech. (1982), 5(3):127-128

## Approval Signatures

Step Description	Approver	Date
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## Applicability

Royal Oak

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