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.

Aniline; moderate skin, severe eye irritant. Sensitizer. Toxic by skin absorption. Possible carcinogen. Combustible liquid.

Eosin Y; possible carcinogen.

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

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:_____

PROCEDURE CARD

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SAFETY: Work in well ventilated area. Carcinogenic

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