

Method Procedure

Document I	Number: SASoM/METHOD/117.v1
Title:	Histology – Martius (yellow) Scarlet (red) Blue stain
Version:	v1
Author:	Peter Mullen

Effective from:	28/04/2020	
Valid to:	27/04/2022	

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.

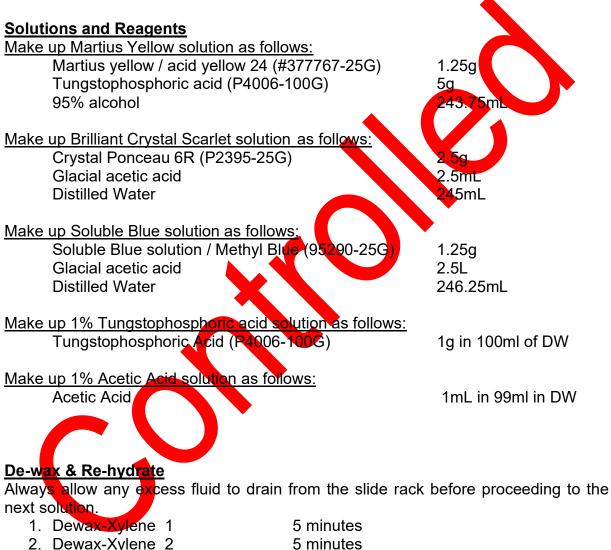


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4.0 Procedure –

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



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

5 minutes

2 minutes

2 minutes

2 minutes



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<u>Staining</u>

- 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)



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9.0 Approval and sign off -

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
Management Appr	oval	
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date:
QA release by:		
Name:	Alex MacLellan	
Position:		
	QA Manager	
Signature:		Date:



STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE