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Title: General Procedure for Dewaxing of Paraffin Sections, Rehydration / Dehydration and Subsequent Mounting of Slides

Version 3.0

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Reviewed annually	

SOP History		
Number:	**Date:**	**Reasons for Change:**
01	01/06/2013	Original
02	24/02/2014	Change to title and purpose
03	17/11/2014	Change to procedure

1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to describe the current procedure for the dewaxing of paraffin sections, rehydration / dehydration and subsequent mounting of slides after staining sections with stain of choice.

2.0 Scope:

This SOP applies to all Histology staff, students, visitors and any other supervised/trained individuals involved in this procedure within the Shared University Research Facilities (SuRF), 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 involved in this procedure are responsible for ensuring that methods are followed in accordance with this SOP after suitable training.

3.2 All staff must have read and signed any relevant risk assessment document(s) relating to this SOP before performing this procedure.

4.0 Procedure:

Control slides must always be used to check staining efficiency.

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 (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.

Unless otherwise directed by the specific staining method employed, follow the procedure outlined below.

4.1 Dewax and rehydrate:

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
• Absolute ethanol (1)	Fume extraction unit	20 seconds
• Absolute ethanol (2)	Fume extraction unit	20 seconds
• Absolute 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

4.2 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

4.3 Staining and expected results:

Follow the standard protocol for the stain or dye being used unless requested otherwise

4.4 Dehydrate and clear:

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
• Absolute ethanol (1)	Fume extraction unit	20 seconds
• Absolute ethanol (2)	Fume extraction unit	20 seconds
• Absolute 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	Prior to mounting

4.5 Mounting slides:

4.5.1 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.

4.5.2 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.

4.6 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-007: General Procedure for Dewaxing of Paraffin Sections, Rehydration / Dehydration and Subsequent Mounting of Slides

- 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 Approval and sign off:

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

7.0 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:

Signature:	Date:

Signature:	Date:

Signature:	Date: