



**Document Number:** SASoM/METHOD/117.v1

**Title:** Histology – Martius (yellow) Scarlet (red) Blue stain

**Version:** v1

**Author:** Peter Mullen

Effective from:	28/04/2020
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SOP History		
Number	Date	Reason for Change
v1	28/04/20	Original

### 1.0 Purpose –

This SOP describes the current procedure for carrying out Martius Scarlet Blue staining on FFPE sections in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

### 2.0 Scope –

This SOP applies to all staff in the SASoM carrying out Martius Scarlet Blue staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

### 3.0 Responsibilities –

All staff performing Martius Scarlet Blue staining on FFPE sections in this manner are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.



#### 4.0 Procedure –

The Martius Scarlet Blue method is a trichrome stain (three dyes are employed) for the selective demonstration of elastica, connective tissue in general, or fibrin. Fibrin (muscle fibre) is stained red using crystal scarlet solution while methyl blue is used to stain the collagen / fibrin and red blood cells (erythrocytes) are stained yellow using Martius Yellow.

Control Tissue: Placenta FIBRIN, Kidney tissue

#### Solutions and Reagents

##### Make up Martius Yellow solution as follows:

Martius yellow / acid yellow 24 (#377767-25G)	1.25g
Tungstophosphoric acid (P4006-100G)	5g
95% alcohol	243.75mL

##### Make up Brilliant Crystal Scarlet solution as follows:

Crystal Ponceau 6R (P2395-25G)	2.5g
Glacial acetic acid	2.5mL
Distilled Water	245mL

##### Make up Soluble Blue solution as follows:

Soluble Blue solution / Methyl Blue (95290-25G)	1.25g
Glacial acetic acid	2.5L
Distilled Water	246.25mL

##### Make up 1% Tungstophosphoric acid solution as follows:

Tungstophosphoric Acid (P4006-100G)	1g in 100ml of DW
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##### Make up 1% Acetic Acid solution as follows:

Acetic Acid	1mL in 99ml in DW
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#### De-wax & Re-hydrate

Always allow any excess fluid to drain from the slide rack before proceeding to the next solution.

1. Dewax-Xylene	1	5 minutes
2. Dewax-Xylene	2	5 minutes
3. Dewax-Xylene	3	5 minutes
4. Rehydration-100% Alcohol		2 minutes
5. Rehydration-100% Alcohol		2 minutes
6. Rehydration-80% Alcohol		2 minutes
7. Rehydration-50% Alcohol		2 minutes
8. Wash in running water		2 minutes



## **Staining**

1. After hydration in water, place the slides in Celestine blue solution for 5min.
2. Wash in tap water.
3. Place the slides in filtered haematoxylin for 2min.
4. Wash in tap water.
5. Blueing in Scott's tap water for 1min.
6. Wash in tap water.
7. RINSE in 100% alcohol.
8. Place the slides in Martius yellow solution for 2min.
9. Wash in tap water.
10. Place the slides in Brilliant crystal scarlet for 10min.
11. Wash in tap water.
12. Wash with 1% Tungstophosphoric acid solution briefly.
13. Wash in tap water.
14. Place the slides in Soluble blue for 2min.
15. Wash in tap water.
16. Dehydrate in 100% EtOH.
17. Clear in Xylene and mount.

### **5.0 Personal protection –**

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

### **6.0 Spillages –**

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

### **7.0 Training –**

All staff should undergo training in this technique before performing the procedure.

### **8.0 Related documents –**

#### **8.1 Risk assessments –**

RA20229 (Histology - Martius, Masson's and Periodic acid-Schiff Staining)



## 9.0 Approval and sign off –

### Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

### Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature: Date:

### QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

