**UW Medicine - Pathology**

 6000-02-04-20

GMS - Fungus

Grocott's Methenamine Silver Nitrate Procedure

Hand Staining Method using Artisan GMS Stain Kit (Manual Backup)

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

PURPOSE

The demonstration of fungal organisms in tissue sections.

PROCEDURE

**Fixation:**

10% Neutral Buffered Formalin, Helly's or Zenker's.

**Technique:**

Cut at 4 microns. Use fungus control slide.

**Procedure:**

1. De-paraffinize to 80% and hydrate in DH2O.
2. Oxidize in Perchloric Acid for 5 min
3. Wash in running tap water.
4. Place in 1% Sodium Bisulfite - 8 min.
5. Wash in running tap water - 5-10 min., then in DH2O.
6. Place in Silver Nitrate solution sections turns yellowish-brown, approximately 1.5min. (Preheat solution at least 10 min in 37° oven). Check under microscope for progress of impregnation, rinse slide in DH2O and examine.) Staining is complete when fungus appears dark brown.
7. Rinse in DH2O - 4-6 changes.
8. Flood in Borate solution for 5 min.
9. Rinse in DH20.
10. Tone in Gold Chloride - 5 min.
11. Rinse DH2O.
12. Remove unreduced silver with 2% Sodium Thiosulfate - 8 min.
13. Wash carefully in running tap water.
14. Rinse DH2O.
15. Counter-stain with Light Green solution until background is pale green, about 20-30 sec.
16. Rinse quickly in DH2O.
17. Dehydrate, clear and mount as usual.

**Results:**

Fungus Sharply delineated in black

Mucin Taupe to dark gray

Inner parts of mycelia and hyphae Old rose

Nuclei Red

Background Pale green

**Solutions - received pre-mixed from Artisan**

**Chromic Acid (not used in our staining protocol)**

**Perchloric Acid**

**1% Sodium Bisulfite**

**Methenamine-Silver Staining Solution**

**Borate Solution**

**Gold Chloride Solution**

**Sodium Thiosufate**

**Light Green Solution**

**Comments:**

**Principle**: Polysaccharides in the fungal cell wall are oxidized to aldehydes by chromic acid. Chromic acid is a strong oxidant, further oxidizing many of the newly released aldehyde groups to breakdown products that will not react; this helps suppress the weaker background reactions of collagen fibers and basement membranes. Only substances that possess large quantities of polysaccharides, such as fungal cell walls, glycogen, and mucins, will remain reactive with the methenamine silver, reducing it to metallic silver. Methenamine gives the solution the alkaline properties necessary for proper reaction and sodium borate acts as a buffer. Gold chloride is a toning solution and the sodium thiosulfate removes any unreduced silver.

**Notes**:

1. Failure to adequately remove the alcohol used during deparaffinization and hydration will result in reduction of the chromic acid solution; this will cause the color of the solution to change from orange to brown. The solution should be discarded when a color change is noted. It usually requires slightly longer for P. carinii to become well stained than it does for the pathogenic fungi; therefore, for optimum staining, it is important to know which organism is suspected and to use the appropriate control. If unknown, use a Pneumocystis control. The fungi will be slightly overstained, but diagnostic. If the reaction is timed with a fungus control, it may not demonstrate any Pneumocystis organisms. However, it is important that organisms not be overstained to the point of obscuring internal structures.
2. **\*\*\*\*\*\*\*\*\*\*\*\*Acid Wash staining dishes.**

REFERENCES

Am. J. Clin. Path., 25:975, 1955.

Written By: Director Approval:

(Signature & Date) (Signature & Date)

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