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Beaumont

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Histology Special Stain - Fite's Acid Fast (AFB) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of mycobacteria. It is the stain of choice for leprosy. It can also be used to demonstrate other mycobacteria, such as those causing tuberculosis, and non-pathogens, Kinyoun is the stain of choice.

II. PRINCIPLE:

The mycobacteria have a cell wall that is different than most other bacteria. While both will stain with the carbol-fuchsin, only the mycobacteria will resist decolorization with acid-alcohol. The xylene-peanut oil mixture coats the mycobacteria, enhancing the acid fastness. 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 mycobacteria will resist brief decolorization with dilute acid, 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. 10% neutral buffered formalin preferred.
3. 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. 60 minutes at 60°C.

E. Type of Slide
    1. Plain slides

IV. REAGENTS:

A. **Xylene-PEANUT OIL**

Xylene	**60.0 mL**
Peanut Oil	**20.0 mL**

Mix together just before use.

B. **Ziehl-Neelsen Carbol Fuchsin Stain**

Basic fuchsin	**1.0 gm**
Phenol crystals, melted	**5.0 mL**
Absolute ethanol	**10.0 mL**
Distilled water	**85.0 mL**

Dissolve together. Filter. Stable at room temperature for months; may be re-used until weak.

C. **2% Sulfuric Acid**

Sulfuric acid, concentrate	**2.0 gm**
Distilled water	**98.0 mL**

Mix together. Stable at room temperature for months.

D. **0.2% Methylene Blue**

Methylene blue	**0.2 gm**
Absolute ethanol	**20.0 mL**
Distilled water	**80.0 mL**
Acetic acid	**1.0 mL**

Dissolve together methylene blue and absolute ethanol. Add distilled water and acetic acid. Stable at room temperature for months; may be re-used until weak.

V. EQUIPMENT:

A. Balance

B. 60°C oven

C. Magnet stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars
D. Forceps
E. Funnel
F. Filter paper

VII. QUALITY CONTROL:

Tissue with mycobacteria or with leprosy, if possible, for demonstrating leprosy.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Basic Fuchsin
    1. Is an irritant and 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. Sulfuric 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.
E. Acetic Acid
    1. Is an acid.
    2. Add drop by drop to solution.
    3. May cause skin and eye burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize in xylene-peanut oil, 2 changes.	5 minutes	Avoid alcohols for dehydrating. Alcohols will cause a false-negative.
2	Drain slides and carefully blot section and slides with filter paper.		

3	Place in Ziehl-Neelsen carbol-fuchsin solution at room temperature.	20-30 minutes	
4	Rinse in running tap water.	1 minute	
5	Decolorize in 2% sulfuric acid until background is pale pink.	10-60 seconds	Keep decolorization time to a minimum, as over-decolorization will give a false-negative.
6	Rinse in running water.	1-5 minutes	
7	Counterstain in 0.2% methylene blue until background is pale blue.	10-30 seconds	Over staining with methylene blue will mask the mycobacteria.
8	Rinse quickly in distilled water, 2 changes.	5 seconds	
9	Blot dry with filter paper.		
10	Allow to air dry completely.	1-5 minutes	
11	Place in one change of xylene.		
12	Coverslip.		

X. LIMITATIONS:

A. The following may influence the validity of test results:
    1. Older mycobacteria may need longer times in the carbol-fuchsin solution. The use of heat (60°C.) will also aid in the staining

XI. RESULTS:

A. Mycobacteria, including leprosy - **magenta**
B. Nocardia, actinomycotic filaments - **magenta**
C. Background - **blue**
D. Other bacteria, fungi - **blue**
E. Red blood cell, sperm heads, lipofuchsin - **magenta**
F. 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

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Applicability

Royal Oak