Document Number:	**SASoM/METHOD/131.v1**
Title:	**Histology – Perls Prussian Blue Staining**
Version:	**v1**
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SOP History		
Number	Date	Reason for Change
v1	20/01/2021	Original

1.0 Purpose –
This SOP describes the current procedure for carrying out Perls Prussian 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 Perls Prussian Blue Staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff performing Perls Prussian 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 –

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Principle:

Dilute mineral acid hydrolysis releases ferric ions from protein bound tissue deposits, which, in the presence of ferrocyanide ions, are precipitated as the highly coloured and highly water-insoluble complex, potassium ferric ferrocyanide, or Prussian blue.

$FeCl3 + K4Fe(CN)6 = KFeFe(CN)6^{-} + 3KCl$

Ferrous ions do not produce a coloured reaction product, thus are excluded from the visualisation. Tissue deposits containing ferric ions are invariably Hemosiderin / haemosiderin.

The original method of Perls applied the ferrocyanide and acid as separate reagents. The “mixed method” (as written here) is well suited for a routine laboratory, but it must be kept in mind that heavy deposits of haemosiderin in tissue sections may lead to leaching of the coloured end product, with subsequent artefactual background staining of collagen.

Asbestos is the name given to a special form of silica which exists in the form of long, thin, crystalline fibres. The fibres become coated with protein which contains haemosiderin and therefore appears brown on unstained and H&E sections, and blue by the perl's prussian blue reaction. The asbestos fibres with their protein are known as "asbestos bodies" and the characteristic birefringence / birefraction is lost.

Technical Points:

1. A known positive control section must be used to ensure correct differentiation has been achieved.
2. Neutral buffered formalin gives good results. Other fixatives may be used, but acidic fixatives, dichromate fixatives, and acidic decalcification fluids should be avoided. These reagents will cause progressive hydrolytic loss of ferric ions from tissues, and a negative result must be viewed with suspicion.
3. (Step 5) - Red background staining results if the neutral red stain is applied directly from tap water.
4. (Step 6) - Differentiation of neutral red staining is achieved during dehydration.

Method:

1. Bring sections to distilled water.
2. On a rack, flood with equal parts mixture of ferrocyanide and hydrochloric acid for 10 min (asbestos bodies for 30 mins)

3. Wash well in distilled water, several changes 5 min
4. Counterstain with filtered neutral red stain 1 min
5. Rinse in distilled water
6. Rapidly dehydrate in absolute alcohol, clear and mount.

Results:

- ferric salts..... deep blue
- nuclei..... ..... red
- erythrocytes..... yellow
- asbestos bodies.....blue/black

Reagent Formulation:

1. aq hydrochloric acid (Analytical Reagent grade)

2. aq potassium ferrocyanide (Analytical Reagent grade)
   CARE - toxic hazard

3. Neutral red stain
   neutral red (Sigma 861251-25g).....1.0 g
   distilled water..... 100.0 ml
   glacial acetic acid.....1.0 ml

Dissolve the dye in the distilled water. Add the acid. Mix well. Filter into the reagent bottle. Keeps well. (The reagent is usually supplied from MedVet / Sigma already made up).

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 – Congo Red Staining, Perls Prussian Blue Staining)

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Peter Mullen Date: 25/01/2021

Management Approval:

Name: Angus Jackson

Position: Lab Manager

Signature: Angus Jackson Date: 25.01.2021

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