# GROCOTT'S METHENAMINE SILVER (GMS) KIT

## DESCRIPTION

The GMS staining procedure is an oxidation/reduction silver stain. The principal of this staining kit is to introduce a strong oxidation agent, in this case chromic acid, into the tissue or specimen. The acid interacts with the polysaccharides of the fungal cell walls and forms aldehydes groups. When "clear" silver ions are applied to the specimen, they reduce and become metallic silver. Visually, this makes the fungal elements appear black under light microscopy. Light green is used as the counter stain and will make the background appear blue green to pale green.

### Kit Components

- 6511-04 Silver Nitrate 5%
- 6512-16 Methenamine Solution
- 6513-04 Gold Chloride 0.2%
- 6514-16 Sodium Borate 5%
- 6516-16 Sodium Bisulfate 1%
- 6517-16 Chromic Acid Solution
- 6518-16 Sodium Thiosulfate 2%
- 7032-16 Light Green 0.5%

### Intended Use

For the detection and demonstration of fungi in aspirates, tissues, and smears.

### Precautions

Refer to SDS for more information. Kit should be used by trained laboratory professionals and performed under a fume hood to minimize exposure.

### Specimen Collection and Storage

Deparaffinized and re-hydrated 4–6-micron tissue sections on positively charged slides. Or alcohol fixed smears/cytospins.

### Quality Control

Ethos Biosciences tests each lot using appropriate tissue controls: positive fungal control. Follow all recommended Quality Control procedures detailed within your laboratory handbook.

### Interpretation of Test

- Fungal Elements- black
- Inner fungal elements- can appear pink-red/rose
- Mucin- dark grey
- Background- pale green
- Bacteria- negative, green

### Limitations

1. Do NOT use metal while working with silver solution or working solution. This will contaminate the solution, and it will turn black. Solution should not be used if milky black in color.
2. It is critical to use acid cleaned glassware or virgin glassware when performing this test.
3. If oxidizer has degraded, it will lead to nonspecific staining of the reticulum and carbohydrates.
4. Not oxidizing long enough will not create enough alde hydes and will not produce enough silver deposits for visualization.
5. Overstaining with silver will cause nonspecific silver deposits
6. Timing is critical during the methenamine silver step. Once the slides have incubated in the warm solution for 10 minutes, check if the tissue sections are turning "toast brown". Rinse with DI water and check microscopically. Slides can be returned to silver solution; check slides every 5 minutes until the fungal elements are appropriately stained brown/black. Use the same slide QC as reference of color.
7. When checking for very small organisms, a coverslip can be carefully placed on wet slide and viewed under high power.
8. During the gold chloride step, slides should be agitated until the tissue turns grey. Do not leave in solution too long.
9. For cytospins and smears, fix in alcohol for 15 minutes, quickly rinse in DI water then place in 10% formalin for 10 minutes. Rinse in DI before continuing to staining process.

### References


Grocott, R.G. 1955, A stain for fungi in tissue sections and smears. *American Journal of Clinical Pathology*. Vol 25, pg 975.

Alturkistani, H., Tashkandi, F., and Mohammedsaleh, Z. 2015. Histological Stains: A Literature Review and Case Study. *Global Journal of Health Science*. Vol 8.

Goldman, Kelsey. "GMS." HistologyOutlines.com, 2 January 2024.

University of Utah. GMS Methenamine Silver Grocott's Modified. Surgical Pathology Staining Manual.

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed,1980, pp243-246, Battelle Press, Ohio.

## Procedure

Methenamine Silver Working Solution: 25 mL of 3% methenamine, 1.25 mL silver nitrate solution, 2mL borax solution, 23mL of DI water. Pre-heat the working solution in an acid cleaned jar with lid, heat for 15 minutes at 60˚C.

| PROCEDURE | | | | | | |
|---|---|---|---|---|---|---|
| # | ACTION | SOLUTION | HEAT | TIME | | DETAILS |
| | | | | MIN | SEC | |
| 1 | DEPARAFFINIZE | XYLENE OR SUB, 2 CHANGES | – | 5 | – | 5 MINUTES EACH CHANGE OR AS REQUIRED |
| 2 | HYDRATE | GRADED ALCOHOLS | – | 1 | – | 1 MINUTE EACH CHANGE OR 10 DIPS PER GRADED ALCOHOL |
| 3 | RINSE | RUNNING DI WATER | – | 1 | – | OR THREE CHANGES OF DI WATER |
| 4 | IMMERSE | CHROMIC ACID SOLUTION | – | 10 | – | |
| 5 | RINSE | RUNNING TAP WATER | – | 5 | – | RINSE IN DI WATER AFTER |
| 6 | IMMERSE | SILVER WORKING SOLUTION | 60˚C | 10-15 | – | SECTIONS SHOULD APPEAR TOAST BROWN |
| 7 | RINSE | DI WATER | – | – | – | RINSE WELL |
| 8 | IMMERSE | GOLD CHLORIDE | – | 0.5-1 | – | UNTIL SECTION TURNS GREY |
| 9 | RINSE | DI WATER | – | – | – | RINSE WELL |
| 10 | IMMERSE | SODIUM THIOSULFATE | – | 1 | – | |
| 11 | RINSE | RUNNING TAP WATER | – | 1 | – | RINSE WITH DI WATER AFTER |
| 12 | IMMERSE | LIGHT GREEN | – | – | 15-30 | |
| 13 | RINSE | DI WATER | – | – | – | QUICKLY |
| 14 | DEHYDRATE | GRADED ALCOHOLS | – | – | – | 1 MINUTE EACH CHANGE OR 10 DIPS PER GRADED ALCOHOL |
| 15 | CLEAR | XYLENE OR SUB, 2 CHANGES | – | 5 | – | 5 MINUTES EACH CHANGE OR AS REQUIRED |
| 16 | COVERSLIP | RESINOUS MEDIUM | – | – | – | |

## PRODUCT INFORMATION

| CAT. # | DESCRIPTION |
|---|---|
| 6264-16 | Grocott Methenamine Silver Stain Kit |
| 6511-04 | Silver Nitrate 5% |
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