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Title: Haematoxylin & Eosin (H&E) Staining & Subsequent Mounting of Slides

Version 5.0

Author: Melanie McMillan

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SOP History		
Number:	**Date:**	**Reasons for Change:**
01	01/06/2013	Original
02	17/03/2014	Title change & change to COSHH hazard listing
03	01/12/2014	Title change & change to procedure
04	01/11/2015	Change to purpose, scope, responsibilities, procedure, related documents / references and appendices
05	01/03/2016	Change to procedure and related documents / references

1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to describe the current procedure for the haematoxylin and eosin (H&E) staining of paraffin embedded tissues and subsequent mounting of slides within the Histology, Immunodetection & Aquila-HistoPlex sections of the Shared University Research Facilities (SuRF), hereafter collectively referred to as 'SuRF Histology'.

H&E staining is a popular method in histology, used widely in medical diagnosis. The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized haematoxylin. This colours nuclei of cells (and a few other objects, such as keratohyalin granules) blue. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colours other eosinophilic structures in various shades of red, pink and orange.

2.0 Scope:

This SOP applies to all staff, including students, visitors and any other supervised / trained individuals involved in this procedure within SuRF Histology, based in the Queen's Medical Research Institute (QMRI), Edinburgh.

3.0 Responsibilities:

This document is a guide only – on site training is essential before use.

3.1 All staff are responsible for ensuring that methods are followed in accordance with this SOP after suitable training, and where relevant, update their SOP Training Record (Standard document QA008) accordingly.

3.2 All staff involved in this procedure must be familiar with the location of any manufacturers manuals, instructions or guidance pertaining to equipment or methodology, and are strongly advised to read and understand this material before performing this procedure.

3.3 All staff must have read any corresponding relevant risk assessment / COSHH documents before performing this procedure.

4.0 Procedure:

Sections to be stained should have been baked in a 55°C oven overnight.

Control slides must always be used to check staining efficiency. Practically every tissue has an internal control so no other control is needed, if a control is desired, lung aorta strips will provide good material.

Validation of the Shandon Varistain Gemini ES Slide Stainer is performed every time the machine is used prior to the first run of the day with a control slide of lung aorta strips. This must be dated and signed.

Tissue that has been dehydrated, embedded in wax and then sectioned must be dewaxed and rehydrated before staining with specific stains or dyes.

The reagents for dewaxing and rehydrating by hand (similarly those for dehydrating and clearing) slides are laid out in the laboratory within the fume extraction cabinet or on downflow benching. When not in use replace the lids on the reagent dishes to minimise the release of fumes.

4.1 Preparation of solutions:

95% ethanol (GP grade):

ethanol (GP grade)	95ml
deionised water	5ml

80% ethanol (GP grade):

ethanol (GP grade)	80ml
deionised water	20ml

70% ethanol (GP grade):

ethanol (GP grade)	70ml
deionised water	30ml

1% acid alcohol:

hydrochloric acid, concentrated (sp.gr.1.19)	1ml
70% ethanol	99ml

Scott's tap water:
Commercially available

sodium bicarbonate	*2g*
magnesium sulphate	*20g*
deionised water	*1000ml*

Dissolve the salts in the water. Store stock solutions at room temperature.

Haematoxylin (Harris'):
Commercially available

haematoxylin	*2.5g*
ethanol	*25ml*
potassium alum	*50g*
deionised water	*500ml*
mercuric oxide	*1.25g or*
sodium iodate	*0.5g*
acetic acid (glacial)	*20ml*

The haematoxylin is dissolved in ethanol, and is then added to the alum, which has previously been dissolved in the warm deionised water in a 2-litre flask. The mixture is rapidly brought to the boil and the mercuric oxide or sodium iodate is then slowly and carefully added. Plunging the flask into cold water or into a sink containing chipped ice rapidly cools the stain. When the solution is cold, the acetic acid is added, and the stain is ready for immediate use. The glacial acetic acid is optional but its inclusion gives more precise and selective staining of nuclei.

Eosin Y:
Commercially available

Note historically there are 2 working solutions of Eosin Y in use in Histology. (1) An aqueous solution from Thermo Fisher Scientific, used in E1.24 and (2) a mix of Eosin Y 515, alcoholic and Eosin Y, 1% aqueous both from Leica Biosystems, used in E1.27 (1 part alcoholic to 3 parts aqueous). Both achieve the same result.

4.2 Dewax and rehydrate by hand:

Note: Allow any excess fluid to drain from the slide rack before proceeding to the next solution.

• Xylene (1)	Fume extraction unit	5 minutes
• Xylene (2)	Fume extraction unit	5 minutes
• Xylene (3)	Fume extraction unit	5 minutes
• Ethanol (1)	Fume extraction unit	20 seconds
• Ethanol (2)	Fume extraction unit	20 seconds
• Ethanol (3)	Fume extraction unit	20 seconds
• 95% ethanol (GP grade)	Fume extraction unit	20 seconds
• 80% ethanol (GP grade)	Fume extraction unit	20 seconds
• 70% ethanol (GP grade)	Fume extraction unit	20 seconds
• Wash in running water	Sink	2 minutes

4.3 Staining and expected results by hand:

- The Haematoxylin used is commercially available ready-made Harris Haematoxylin. This should be filtered before use and replaced every two weeks. Although slides should always be quality controlled by eye and solution changed sooner if there is a problem.
- Place the slides into haematoxylin for 5 minutes.
- Remove and wash in running water for 20 seconds.
- Differentiate for 5 seconds maximum in 1% acid alcohol.
- Transfer to a dish of Scott's tap water substitute for 2 minutes until the tissue sections turn blue.
- Place slides in the eosin solution and stain for 2 minutes.
- Remove and wash in running water for 20 seconds.
- Control slides must always be used to check staining efficiency.
- **Proceed to section 4.4** dehydrate, differentiate in ethanol and clear in xylene. Then **proceed to section 4.6** and mount either by hand or by using Shandon ClearVue Coverslipper.

4.4 Dehydrate and clear by hand:

Note: Allow any excess fluid to drain from the slide rack before proceeding to the next solution.

• 70% ethanol (GP grade)	Fume extraction unit	20 seconds
• 80% ethanol (GP grade)	Fume extraction unit	20 seconds
• 95% ethanol (GP grade)	Fume extraction unit	20 seconds
• Ethanol (1)	Fume extraction unit	20 seconds
• Ethanol (2)	Fume extraction unit	20 seconds
• Ethanol (3)	Fume extraction unit	20 seconds
• Xylene (1)	Fume extraction unit	5 minutes
• Xylene (2)	Fume extraction unit	5 minutes
• Xylene (3)	Fume extraction unit	5 minutes

Results:	nuclei	blue
	background	pink to red

4.5 Using Shandon Varistain Gemini ES Slide Stainer, Protocol 2 see appendix 1

The Shandon Varistain Gemini ES Slide Stainer is designed for high throughput, precision, safety, durability and ultimate flexibility in histology and cytology staining applications. This Shandon stainer features load-on-demand multiple baskets and programs, power outage backup and built in fume control to make staining easier, faster and safer. Training and guidance will be provided prior to initial usage.

NOTE: this machine is pre-programmed so there is NO need to change any of the settings. An access code is now in place and no program can be changed.

- **In summary:**
- Check the levels of all the reagents in the baskets and, if low, top these up.
- Turn the cold water tap on (until the black lines on the tap align).
- Switch on the electrical supply on the front of the control box (located underneath the staining machine).
- Wait until the machine completes its test cycle with the validation control slide of spleen.
- Place the microscope slides into the special slide racks with the label end next to the arrow on the rack and sections facing in the direction of this arrow.
- Lock the slides in place by moving the locking bar on the rack away from the arrow.
- Push the TOP clear window to the right (the monitor display will read "H&E other" and place the rack of slides into the "LOAD" basket.
- Close the window and press the "URGENT START" button on the control box. Press the "OVERIDE" button if the machine alarms.
- The machine will start automatically.
- Up to 5 racks may be loaded at any one time.
- Switch off in reverse order.
- **Proceed to section 4.6** and mount either by hand or by using Shandon ClearVue Coverslipper.
- If an error message occurs then refer to the key instructions in the manufacturers manual.
- Cleaning and maintenance should be carried out every 15 cycles as per manufacturer's instruction manual.

Results:	nuclei	blue
	background	pink to red

4.6 Mounting slides:

4.6.1 by hand:

- Slide mounting should **always** be performed under the fume extraction unit within the main laboratory.

- Unless otherwise stated slides should be mounted directly from xylene using Pertex.
- Nitrile gloves are available for use when mounting sections.
- Place appropriately sized coverslips onto the blotting paper under the fume extraction unit.
- Using a pastette, place a drop of mountant onto each coverslip.
- 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.
- 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.
- Turn the slide over so that the coverslip is now on top of the slide. If necessary, center the coverslip over the tissue section and remove any air bubbles by pressing down gently with a finger. Wipe away any excess mountant using a clean cloth.
- 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.)
- Continue until all slides are mounted.
- Once slides are mounted they can be left on cardboard slide trays in the fume hood to dry.

4.6.2 using Shandon ClearVue Coverslipper:

The Shandon ClearVue Coverslipper provides a fast and accurate automated method for coverslipping glass slides. Training and guidance will be provided prior to initial usage.

- **In summary:**
- Switch on the electrical supply at the mains plug & on the side of the machine.
- Wait until the machine completes its test cycle.
- Check the mountant level in the glass bottle at the front of the machine and, if low, top up by unscrewing the plastic lid and add more mountant to the level marked on the bottle.
- Place the microscope slides into the special slide racks with the label end next to the arrow on the rack and sections facing in the direction of this arrow.
- Lock the slides in place by moving the locking bar on the rack away from the arrow.
- Place the rack into the coverslipper (open the lid on the lower left hand of the coverslipper and hook the rack on the metal ridge with the sections facing upwards)
- Close the lid and the machine will start automatically.
- Once complete, the mounted slides will be dispensed into the top left hand chamber. These can be left there to dry or removed by opening the lid and lifting out the rack of slides.
- Up to 5 racks may be loaded on the machine at any one time.
- Switch off in reverse order.
- If an error message occurs then refer to the key instructions in the manufacturers manual.

- Cleaning and maintenance should be carried out as per manufacturer's instruction manual once a week.

4.7 Changing reagents:

- All working reagents are changed weekly but individual reagents may be changed more often if required. Stock reagents are changed every 2 months or as according to expiry date on bottle. 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 mild detergent solution.
- Clean the set of staining dishes and fill with fresh reagents.
- Place clean lids on the dishes. Complete and sign the reagent change record.

5.0 Related documents / references:

- COSHH: SuRF-COSHH-052: Haematoxylin and Eosin (H&E) Staining and Subsequent Mounting of Slides

- QA008 – SOP Training Record
- QA013 – Shandon Varistain Gemini ES – Reagent Change Form

- Manufacturer's manuals / instructions

- The University of Edinburgh Health & Safety Policy / Codes of Practice (available on University's Health and Safety Department website)

- College of Medicine and Veterinary Medicine Health and Safety Manual (available on University's Health and Safety Department website)

- Bancroft J.D., and Gamble M. Theory and Practice of Histological Techniques 6th Edition (2008) Churchill Livingstone, Elsevier Limited

6.0 Appendices

Appendix 1 Shandon Varistain Gemini ES – Protocol 2

Step	Reagent	Conc. (%)	Uses	Time	Limit	Agitate
1	Dry Storage		0	00:00	No Maximum	Initial
2	Xylene		20	05:00	No Maximum	Initial
3	Xylene		20	05:00	No Maximum	Initial
4	Xylene		20	05:00	No Maximum	Initial
5	Xylene		20	05:00	No Maximum	Initial
6	Ethanol	100	20	02:00	No Maximum	Initial
7	Ethanol	100	20	02:00	No Maximum	Initial
8	Ethanol	95	20	02:00	No Maximum	Initial
9	Running Water Wash		20	01:00	No Maximum	Initial
10	Haematoxylin		20	05:00	Standard	Initial
11	Running Water Wash		20	01:00	Standard	Initial
12	Acid Alcohol		20	00:05	Critical	Initial
13	Running Water Wash		20	01:00	No Maximum	Initial
14	Scotts Tap Water Sub.		20	01:00	Standard	Initial
15	Running Water Wash		20	01:00	No Maximum	Initial
16	Aqueous Eosin Y		20	01:00	Standard	Initial
17	Running Water Wash		20	01:00	Standard	Initial
18	Ethanol	95	20	00:30	No Maximum	Initial
19	Ethanol	100	20	00:30	No Maximum	Initial
20	Ethanol	100	20	00:45	No Maximum	Initial
21	Xylene		20	01:00	No Maximum	Initial
22	Xylene		20	01:00	No Maximum	Initial
23	Xylene		20	01:00	No Maximum	Initial
24	Xylene		20	01:00	No Maximum	Initial

7.0 Approval and sign off:

Author:		
Name:	Melanie McMillan	
Position:	Principal Investigator	
Signature:		Date:
Management Approval by:		
Name:	Mike Millar	
Position:	Facility Manager	
Signature:		Date:
QA Release by:		
Name:	Robin Sellar	
Position:	QA Manager	
Signature:		Date:

8.0 Verification by users:

Sign below to indicate you have read and understood the activity outlined above and the risk control measures that you must implement, use and / or wear. Ensure you have received sufficient information, instruction and training to enable you to conduct this activity with the minimum of risk to yourself and others.

Signature:	**Date:**