AMOEBA - GRIDLEY'S METHOD

PURPOSE: To identify amoeba.

PRINCIPLE: The procedure does not stain amoebae differentially but is useful in that it demonstrates the ingested erythrocyte which is stained by the eosin.

CONTROL: A known control containing amoeba.

FIXATION: 10% formalin

TECHNIQUE: Cut paraffin sections at 5µ.

REAGENTS:

**Aniline-Eosin Solution:**
- Eosin Y: 1.5 gm
- Alcohol, 80%: 100.0 ml
- Aniline: 3.0 ml
- Glacial acetic acid: 1.0 ml

Mix well. Stable for 6 months.

CAUTION: Carcinogen, flammable.

**Naphthol Green B Solution:**
- Naphthol green B: 1.0 gm
- Distilled water: 100.0 ml
- Glacial acetic acid: 1.0 ml

Mix well, stable for 6 months.

CAUTION: Avoid contact and inhalation.

**Hematoxylin:**
- Purchased

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation.


Eosin Y; possible carcinogen.

Glacial acetic acid; severe irritant to skin and eyes. Target organ effects on the respiratory system. Corrosive.
MICROORGANISMS
AMOEBA

PROCEDURE:
1. Deparaffinize, hydrate to distilled water.
2. Hematoxylin, 5 minutes.
3. Decolorize and blue hematoxylin, rinse in distilled water.
4. Aniline-eosin solution, 5 minutes.
5. Distilled water.
6. Naphthol green B solution, 5 minutes.
7. Differentiate in 95% alcohol until erythrocytes are bright rose, check microscopically.
8. Dehydrate in absolute, clear and mount in Permount.

RESULTS:
- amoeba: blue-green
- nuclei of amoeba: deeper blue-green
- ingested erythrocytes: deep rose
- connective tissue: green

REFERENCES:
Luna, L. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd E. 1980, pp 228-229
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: ___________________ By: _______________
Approved: ___________________ By: _______________

Downloaded from WebPath: Internet Pathology Laboratory
http://www-medlib.med.utah.edu/WebPath/webpath.html
PROCEDURE CARD

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SAFETY: Work in well ventilated area. Carcinogenic

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| DATE: |  
| TECH: |  
| EXPIRATION: |  

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- Naphthol green B 1.0 gm
- Distilled water 100.0 ml
- Glacial acetic acid 1.0 ml

Mix well, stable for 6 months.

**CAUTION:** Avoid contact and inhalation.

| DATE: |  
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