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Beaumont

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Histology Special Stain - Kinyoun Acid Fast Bacteria - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of acid-fast mycobacterium, such as those causing tuberculosis, plus all non-pathogenic mycobacterium. Several fungal species, such as Nocardia, will also stain positive. It is not the stain of choice for leprosy.

II. PRINCIPLE:

The mycobacterium has a cell wall that is different than most other bacteria. While other will stain with the carbol-fuchsin, only the mycobacterium will resist decolorization with acid-alcohol. Basic fuchsin is the dye, which combines with the phenol (carbolic acid) to stain the cell wall. Possibly due to the thickness of the cell wall and its mycolic content, the mycobacterium will resist brief decolorization with acid alcohols, while all other structures will lose the red color. Methylene blue is the counterstain and may demonstrate other bacteria or fungus.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. Avoid Carnoy as it may dissolve the lipid in the cell walls, resulting in a false-negative.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens-5µ.

D. Slide Drying

1. 30 minutes at 60°C.

E. Type of Slide

1. Plain slides.

IV. REAGENTS:

A. **Kinyoun Carbol-Fuchsin Solution**

Basic fuchsin	**4.0 gm**
Phenol crystals, melted	**8.0 mL**
95% ethanol	**20.0 mL**
Distilled water	**100.0 mL**

Dissolve together. Filter. Stable at room temperature for months; may be reused until weak.

B. **2% Acid-Alcohol**

Hydrochloric acid, concentrate	**2.0 mL**
95% alcohol	**98.0 mL**

Mix together; stable at room temperature for months.

C. **Methylene Blue Stock**

Methylene blue	**1.4 gm**
95% alcohol	**100.0 mL**

Dissolve using mechanical stirrer; stable at room temperature for months.

D. **Methylene Blue Working Solution**

Methylene blue stock	**10.0 mL**
Tap water	**90.0 mL**

Mix together; stable at room temperature for months.

V. EQUIPMENT:

A. Balance

B. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Funnel, filter paper

D. Coplin jars

VII. QUALITY CONTROL (QC):

Tissue with mycobacterium. Tissue with leprosy, if demonstrating leprosy.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Basic Fuchsin
    1. Is an irritant
    2. Is a suspected carcinogen.

B. Phenol
    1. Is a poison.
    2. May be fatal if absorbed through skin.
    3. May cause liver or kidney damage.
    4. May cause skin and eye burns.
    5. Is an irritant to eyes and respiratory tract.
    6. Combustible.

C. Hydrochloric Acid
    1. Is an acid.
    2. Add drop by drop to solution.
    3. May cause severe skin and eye burns.

D. Methylene Blue
    1. Needs to be kept away from heat, as it will decompose.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place in Kinyoun carbol-fuchsin solution.	20-60 minutes	Incubate at room temperature
3	Rinse in 70% ethanol, 3-5 changes.	5-10 seconds	
4	Decolorize in 2% acid-alcohol until background is pale pink.	1-2 minutes	Keep decolorization time to a minimum, as over decolorization will give a false-negative.
5	Rinse in running water.	1-5 minutes	This will stop the further decolorization of the mycobacterium by the acid-alcohol and will allow the methylene blue to stain the tissue
6	Counterstain in 0.2% methylene	1-5	Over staining with methylene blue will mask the

	blue until background is pale blue.	minutes	mycobacterium.
7	Rinse quickly in distilled water, 2 changes.	5 seconds	
8	Dehydrate through graded alcohols, clear with xylene.		
9	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:

1. Older mycobacterium may need a longer time in the carbol-fuchsin solution. The use of heat (60°C) will also aid in the staining.
2. The longer time (1 hour) and use of heat (60°C) is usually needed to demonstrate lipofuchsin.

XI. RESULTS:

A. Mycobacterium - **magenta**

B. Nocardia, actinomycotic filaments - **magenta**

C. Background - **blue**

D. Other bacteria, fungi - **blue**

E. Red blood cell, sperm heads - **magenta**

F. Lipofuchsin - **magenta**

G. Keratin, hair, hooklets of E. granulosus - **magenta**

XII. REFERENCES:

A. Bancroft JD and Steven A: Theory and Practice of Histological Techniques, 3rd edition, New York, NY. Churchill-Livingstone, 1990.

B. Carson FL: Histotechnology: A Self-Instructional Text. Chicago, IL, ASCP Press, 1990.

C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

Step Description	Approver	Date

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Applicability

Royal Oak