GROCOTT
for fungi
04 – 043823

In-vitro diagnostic medical device
CND Code: W01030799

number of tests ......................... 60 preparations (120 tests maximum)
procedure time ....................... 1 hour and 50 minutes
product validity ....................... 1 year
storage temperature .................. 2-8 °C
complementary equipment.......... graduated cylinder, glass stick, oven

Expected aim
Product for the preparation of cyto-histological samples for optical microscopy.

Application
To demonstrate fungi in tissue sections.

Principle
In most fungi, cell walls are made of chitin, a polymer of N-acetylglucosamine together with polymers of D-glucose and D-mannose, proteins and lipids. Chromic acid reacts with glycolic and glycoaminic groups in polysaccharide chain oxidating them to aldehydic groups and thus breaking the chain itself. These newly-formed aldehydic groups reduce silver chloride, which is part of the silver-methenamine complex, to metallic silver and make it visible.

Method
1) Bring section to distilled water.
2) Put on the section 10 drops of reagent A: leave to act 20 minutes.
3) Wash in running tap water for a few seconds.
4) Put on the section 10 drops of reagent B for 1 minute.
5) Wash in tap water for 5 minutes.
6) Rinse in 4 changes of distilled water.
7) In one of the enclosed slide-holder put 17 ml of distilled water and add: 20 drops of reagent C, 10 drops of reagent D and 20 drops of reagent E. Stir briefly with a glass stick previously washed in distilled water.
8) Put the section in the holder and incubate in oven at 60 °C for 1 hour.
9) Take out the holder from oven and let it cool for 10 min-utes.
10) Wash in 6 changes of distilled water.
11) Put on the section 10 drops of reagent F: leave to act for 3 minutes.
12) Wash in distilled water.
13) Put on the section 10 drops of reagent G: leave to act 5 minutes.
14) Wash in tap water.
15) Put on the section 10 drops of reagent H for 30 seconds.
16) Dehydrate through ascending alcohols, clear in xylene and mount

Results
Fungi ................................................................. sharply delineated in black
Mucines ........................................................................................................ dark grey
Background ................................................................................................. green

Reagents
A) Chromic acid solution ................................................................. 30 ml
B) Sodium bisulfite solution ............................................................ 30 ml
C) Hexamethylenetetramine solution .................................................. 30 ml
D) Silver nitrate solution ................................................................. 30 ml
E) Sodium borate solution ................................................................. 30 ml
F) Gold chloride solution ................................................................. 30 ml
G) Sodium Thiosulfate solution ....................................................... 30 ml
H) Light green solution ................................................................. 30 ml
Warning and precaution

For good results follow these rules:
- Avoid to infect section and slide with no pathogenic fungi (handle only with gloves, do not expose preparation to air)
- Use always fresh distilled water.
- Use only clean glasswork.
- Avoid deposit of dust on section.
- Avoid contact between solutions and metal objects (tweezers..)

The product must be used exclusively by specialized technical operators. The product is classified as hazardous. Read with attention the information written on the label (dangerous symbols, risks and safety phrases). Consult always the safety data sheet where the information about the risks of the preparation, precautionary measures during use, first aid and disposal are available. Do not use if primary packaging is damaged.

Storage
Store the preparation at 2-8°C. Keep the containers tightly closed.

Stability
After the first opening, the product is usable until the expiry date, if correctly stored.

Disposal
Hazardous preparation: observe all state and local environmental regulations regarding waste disposal.

References

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