

GORDON - SWEET
method for reticulin fibers
04 – 040802

IVD In-vitro diagnostic medical device **CE**

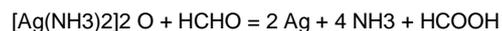
CND Code: W01030799

number of tests 100
procedure time 40 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.

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 diamminic 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 - $[Ag(NH_3)_2]_2O$. 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.



Any excess silver in the unprecipitated state is removed by treating with gold chloride
Unreduced silverdiamine cation is then removed by sodium thiosulfate ($Na_2S_2O_3$). Both form a complex which is highly soluble but cannot be oxidized any more. A nuclear red staining completes the method.

Warning

For good results follow this 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	<ol style="list-style-type: none">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 1 minute.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 2 minutes13) Wash in distilled water.14) Put on the section 10 drops of reagent H: leave to act 2 minutes15) Wash in distilled water16) Put on the section 10 drops of reagent I: leave to act 5 minutes17) Dehydrate through ascending alcohols: clear in xylene and mount.
Results	Reticular and nervous fibersblack Nucleired
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) Formalin solution 30 ml G) Gold chloride solution 30 ml H) Sodium thiosulphate fixing solution 30 ml I) Nuclear Fast Red 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	<ul style="list-style-type: none">• Gordon and Sweet H. A rapid method for the silver impregnation of reticulum. 1936, Am. J. Path., 12: 545-551.

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