Document Number: **SASoM/METHOD/041.v6**

Title: **Haematoxylin and Eosin (H&E) Staining of Tissues and Mounting of Slides.**

Version: **v6**

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SOP History		
Number	Date	Reasons for Change
V1	07/02/2013	Original
v2	19/09/2013	Correct spelling mistake
V3	19/09/2015	Update
V4	19/09/2017	Update
V5	19/09/2019	Update
V6	19/09/2021	Update

1.0 Purpose:

This Standard Operating Procedure (SOP) describes the current procedure for the H&E staining of paraffin embedded tissues in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope:

This SOP applies to all staff in the SASoM involved in H&E staining of paraffin embedded tissues in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities:

All staff involved in the staining of paraffin embedded cells and tissues are responsible for ensuring that methods are followed in accordance with this SOP after suitable training.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure:

4.1 Sections to be stained are put on slides and baked either in a 37°C oven for overnight or in a 55°C oven for 2 hours or in a 80°C oven for 1 hour.

4.2 Layout of H&E staining dishes

The reagents for de-waxing and re-hydrating (similarly those for de-hydrating and clearing) slides are laid out in the lab within the fume extraction cabinet. Always use these reagents under the fume extraction unit. When not in use replace the lids on the reagent dishes to minimise the release of fumes.

The reagents for performing H&E staining are laid out next to the fume cupboard

	FUME HOOD		**BENCH**
Dish No	Reagent	Dish No	Reagent
1	Dewax-Xylene	4	Rehydration-100% Alcohol
2	Dewax-Xylene	5	Rehydration-100% Alcohol
3	Dewax-Xylene	6	Rehydration- 80% Alcohol
16	Clearing-Xylene	7	Rehydration-50% Alcohol
17	Clearing-Xylene	8	Haematoxylin (Harris)
18	Clearing-Xylene	9	1% Acid Alcohol (optional)
		10	Scott's Tap Water
		11	Eosin (Aqueous or Alcohol)
		12	Dehydration-50% Alcohol
		13	Dehydration-80% Alcohol
		14	Dehydration-100% Alcohol
		15	Dehydration-100% Alcohol

4.3 Staining Procedure

Follow the Standard Protocol unless requested otherwise.

The Haematoxylin used is Shandon 's ready-made Harris Haematoxylin. This **should be filtered** before use and discarded after every 200 slides have been stained. Although slides should always be quality controlled by eye, solutions can be changed sooner if there is a problem.

Eosin used is supplied by Shandon as EosinY (aqueous solution).

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

9. Place the slides into haematoxylin for minimum 30secs to maximum 10 minutes (3 minutes is probably about right).
10. Remove and wash in running water for 2 minutes. (slides can be dipped in Acid Alcohol at this point to reduce Haematoxylin colour if required – see trouble shooting)
11. Transfer to a dish of *Scott's tap water substitute for 1 minute until the tissue sections turn blue (can be anything from 30secs to maximum 10 minutes). *(3.5g sodium bicarbonate, 20g magnesium sulphate, dissolve and make up to 1 litre with Elga water)
12. Place slides in the Eosin Y aqueous solution for 5 minutes - if using Eosin Y 'alcoholic', staining should be reduced to only 2 minutes.
13. Remove and wash in running water for 2 minutes.

De-hydrate, Clear, Mount

14. Allow any excess fluid to drain from the slide rack before proceeding to the next solution
15. Dehydration-50% Alcohol — 30 seconds
16. Dehydration-80% Alcohol — 30 seconds
17. Dehydration-100% Alcohol — 2 minutes
18. Dehydration-100% Alcohol — 2 minutes
19. Clearing-Xylene 16 — 5 minutes
20. Clearing-Xylene 17 — 5 minutes
21. Clearing-Xylene 18 — 5 minutes
22. Mount in DPX as follows:

Mounting Slides

23. Slide mounting should always be performed under the fume extraction unit within the main laboratory.
24. Unless otherwise stated slides should be mounted directly from xylene using an appropriate mountant.

25. Nitrile gloves are available for use when mounting sections.
26. Place appropriately sized coverslips onto the blotting paper under the fume extraction unit.
27. Using a pastette, place a drop of mountant onto each coverslip.
28. Remove a slide from the xylene and align the long edge of the slide with the coverslip, ensuring that the section is facing towards the coverslip.
29. Tilt the slide towards the coverslip until it touches the mountant. Gently release the slide allowing the mountant to spread between the coverslip and the slide.
30. Turn the slide over so that the coverslip is now on top of the slide. If necessary, centre the coverslip over the tissue section and remove any air bubbles by pressing down gently with a yellow tip. Wipe away any excess mountant using a cotton bud dipped in xylene and carefully wipe away xylene residue.
31. If unsuccessful, place the slide and coverslip into the xylene and slide the coverslip away from the section, then remount using a new coverslip. (Do not pull the coverslip off.)
32. Continue until all slides are mounted.
33. Once slides are mounted they can be left on cardboard slide trays in the fume hood to dry.

Changing reagents

All the reagents are changed every 200 slides but individual reagents may be changed more often if required. A record of this is kept on the wall next to the reagent dishes and should be filled in whenever reagents are changed.

Discard the reagents into a properly labelled waste container.

Clean work area with water and 70% alcohol..

Clean the set of staining dishes and fill with fresh reagents. (NB. The haematoxylin **must be filtered prior to use**.)

Place clean lids on the dishes. Complete and sign the reagent change record.

Troubleshooting

If at any time during the staining run, the staining does not look right there are steps that can be taken to correct this:

- If after staining the haematoxylin looks too dark macroscopically, the slides can be washed in water and placed into a dish of 1% Acid Alcohol for either (i) 5 - 10seconds to remove some of the haematoxylin and then 'blued' again in Scott's Tap Water substitute, or (ii) the slides can be left in 1% Acid Alcohol until all the haematoxylin has been

removed and the slides can be washed and placed back into haematoxylin for a reduced amount of time.

- If during dehydrating steps the eosin leaches out of the sections the slides can be taken back through the alcohols to water to re-hydrate the sections and placed back into eosin for a further 5 minutes before completing the staining protocol.
- If after completing the staining protocol, and the slides have been mounted a problem is found, the slides can be placed into a dish of remounting xylene overnight to remove the coverslip and then re-hydrated and re-stained as required.

If after completing the staining run, the slide does have bubbles between the slide and the coverslip, the slide can be placed into a dish of remounting xylene overnight to remove the coverslip and then re-mount it.

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
RA18344 - Haematoxylin and Eosin Staining

9.0 Approval and sign off:

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 09/09/2021

Management Approval by:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 09/09/2021

QA Release by:

Name: John O'Connor

Position: QA Manager

Signature:  Date: 09/09/2021

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