

Method Procedure

SASoM/METHOD/120.v1
ogy – Mallory's (PTAH) Staining
/lullen

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 Mallory's 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 Mallory's Staining on FFPE sections in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities -

All staff performing Mallory's 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 Mallory's Phosphotungstic Acid - Hematoxylin (PTAH) staining method is used for visualising muscle cross-striations and fibrin. This protocol includes the use of Potassium dichromate which is an inorganic chemical reagent most commonly used as an oxidizing agent in various laboratory applications, including histology. As with all hexavalent chromium compounds, it is acutely and chronically harmful to health and therefore has the highest CHARM Hazard rating of 5, C,O, T.

Control Tissue: Use skeletal or cardiac muscle, tissue containing fibrin, or cerebral cortex for glial fibers.

Solutions and Reagents

Make up 1% Oxalic acid solution as follows: Oxalic acid (#75688-250G)

1q in 100mL DW

<u>Make up 0.5% Potassium Permanganate (FILTER it before use)</u> Make up 2.5g of potassium permanganate (#223468-500G) in 500ml of DW

Make up Mordant / Acid Fixative (make up fresh)

Make up 10% Hydrochloric Acid (slowly add 10mL of HCl to 90mL of DW). Make up 3% Potassium Dichromate (#207802-100G) by dissolving 3g in 100mL of water, <u>**OR**</u>, purchase 4.2% Potassium Dichromate solution (#1091181000) and then dilute further to a final concentration of 3% - this is the preferred option as it takes away the need to weigh out a nasty chemical powder.

Mix 1 part of 10% HCl and 3 parts of 3% potassium dichromate.

<u>Make up Phosphotungstic Acid-Hematoxylin (PTAH)</u> - make up fresh but it will keep for several days if filtered before use each time.

[A] Add 0.1g of haematein* (#51230-10G) and grind to a chocolate-coloured paste with 1mL of distilled water. An unsatisfactory batch of haematein will appear lighter in colour and should be discarded.

[B] Dissovle 1.0g of Phosphotungstic Acid (P4006-100G) in 100mL of distilled water and mix with the ground haematein solution. Bring to the boil, cool and filter.

(*haematoxylin is the oxidised form of haematein)



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

5 minutes 5 minutes

5 minutes

- 2 minutes
- 2 minutes
- 2 minutes

- 2 minutes 2 minutes

- Staining
 - 1. After hydration in water, Place in 0.5% Potassium Permanganate solution to beach for 5 minutes.
 - 2. Wash well in tap water.
 - 3. Treat with 1% Oxalic Acid for 2 minutes.
 - 4. Wash well in water.
 - 5. Place in Mordant / Acid Fixative for 30 minutes,
 - 6. Wash well in water.
 - 7. Stain in Phosphotungstic Acid-Hematoxylin (PTAH) solution overnight.
 - 8. 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 S<mark>pi</mark>llages –

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 -RA20230 (Histology - Mallory's 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:		

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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
	X		