

GROCOTT'S METHOD FOR FUNGI (GMS)

Purpose The demonstration of fungal organisms.

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.

Fixative 10% neutral buffered formalin.

Reagents

10% Chromic Acid

Chromic acid ... 10.0 g
Distilled water ... 100.0 ml

5% Silver Nitrate

Silver nitrate ... 5.0 g
Distilled water ... 100.0 ml

3% Methenamine

Hexamethylenetetramine (methenamine) ... 3.0 g
Distilled water ... 100.0 ml

5% Borax

Borax (sodium borate) ... 5.0 g
Distilled water ... 100.0 ml

Methenamine-Silver Nitrate (stock)

5% Silver nitrate ... 5.0 ml
3% Methenamine ... 100.0 ml

A white precipitate forms but immediately dissolves upon shaking. Clear solution remains usable for months. Store in refrigerator.

Methenamine-Silver Nitrate (working)

Methenamine-silver nitrate (stock) ... 25.0 ml
Distilled water ... 25.0 ml

5% Borax ... 2.0 ml

Working solution should be made fresh.

1% Sodium Bisulfite

Sodium bisulfite ... 1.0 g
Distilled water ... 100.0 ml

0.1% Gold Chloride

1% Gold chloride ... 10.0 ml
Distilled water ... 90.0 ml

2% Sodium Thiosulfate (hypo)

Sodium thiosulfate ... 2.0 g
Distilled water ... 100.0 ml

0.2% Light Green (stock)

Light Green (stock) ... 10.0 ml
Distilled water ... 50.0 ml

Quality Control Use a control slide with a known fungi.

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Procedure

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in 10% Chromic acid solution for 10 minutes.
3. Wash in water for a few seconds.
4. 1% Sodium Bisulfite solution for 1 minute to remove any residual chromic acid.
5. Wash in running water for 5-10 minutes.
6. Rinse in 3-4 changes distilled water.
7. Place in freshly mixed working Methenamine-silver nitrate solution in oven at 58-63°C for 30-60 minutes or until sections turn yellowish-brown.
8. Rinse in 6 changes of distilled water.
9. Tone in 0.1% gold chloride solution for 2-5 minutes.
10. Rinse in distilled water.
11. Remove unreduced silver with 2% sodium thiosulfate (hypo) for 2-5 minutes.
12. Wash thoroughly in tap water.
13. Counterstain with working 0.2% light green solution for 30-45 seconds.
14. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
15. Mount with synthetic resin.

Results

Fungi ... sharply delineated in black

Mucin ... taupe to dark gray

Inner parts of mycelia and hyphae ... old rose

Background ... pale green

References

Manual of Histologic Staining Methods, AFIP, 3rd Edition, pp. 230-231.