
Brief communication

A modified, inexpensive method for performing the reticulin stain

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Introduction

Reticulin stain is a commonly used special stain to demonstrate reticulin fibers. Reticulin is a type III collagen found in the basement membrane of many organs and provides structural integrity. It is found commonly in the liver, kidneys and the spleen. Reticulin staining method is a metal impregnation technique where ammoniacal silver initially binds to the tissue component of interest. The reducing agent (formalin) produces a dark insoluble precipitate which is then treated by toning and fixing in sodium thiosulphate¹ (Reticulin stain kit)

This stain is useful in distinguishing reticular fibers present in a range of non-neoplastic and neoplastic conditions such as liver nodules and in FNAB cell block preparations^(2,3)

Justification

In routine practice, Gordon and Sweet's method is used for performing the reticulin stain.

Gold chloride is a chemical that is required to perform the standard method of reticulin stain which is very expensive, making this stain among one of the most expensive special stains in routine use especially in a developing country like Sri Lanka. Hence, a less expensive method by modifying the standard method that would give the same results will reduce the reagent cost significantly.

Objectives

General objective:

- To develop an easy, effective and a cost effective method to perform reticulin stain by modifying the standard method used currently in routine histopathology practice.

Specific objectives

- To develop an easy method for performing reticulin stain on routine histopathology practice by modifying the currently used method

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- To compare the cost effectiveness of the standard method against the modified method.

Method

- Liver resection specimens to be discarded were used to perform the stain due to the rich reticulin framework of this tissue.
- Standard method (S) - A control section was stained by using the standard method including the step that requires toning in 0.2% gold chloride solution for 03 minutes.
- Silver solution was prepared using the following method.
 - To 05ml of 10% aqueous silver nitrate solution add concentrated ammonia drop by drop while mixing.
 - A precipitate appears and dissolves on mixing. Once the end point is reached add 05ml of 03% sodium hydroxide solution and mix the solution. A second precipitate will appear.
 - Add ammonia drop by drop while mixing to re-dissolve the precipitate.
 - When the precipitate is completely dissolved, stop adding ammonia and adjust the volume of the mixture to 50ml with distilled water.

- Filter and store in a dark bottle at 40C.

- Standard method for reticulin stain was performed by the Gordon & Sweet method for reticulin fibers on the control section (**Gordon & Sweet 1936**)

Standard method for reticulin stain

1. Deparaffinize sections and bring to water.
2. Treat with 1% potassium permanganate solution, 5 minutes.
3. Rinse in tap water.
4. Bleach in 1% Oxalic acid solution.
5. Rinse in tap water.
6. Treat with 2% iron alum, 15 minutes.
7. Wash in several changes of distilled water.
8. Place in coupling jar in silver solution, 2 minute.
9. Wash in several changes of distilled water.
10. Reduce in 10 % aqueous formalin solution, 2 minutes.
11. Rinse in tap water.
12. Tone in 0.2% gold chloride solution, 03 minutes.
13. Rinse in tap water.
14. Treat with 5% Sodium thiosulphate solution, 3minute.
15. Rinse in tap water.
16. Treat with 2% sodium thiosulfate solution, 1 minute.
17. Rinse in tap water.
18. Counter stain as desired (Van Gieson or eosin is suitable)

- **Modified method (M)** - Gordon & Sweet method for reticulin fibers was performed on sections M01-M09 by deleting the step that requires toning in 0.2% gold chloride solution for 02 minutes (Step 12). The time that the section was stained in silver solution was increased systematically from 1 minute to 9 minutes and the stained sections were compared with the control section.

Modified staining method for reticulin

1. Deparaffinize sections and bring to water.
2. Treat with 1% potassium permanganate solution, 5 minutes.
3. Rinse in tap water.
4. Bleach in 1% Oxalic acid solution.
5. Rinse in tap water.
6. Treat with 2% iron alum solution for 15 minutes.
7. Wash in several changes of distilled water.
8. Place in coupling jar of silver solution, 2 minute.
9. Wash in several changes of distilled water.
10. Reduce in 10 % aqueous formalin solution, 2 minutes.

11. Rinse in tap water.
12. Treat with 5% sodium thiosulphate solution, 3 minutes.
13. Rinse in tap water.
14. Counter stain as desired.
15. Dehydrate through alcohol.
16. Clear in xyline and mount with DPX

- The reticulin fibers were stained black in colour, nuclei were stained black in colour and the connective tissue was stained pink/red.
- The sections stained by the standard and the modified method with different time durations in silver solution were also scored according to the following scoring system.
 - Score 0- Diffuse stain particles present; over stained with silver; requires repeat staining
 - Score 1- Diffuse stain particles present; limiting interpretation
 - Score 2- Focal stain particles present; good staining intensity
 - Score 3- Stain particle not present; clear staining intensity

Results

Table 01. Different times used in the modified method compared the standard method and the score for staining intensity			
Slide no.	Time duration for Silver solution	Time duration in gold chloride	Score for staining intensity
S - 01	02 minutes	02 minutes	03
M-01	02 minutes	0	01
M 02	03 minutes	0	01
M 03	04 minute	0	02
M 04	05 minutes	0	03
M 05	06 minutes	0	03
M 06	07 minutes	0	02
M 07	08 minutes	0	02
M 08	09 minutes	0	02
M 09	10 minutes	0	02

S- Section stained by the standard method. M- Sections stained by the modified method

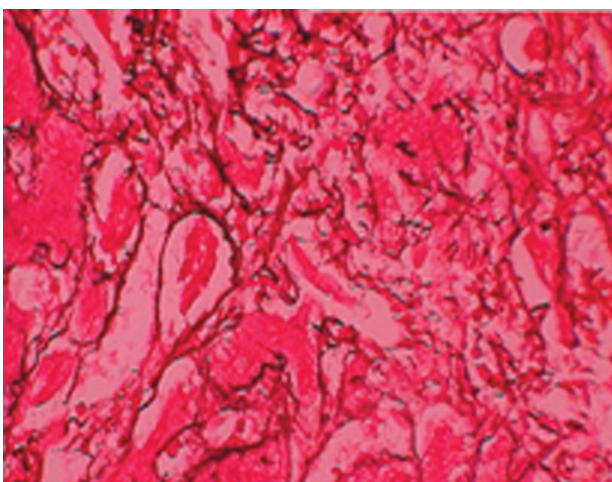


Fig.1. S 01 - Reticulin x400

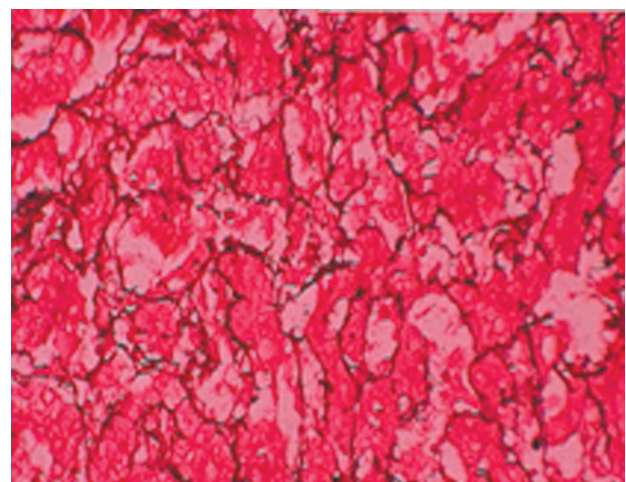


Fig.2. M 04 - Reticulin x400

Table 02. Cost for each section stained by the standard and modified method.

	Standard method (S)	Modified method (M)
Cost	LKR.168.00	LKR.82.00

Discussion and conclusions:

The best scores were observed in sections M4-M5 which included a longer toning period where addition of gold chloride was not required to perform a satisfactory stain to allow interpretation. In comparison with the S1 (Standard procedure), the quality of staining was satisfactory. When considering the cost effectiveness, the modified method was found to be less than 50% of the cost of the standard method used routinely (eg: Gordon and Sweet's Method). Hence the modified method for staining reticulin fibers without the toning step in gold chloride used here can be recommended and is of particular value in the local setting due to the low cost.

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