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Refrigerated Silver Solution is as Good as Fresh Preparation in the Demonstration of Reticulin Fibres

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Abstract

The objective of this study is to investigate the effect of refrigeration on the silver solution prepared for reticulin demonstration. Tissue blocks of Wistar Rat liver were retrieved from the Animal Tissue Block Archive of the Department of Pathology, University of Ilorin Teaching Hospital, Ilorin. Five normal blocks were randomly selected and Five (5) serial sections labeled A to E were made from each block. The group A sections were stained for reticulin fibres using fresh silver bath (Gordon and Sweet method), and the other groups (B-E) were stained with the remaining silver bath refrigerated at $4 \pm 2^\circ\text{C}$. This was done every two weeks for eight weeks. The histological evaluations revealed no morphological difference in the reticular fibres staining using fresh and refrigerated silver bath for up to a period of eight weeks. Contrary to some authors and the general belief that silver bath must be prepared fresh at every point of use for reticulin demonstration. This study has proven that Medical Laboratory Scientists can prepare silver bath and use same for eight weeks with good reticulin fibres staining. This finding can potentially prevent the stress of having to prepare the solution fresh at every point of use and wastage of the remnant solution after use.

Keywords: Reticulin, Refrigerated silver bath, Histological evaluation

Introduction

Reticulin is a type of fiber in connective tissue that composed of type III collagen secreted by reticular cells (Strum et al., 2007). Reticular fibers crosslink to form a fine meshwork (reticulin). This network acts as a supporting mesh in soft tissues such as liver, bone marrow, and the tissues and organs of the lymphatic system (Burkitt et al., 1993). The term reticulin was coined by Siegfried (1892). Today, the term reticulin or reticular fiber is restricted to referring to fibers composed of type III collagen.

However, during the pre-molecular era, there was confusion in the use of the term 'reticulin', which was used to describe two structures:

- The argyrophilic (silver staining) fibrous structures present in basement membranes.
- The histologically similar fibers present in developing connective tissue (Jackson and Williams, 1956).

Maresch (1905) introduced Bielschowsky's Silver Impregnation Technique for neurofibrils as a stain for reticulum fibers, but emphasized the non-specificity of such procedures. Since the 1920s the definition of "reticulin" and studies of its distribution were based solely on silver impregnation techniques. Application of Gomori's and Wilder's methods to human tissues showed variations of staining patterns with the fixatives and techniques employed. Besides reticulin fibers, various other tissue structures (I bands of striated muscle, fibers in nervous tissues, and model substances such as polysaccharides, egg white and gliadin) were also stained. Deposition of silver compounds on reticulin fibers was limited to an easily removable substance; the remaining collagen component did not bind silver (Puchtler et al., 1978).

Reticular fiber is composed of one or more types of very thin and delicately woven strands of type III collagen, these strands build a highly ordered cellular network and provide a supporting network, and many of these types of collagen have been combined with carbohydrate (Miyata et al., 1993). Thus, they react with silver stains and with Periodic Acid-Schiff reagent but are not demonstrated with ordinary histological stains such as those using Haematoxylin (Miyata et al., 1993). It was concluded that the reticular and regular collagenous

materials contains the same four sugars—galactose, glucose, mannose, and fucose—but in a much greater concentration in the reticular than in the collagenous material (Miyata et al., 1993).

The liver is one of the organs in which the cells are supported by a network of reticulin fibres (Burkitt et al., 1993). They appear as fine black lines in silver stained preparation. The fibres surround the individual sheets of liver cells (hepatocytes) and are the only fibrous connective tissue components supporting the cells. While providing support, the fine, open meshwork of reticular fibres facilitates the exchange of substances between the hepatocytes and the blood, which circulates in the irregularly shaped blood vessels (sinusoids) between the hepatocytes. Reticular fibres are also present in the connective tissue surrounding the larger vessels, which penetrate the parenchyma of the liver (Burkitt et al., 1993).

Reticulin stains are silver stains based on the argyrophilic properties of reticulin fibers. The two most common reticulin stains are the Gomori's stain and Gordon and Sweet's stain. The first step in the staining procedure consists of oxidation of the hexose Sugars in reticulin fibers to yield aldehydes. The second step is called "sensitization" in which a metallic compound such as ammonium sulphate is deposited around the reticulin fibers, followed by silver impregnation in which an ammonical or diamine silver solution is reduced by the exposed aldehyde groups to metallic silver. Further reduction of the diamine silver is achieved by transferring the sections to formaldehyde; this step is called "developing". The last step consists of "toning" by gold chloride in which the silver is replaced by metallic gold and the colour of the reticulin fibers changes from brown to black.

Materials and Methods

Formalin fixed, paraffin embedded tissue blocks of Wistar Rat Liver were retrieved from the Animal Tissue Block Archive of the Department of Pathology, University of Ilorin Teaching Hospital Ilorin, Nigeria. Five normal blocks were randomly selected and Five (5) serial sections (4µm in thickness) labeled A to E were made from each block. The group A sections were stained for reticulin fibres using freshly prepared silver bath (Gordon and Sweet, 1936). Other groups (B-E) were stained with the same silver solution refrigerated at 4°C ±2°C on the following days: Group B on the 14th day; Group C on the 28th day; Group D on the 42nd day and group E was stained on the 56th day.

Gordon and Sweets Method for Reticulin Fibres (Bancroft and Cook, 1994)

Principle

Silver from silver oxides is selectively deposited on the reticulin fibres, which appear black after conversion to reduced silver, by the reducing agent (formalin). Gold chloride is used as a toner to give a clearer background and unreduced silver is removed by treatment with sodium thiosulphate.

Reagents

(A) Silver Solution

To 5ml of 10% Silver nitrate solution add strong ammonia until the precipitate formed is just dissolved. Avoid excess of ammonia. Add 5ml of 3% sodium hydroxide solution. Re-dissolve the precipitate by adding strong ammonia drop by drop until the resultant solution retains a faint opalescence. If at this stage any excess ammonia is present, indicated by the absence of opalescence, add a few drops of 10% silver nitrate solution to produce a light precipitate. Make up to 50 ml with distilled water.

(B) Acidified Potassium Permanganate
0.5% Potassium Permanganate 190 ml, 3% Sulphuric Acid 10 ml.

(C) 1 % Oxalic Acid

(D) 4 % Iron Alum (Ammonium Ferric Sulphate)

(E) 10 % Formalin dilute in tap water not distilled

(F) 0.2 % Gold Chloride (Sodium Chloro-Aurate)

(G) 5 % Sodium Thiosulphate

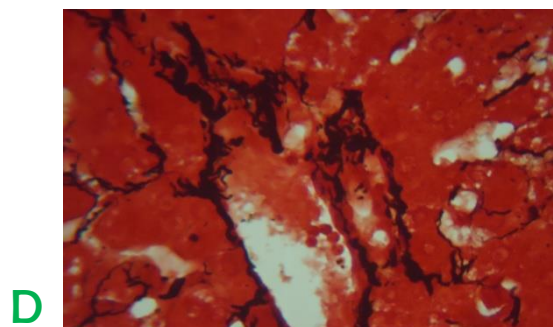
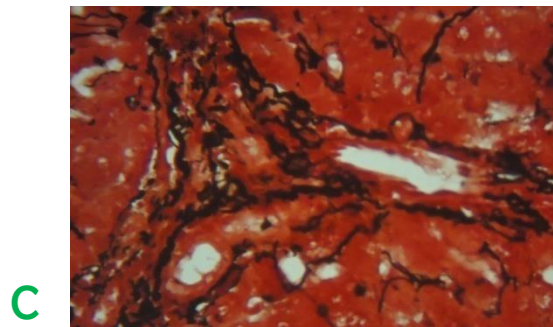
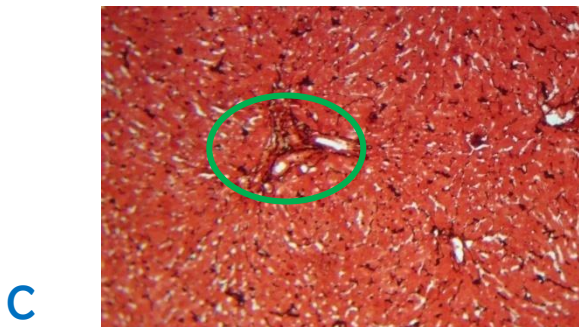
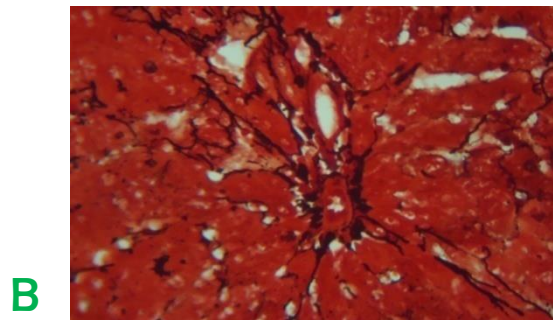
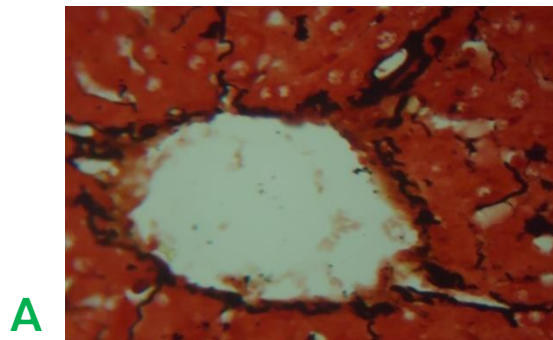
(H) 1 % Neutral Red

Procedure

1. Sections were taken to distilled water
2. Put in acidified Potassium Permanganate for 2 minutes
3. Washed in water
4. Put in 1 % Oxalic Acid for 2 minutes
5. Washed well in distilled water
6. Put in 4 % Iron Alum for 10 minutes
7. Washed in distilled water
8. Put in Silver solution for 1 minute
9. Rinsed in distilled water
10. Put in 10 % Formalin for 2 minutes
11. Washed in water followed by distilled water
12. Put in 0.2 % Gold Chloride for 2 minutes
13. Rinsed in distilled water
14. Put in 5 % (Sodium Thiosulphate) for 2 minutes
15. Washed in water
16. Counterstained with 1 % Neutral Red for 2 minutes
17. Dehydrated rapidly, cleared and mounted with DPX

Results

Histological Illustration



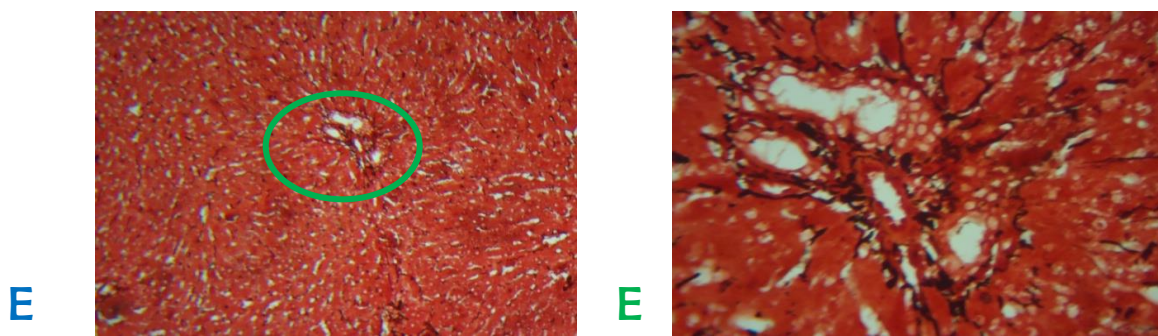


Fig. 1: Photomicrographs of the Liver sections of group A-E (H&E, X64 (left), 640 (right)

Note: The panoramic view of the hepatic blood vessels in the green bordered region is presented on the right. The reticulin fibres are coloured **Black**

Discussion

Reticulin demonstration is a common procedure in diagnostic histopathology laboratory. It is in fact a parameter among the set of the Liver panel. However, the titration to arrive at a reliable solution is usually difficult, especially to a new user. The practice and believe in this part of world and the opinion of some authors regarding the point of use preparation of silver bath; an impregnator, from which silver oxides is selectively deposited on the reticulin fibres, was tested in this work.

The hepatic blood vessel was chosen because of the conspicuous presence of reticulin fibres as compared to the parenchyma of the liver (Burkitt et al., 1993). The liver reticulin fibres were well stained with clear inter-hepatic boundary, nuclei and background stains; there was consistency throughout the eight weeks of the experiment. The finding from this study is contrary to the report of some authors who prescribed and emphasized fresh preparation of silver solution on daily use (Wallington, 1965; Sheehan and Hrapchak, 1980; Culling et al., 1985; Carson, 1990). Our finding is also not in line with the practice in most Nigeria Histopathology Laboratories where point of use preparation is a routine and the remnant solution discarded after use. The result from this study is clearly an indication that the potency of prepared silver bath can be maintained for up to eight weeks at $4 \pm 2^\circ\text{C}$ in the demonstration of reticulin fibres.

Conclusion and Recommendation

The eight weeks research simply established the fact that silver bath can be prepared and stocked for further use. Finding from this study indicates that it is not necessary to prepare silver solution for the demonstration of reticulin fibres at the point of use. It is therefore recommended that Medical Laboratory Scientist should save themselves of the stress of point of use preparation of silver solution especially where constant supply of electricity is available in

the laboratory. This finding can potentially prevent wastage of the remnant solution after use and help in the optimum utilization of resources in the laboratory.

Conflict of Interest

The authors hereby declare that no conflict of interest exists.

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