**UW Medicine - Pathology**

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**AFB**

Ziehl-Neelsen for Acid-Fast Bacteria Procedure

Hand Staining Method (Manual Backup)

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| Adopted Date: 07/15/13  Review Date:  Revision Date: |

PURPOSE

To identify the method for special stain of Ziehl-Neelsen for acid-fast bacteria.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Paraffin sections cut at 4 microns.

**Procedure:**

1. De-paraffinize and hydrate to water.
2. Satin with Carbol fuchsin from Artisan Stain Kit (1 squirt) for 30 minutes.
3. Wash in tap water.
4. Decolorize individually in 0.5% acid alcohol (from renal service Jones Stain) until sections are pale pink and runs clear.
5. Wash thoroughly in running water for 8 minutes.
6. Counter stain by dipping one slide at a time in methylene blue 8-10 dips. Sections should be pale blue.
7. Wash in tap water.
8. Dehydrate in 95% and 100% ETOH.
9. Clear in xylene and mount.

**Results:**

Acid-fast bacilli bright red

Erythrocytes yellowish orange

Other tissue elements pale blue

**Solutions:**

Use Type II de-ionized water for all solution preparation.

**Carbol Fuchsin - premade from Artisan AFB Stain kit**

**1% Acid Alcohol**

Ethyl alcohol, 70% 990mls

Hydrochloric acid, conc 10mls

Stable for six months. Discard after use.

**Methylene Blue**

Methylene blue, (CI# 52015) 0.5gms

Tap water 100.0mls

Glacial acetic acid 0.5mls

Stable for three months.

**Comments:**

Acid-fast stains involve the application of a phenylmethane dye in a phenol solution. Phenol enhances the staining and appears to combine with the fuchsin dye within the acid-fast bacilli. It also functions to dissolve the fuchsin dye. Alcohol is usually added to the carbol fuchsin solution both to enhance the staining and dissolve the dye. When the carbol fuchsin solution is applied, all cells, including the normally hard to stain acid-fast varieties, are colored red. The next step in the procedure involves the application of the acid alcohol decolorizer.

At the de-colorization stage, all cells, except the acid-fast ones, are rendered colorless. this is the classic acid-fast phenomenon; that is, acid-fast cells will retain a carbol fuchsin stain and resist de-colorization with acid treatment. The property of acid fastness is one of degree as there are differences in the resistance to de-colorization depending on the amount of acid use in the decolorizing agent. Acid-fast cells may also appear beaded rather then homogeneously colored, and this beading is believed to be a staining artifact. It may be avoided by using pure dyes and the chloride rather than the acetate salt of the basic fuchsin in the staining solution.

Drying of a section after the carbol fuchsin staining produces a compound that is resistant to de-colorization. Attempts to remove this compound with repeated exposure to the acid alcohol will render the acid-fast organisms completely unstained.

There are several opinions as to why the acid-fast cell stains and resists de-colorization as it does. One concept states that acid fastness is determined by selective permeability of the cell wall, and should the cell be mechanically disrupted, the acid fast property will be lost. There also seems to be a correlation between the lipid content of the acid-fast cell and the ability to stain.

HREFERENCES

*Manual of Histologic and Special Staining Techniques,* McGraw-Hill Book co., 1960, pg. 176. Modified by Histology Laboratory, Harborview Medical Center, Seattle, WA.

Sheehan, d. C. and Hrapchak, B. B.: *Theory and Practice of Histotechnology,* The C. V. Mosby co., 1980, pg. 236-237.

Written By: Director Approval:

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