**UW Medicine - Pathology**

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Fite's Leprosy and Legionnaires for Acid-Fast Bacilli Procedure

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PURPOSE

To identify the method for performing the special stain of Fite's Leprosy and *legionniares* for Acid-Fast Bacilli.

PROCEDURE

**Fixation:**

Any well fixed tissue.

**Sectioning:**

Paraffin sections at 4 microns.

**Solutions:**

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

**Peanut Oil**

Sigma-Aldrich

**Xylene - Peanut Oil**

Peanut oil 50.0 ml

Xylene 50.0 ml

Stable for one month.

**Carbol-Fuchsin**

Sigma-Aldrich. Ready made.

**1% H2 SO4**

Distilled water 495.0 ml

Sulfuric Acid, conc 5.0 ml

Stable for six months. Discard after use.

**Stock Methylene Blue**

Methylene blue 0.7 gm

95.0% Ethanol 50.0 ml

*Working:*

**Methylene Blue**

Stock Methylene blue 5.0 ml

Distilled water 45.0 ml

**Procedure:**

Use control slide.

1. De-paraffinize with xylene-peanut oil mixture, 2 changes for 10 minutes each.
2. Drain and blot to opacity. The residual oil helps to prevent shrinkage and injury of the section.
3. Stain in carbol fuchsin for 30 minutes.
4. Wash in tap water.
5. Differentiate slides individually in of 1% H2SO4 until sections are pale pink.
6. Wash in running tap water for five minutes.
7. Counter stain in methylene blue for 30 seconds. Sections should be pale blue.
8. Wash in tap water.
9. Blot slides and air dry.
10. Dip in xylene and cover slip.

**Results:**

The combination of peanut oil and xylene provides protection by coating the microorganisms. The oil coating, applied around the microorganisms, is not dissolved due to the elimination of alcohol from the hydration process.

The acid-fast stain involves the application of pheylmethane dyes 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 de-colorizer. 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 used. in the decolorizing agent.

Acid-fast cells may also appear beaded rather than 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 1% H2SO4 will render the acid-fast organisms completely unstained.

If the stained slides remain in xylene for a prolonged time before cover slipping, the acid-fast bacteria are not demonstrated as well.

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.

REFERENCES

*Manual of Histologic and Special Staining Techniques,* McGraw-Hill Book co., 1960, pg. 177. Modified by Histopathology Laboratory, Harborview Medical center, Seattle, WA.

Sheehan, D.C. and Hrapcphak, B.B.: *Theory and Practice of Histotechnology*, The C.V. Mosby Co., 1980, pg. 236.

Written By: Director Approval:

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Histology Supervisor