




Grocott GSM for fungi technical information  
 Technical card code 14-113  
 Product code 14-113  
 Pack 1kit. Number of tests 100 or on request  
 Stability of product properly conserved at 4°C 12 months

Produce in Italy by  
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in case of emergency UE number		112
in case of emergency UK number		999
en cas d'urgence Suisse		145

### Principle

Chromic acid oxidation forms aldehydes from fungal cell wall polysaccharide components, which are subsequently demonstrated by reduction of an alkaline hexamine-silver complex. The reaction may be compared to that of the periodic acid Schiff reaction, (see PAS). Grocott's alkaline hexamine-silver solution represents a vehicle which, upon reduction, precipitates nascent silver ions, thus blackening the site. This is known as an "argentaftin reaction". Argentaftin reaction - the ability of a silver complex solution to blacken a tissue element without the need of a reducing bath. The term is adjectival and is applied to many methods, (eg von Kossa). The term "argentaftin reaction" should therefore not be used as a proper name.

In most developed countries fungal infections are becoming more common in the form of opportunistic infections in patients with suppressed immune systems. Fungi stain weakly with haematoxylin; they are also PAS positive due to carbohydrate found in fungal cell walls.

Fixative: nbf acetate buffer not included. Sections: wax 6 µm.

### Procedure. Use acid cleaned glassware. Deparaffinize and dehydrated to distilled water

Oxide in chromic acid solution 60'. (solution A). Wash in tap water for few second  
 Place in sodium bisulfite solution for 1' to remove any residual chromic acid solution. (solution B)  
 Wash in running water for 5 to 10'. Rinse whit 3 or 4 change of distilled water  
 Place in freshly mixed methenamine silver nitrate working solution in oven at 58° to 60°C for 50-60' until section turns yellowish-brown (Check microscopically after washing in distilled water fungi should be dark brown).  
 Rinse in 6 change of distilled water.  
 Tone in gold solution for 2 to 5'. (solution E). Rinse in distilled water.  
 Place in sodium thiosulfate solution for 2 to 5'. (solution F). Wash thoroughly in tap water.  
 Counterstain whit light green working solution for 30 to 45" (solution G)  
 Green light solution to be reconstituted and acidified whir acetic acid (acidification is very subjective)  
 Dehydrate and clear trough 95% ethyl alcohol, absolute ethyl alcohol, and xylen, 2 changes each, 2' each.  
 Mount with DdMount

### Results

Fungi:	Sharply delineated in black
Mucin:	Dark grey
Mycelia and hyphae:	Gray rose
Background:	Green

### Reconstitution of silver nitrate methanamine working solution.

To 25 ml of dH<sub>2</sub>O add 2 drop of silver nitrate solution, 6 drop of methanamine solution, and 4 drop of borace solution. Shake briefly with a glass rod previously washed with distilled water. Please note. If distilled water is not available, use sterile water.

### Start checking for very light brown or tan colour at 50 minutes, then at 55 minutes.

Section should be golden brown sometime between 55 to 60 minutes. Remove from oven immediately once the section have started to turn a golden brown. Cool for several minutes before proceeding to the next step.

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#### Reagent

A chromic oxide acid solution	30 ml
B sodium bisulphite solution	30 ml
C methanamine stock solution	30 ml
D silver nitrate	30 ml
E gold chloride	30 ml
F sodium thiosulfate solution	30 ml
G light green working solution (not acidified)	30 ml
H borax (sodium tetraborate)	30 ml

\* Technical's note: staining time vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183). Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96°C, if the cytoplasmic staining was too strong. Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

\* Risk and Safety Statements outside the EU.

The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Target organs: eyes and nerves. Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based haemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

\* Risk and Safety Statements (U.E.)

The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution. Caution: Substance not yet fully tested. Solution of haemallum. Do not breathe vapors. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

#### Endnotes

- 1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.

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7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.

8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data

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