GROCOTT'S METHENAMINE SILVER (GMS) STAINING PROCEDURE

When staining with GMS wear a lab coat, gloves and goggles.

1. Deparaffinize cell block sections, tissue sections and control sections
   -Place sections in xylene - 3 changes, 5 minutes each.
   -Place in 100% EtOH - 3 changes, 10 dips each.
   -Place in 95% EtOH - 3 changes, 10 dips each.

2. Air dry cytology material, then fix in 95% EtOH or formalin for 15 minutes.

3. Hydrate cytology preparations and deparaffinized sections through several changes of distilled water.

4. Place slides in 10% chromic acid for 10 minutes.

5. Remove chromic acid from sections by washing in running tap water until the yellow color is gone.

6. Rinse in several changes of distilled water.

7. Incubate slides in 80℃ water bath for approximately 5-20 minutes in the following solution.
   Methenamine silver(s).....20.0 ml (refrigerator)
   Deionized water.....20.0 ml
   Borax 5%(s).....3.0 ml

   Slides should be checked after 5 minutes, then every 1-2 minutes, and removed when the sections have become a light tobacco-brown color.

8. Rinse in several changes of distilled water.

9. Tone for 5 minutes in gold chloride.

10. Rinse in distilled water.

11. Remove unreacted silver by placing slides in sodium thiosulfate, 5% for 5 minutes.

12. Wash in running water for 5 minutes.

13. Counter stain in light green for 1 minute.

14. Dehydrate through alcohols; 95% EtOH - 3 changes, 10 dips each.
100% EtOH - changes, 10 dips each.

15. Clear in xylene - 3 changes, 10 dips each.

16. Mount in permount.

Results:

Fungi, bacteria, nocardia, mucin, glycogen are black.
Background is green.

Notes:

When Nocardia is a suspected pathogen, a Nocardia control should be stained along with the usual fungus control.

When any new GMS reagents are made, they are first used in this staining procedure with a confirmed fungal control to assess validity.

References:

See Sections 5.2 and 5.4

Approved:_____ Date:_____
Revised:_____ Date:_____