



<b>Document Number:</b>	<b>SASoM/METHOD/123.v1</b>
<b>Title:</b>	<b>Histology – Picro Sirius Red Staining</b>
<b>Version:</b>	<b>v1</b>
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Effective from:	28/04/2020
Valid to:	27/04/2022

<b>SOP History</b>		
Number	Date	Reason for Change
v1	28/04/20	Original

### 1.0 Purpose –

This SOP describes the current procedure for carrying out Picro Sirius Red Staining 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 Picro Sirius Red Staining Staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

### 3.0 Responsibilities –

All staff performing Picro Sirius Red 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 Picro Sirius Red Staining method is used for visualising Collagen I/III and muscle fibres. Picrosirius red is one of the most important stains to study collagen networks in different tissues. Under polarized light, collagen bundles appear green, red or yellow, and are easily differentiated from the black background, thus allowing for quantitative morphometric analysis. This method shows what is known as birefringence of collagen fibres.

#### 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 Celestine Blue solution as follows:

Celestine Blue (#206342-5G)

Ammonium ferrous sulfate (#221260-25G)

DW

0.5g

5g

100mL

Boil while stirring and then allow to cool before filtering.

Store at 2-8° (stable for 6month)

##### Make up 1% Picro-Sirius Red F3B\* Solution (freshly mixed up) as follows:

Dissolve 2.5g of Picro-Sirius Red / Direct Red 80 (#365548-5G) in 250ml of DW.

Mix 1 part of 1% Picro-Sirius Red / Direct Red 80 and 9 parts of Aqueous Picric Acid.

(\*either Picro-Sirius Red F3B or Direct Red 80 can be used)

##### Make up 0.5% Acidified Water as follows:

Add 5mL acetic acid (glacial) to 1 litre of water (tap or distilled).

#### 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 sections in Celestine Blue for 5 minutes.
2. Stain in Haematoxylin for 2 minutes.
3. Wash in water for 10mins.
4. Rinse briefly in 1% Aqueous Picric Acid solution.
5. Stain in 1% Picro-Sirius Red Solution for 3 minutes.
6. Rinse in 70% alcohol.
7. Dehydrate, clear 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 –  
RA20232 (Histology – Miller's, Carstair's and Picro Sirius Red Staining)



## 9.0 Approval and sign off –

**Author:**

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Signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Name: Peter Mullen  
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Name: Alex MacLellan  
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