SILVER IMPREGNATION
for reticulum
04 – 040801

[IVD] In-vitro diagnostic medical device
CND: Code W01030799

- number of tests: 100
- procedure time: 35 minutes
- product validity: 1 year
- storage temperature: 2-8 °C
- complementary equipment: not requested

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

Application
Recommended method to show argyrophilic reticular fibers in connective tissue, especially to differentiate collagen fibers from connectivum.

Principle
This method produces a selective evident impregnation in a very short time thanks to 2 factors: the preliminary impregnation with an iron salt and the use as silver source of an unstable diaminic complex (ammoniacal solution), which is more reactive than silver nitrate.
1) Pre-treatment with trivalent iron.
   After a preparatory oxidation with potassium permanganate, the section is treated with trivalent iron (ferric ammonium sulfate). Iron ions, more reactive than silver ions, quickly bind affine functional groups in argyrophilic structures.
2) Treatment with ammoniacal solution.
   Silver is present in ammoniacal solution in the form of complex hydrosoluble oxide - [Ag(NH3)2]2 O. This complex silver cation replaces iron previously bound to tissues. In the next step formic aldehyde acts as reducing agent: it removes oxygen from the complex and releases metallic silver that deposits on argyrophilic structures.

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[Ag(NH3)2]2 O + HCHO = 2 Ag + 4 NH3 + HCOOH
\]

Unreduced silverdiamine cation is then removed by sodium thiosulfate (Na2S2O3). Both form a complex which is highly soluble but cannot be oxidized any more.

Warning
For good results follow these rules:
- Always use excellent and absolutely chlorine-free distilled or deionized water.
- Use only perfectly clean glassware.
- Avoid deposit of dust on sections. Never touch solutions with metallic objects (tweezers etc.)

Method
1) Bring the section to distilled water.
2) Put on the section 5 drops of reagent A and 5 drops of reagent B: leave to act 5 minutes.
3) Wash the slide in distilled water.
4) Put on the section 10 drops of reagent C: leave to act 3 minutes.
5) Double washing in distilled water.
6) Put on the section 10 drops of reagent D: leave to act 3 minutes.
7) Double washing in distilled water.
8) Put on the section 10 drops of reagent E: leave to act 3 minutes.
9) Wash in distilled water.
10) Put on the section 10 drops of reagent F: leave to act 5 minutes.
11) Double washing in distilled water.
12) Put on the section 10 drops of reagent G: leave to act 5 minutes.
13) Wash in tap water 5 minutes.
14) Dehydrate through ascending alcohols: clear in xylene and mount.
Results

Reticular and nervous fibers ................................................................. black
Connective tissue ................................................................................. tobacco brown
Collagen ............................................................................................. gold yellow

Reagents

A) Potassium permanganate solution .................................................. 18 ml
B) Acid activation buffer ..................................................................... 18 ml
C) Oxalic acid solution ......................................................................... 30 ml
D) Ferric ammonium sulphate solution ............................................... 30 ml
E) Ammoniacal silver solution ............................................................. 30 ml
F) Neutral formalin solution ............................................................... 30 ml
G) Sodium hyposulphite fixing solution ............................................. 30 ml

Warning and precaution

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

• Peters A. A general purpose method of silver staining (part I impregnation, part II development, part III quantitative studies). 1955, Quart. J. micr. sci..

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