

**Document Number: SASoM/METHOD/121.v1****Title: Histology – Carstair's Staining****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 Carstairs 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 Carstairs Staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing Carstairs 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.

***NB. Be extra careful when dealing with picric acid!
ALL USERS MUST SIGN THE RISK ASSESSMENT FOR PICRIC ACID BEFORE
CARRYING OUT THIS PROCEDURE.***



4.0 Procedure –

The Carstairs staining method is used for visualisation of Fibrin & Platelets. Interpretation is summarised as below:

Fixation Time:	48 Hours or more	Less than 48 hours
Fibrin	Bright Red	Orange to Orange Red
Platelets	Gray Blue or Navy	Light Gray
Collagen	Bright Blue	Bright Blue
Muscle	Red	Red
Red Blood Cells	Clear Yellow	Red, Green or Yellow

Solutions and Reagents

Aqueous Picric Acid (1%):

Aqueous Picric Acid is purchased as a ready-to-use solution (VWR; #87897.180) supplied at 10g/Litre (1% concentration).

Make up 5% aqueous ferric alum (Ferric Ammonium Sulfate) Solution as follows:

Iron alum (ammonium ferric sulfate) should consist of violet crystals that dissolve completely in H₂O to make a light orange-brown solution. If the crystals are large, it may be necessary to leave the mixture overnight on a magnetic stirrer. There are many bad batches of this compound, which contain insoluble white or light brown material. Even with clean iron alum, a precipitate forms after several months, and the solution should then be discarded.

5% Ferric Ammonium Sulfate

5g in 100mL of DW

Make up 'Ponceau Fuchsin' solution (freshly make up and filtered) as follows:

[A] 1% Acetic Ponceau 2R solution as follows:

Ponceau 2R (xylylene Ponceau) (#P2395-25G)

1g

Glacial acetic Acid

1mL

DW

99mL

[B] 1% Acetic Fuchsin solution as follows:

Fuchsin Acid (acid fuchsin) (#1052310025)

1g

Glacial Acetic Acid

1mL

DW

99mL

Freshly combine 1 part of 1% acetic ponceau, 1 part of 1% Acetic Acid Fuchsin, and 2 parts of DW.

Make up 1% Acetic Acid Solution as follows:

Glacial Acetic Acid

1mL in 100mL of DW

Make up 1% aqueous Phosphotungstic Acid Solution as follows:

Phosphotungstic Acid (#P4006-100G)

1mL in 100mL of DW



Method Procedure

Make up Aniline Blue Solution as follows:

Aniline Blue / Methyl Blue (#95290-25G)	1g
1% Acetic Acid	100mL

Make up Picric Acid - Orange G Solution as follows:

Make up 2% Orange-G (#861286-25G) 2g in 100ml DW
Freshly dilute 1 part of 2% orange-G in 9 parts of 1% Aqueous Picric Acid.

NB. Be extra careful when dealing with picric acid!
ALL USERS MUST SIGN THE RISK ASSESSMENT FOR PICRIC ACID BEFORE CARRYING OUT THIS PROCEDURE.

Mayer's Haematoxylin

This is supplied ready to use

De-wax & Re-hydrate

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

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|-----------------------------|---|-----------|
| 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, mordant (fix) the slides in 5% Ferrous Alum / Ferric Ammonium Sulfate for 5 minutes.
2. Rinse in running tap water.
3. Stain with Mayer's Haematoxylin for 5 minutes.
4. Rinse in running tap water.
5. Stain in Picric Acid - Orange G solution for 30-60minutes.
6. Rinse once in distilled water.
7. Stain in Ponceau-Fuchsin solution for 2-4 minutes.
8. Differentiate in 1% Phosphotungstic Acid solution until muscle is red and background is pale pink.
9. Rinse in distilled water.
10. Stain in Aniline Blue solution for 1 hour.
11. Rinse in several changes of distilled water.
12. Dehydrate, clear, coverslip using a synthetic mounting medium.



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 –
RA20232 (Histology – Miller's, Carstair's and Picro Sirius Red Staining)

9.0 Approval and sign off –

Author:

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Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature: Date:

QA release by:

Name: Alex MacLellan

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