



**FACULTY OF
HEALTH SCIENCE**

**School of
Human Life Sciences**

**HISTOLOGY/HISTOPATHOLOGY
SAFETY BOOKLET**

CONTENTS

LABORATORY RULES	1
EMERGENCY PROCEDURES	2
CLEANING AND WASTE DISPOSAL	3
CLEAN-UP PROCEDURE FOR A BIOHAZARD	4
PERSONAL PROTECTIVE EQUIPMENT and PROCEDURES	5-6

APPENDIX

PROCEDURE – ROTARY MICROTOME	7
PROCEDURE – SHANDON DUPLEX PROCESSOR	8
PROCEDURE - SHANDON HISTO CENTRE	9

ENSURE THAT YOU HAVE READ THIS BOOKLET, AND UNDERSTAND THE CONTENTS. THEN PLEASE COMPLETE THE ON-LINE STUDENT SAFETY MODULE.

1 LABORATORY RULES

1.1 General Conduct - Every individual that enters the laboratory has a duty of care to other users, and is expected to behave in a manner that does not compromise the safety of others.

1.2 Eating or drinking are not allowed anywhere within the laboratories.

1.3 Regard all organisms and biological materials used in this laboratory as potentially infectious and pathogenic to humans.

1.4 Laboratory coats must be worn for each practical session.

1.5 Long hair should be tied back neatly, away from the shoulders.

1.6 Enclosed footwear must be worn - (thongs and open sandals are not allowed).

1.7 Avoid placing any object in your mouth - (pencils, pens, fingers etc).

1.8 Cover any open cuts on hands and other exposed skin surfaces and/or wear gloves.

1.9 When working with biological substances such as blood or urine, ensure that all work is carried out in the designated working area as instructed by the practical demonstrator. It is essential that all books, writing materials and other personal belongings are kept separate from this working area for the duration of the practical session.

1.10 Carry out procedures to minimise the risks of spills, splashes and the production of aerosols. If you have an accident of any kind, call the instructor immediately. For minor spills, put on gloves, cover spill with paper towel and pour on disinfectant. Leave for 10 minutes and then mop up.

1.11 After completion of practical sessions involving biological substances such as blood or urine, always wash your hands thoroughly before leaving the laboratory.

No slides or cultures are to be removed from, or brought into the laboratory without permission of the Laboratory Safety Officer or the Histology teaching staff.

Students will not use any histology equipment in the laboratory except under the direction and supervision of the teaching staff.

1.12 Familiarise yourself with the location and operation of the following emergency items for each laboratory;

First aid kit

Fire extinguisher

Fire Blanket

Eyewash station

Spill Kit

Emergency Shower

Respirator – Formalin and Organic Vapour

2.0 EMERGENCY PROCEDURES

Each laboratory has a posted evacuation procedures sheet. Please ensure that you read and understand these documents before working within the histology laboratory.

2.1 Designated Building/Area Wardens

Designated Floor Wardens for Building C, Level 2:

Ms. Hetty Binns Room C208 Ext. 5470

Mrs Merrilyn Johnson C208 Ext. 5471

First Aid Officers:

Ms. Hetty Binns Room C208 Ext. 5470

Mrs Merrilyn Johnson C208, Ext 5471

Mrs Laura Maddock, C208, Ext 5463

2.2 Be familiar with the location of the emergency eye wash station at the rear of the laboratory and in the adjacent Haematology laboratory C219. Use eye wash immediately if any material or reagent is splashed into the eye.

2.3 All accidents and spillages must be reported to the staff member in charge.

2.4 Be familiar with the location and use of fire extinguishers and fire blankets. These are to be used only in the event of small, localized fires.

2.5 Emergency Exits

There are illuminated exit signs leading to external doors from the histology laboratories.

In the case of a large fire and/or the sounding of the fire alarm, all students and staff, except those with designated duties, must proceed to the designated muster point outside the administration block. Move rapidly but calmly via the closest exit.

3.0 CLEANING AND WASTE DISPOSAL

Appropriate cleaning and waste disposal is designed to separate different types of waste and minimise risks when handling hazardous products or byproducts.

Please follow the instructions carefully and if in doubt - **ASK!**

3.1 Sharps

Sharps are to be placed in the sharps containers located around the histology laboratory; scalpel blades are to be disposed of in the blade removal system, which are made available when required. Please read the instructions on the blade removal systems to avoid damaging the unit.

DO NOT WALK AROUND WITH SHARPS. TAKE THE CONTAINER TO THE SHARPS.

3.2 Broken Glass

When any glassware is broken, notify the instructor immediately for assistance with disposal. Non-contaminated broken glass can be disposed of in the glass bin, which is located at the front of the laboratory or in a sharps container that is available for that purpose.

3.4 Biological Material

Biological material of animal origin (i.e. rat or fish) is used in certain practicals. Waste from these practicals should be disposed of into the Biohazard bags provided, or as instructed by the demonstrator. Any materials used that are of a biological nature must be disposed of in the appropriate receptacles.

3.5 General Waste

There is a bin located in the histology laboratory and gowning room for disposal of general waste, including non-contaminated paper towel and all other waste that has not already been discussed above.

3.6 Cleaning of General Work Areas

General work areas are to be kept tidy and free of unnecessary equipment. Any tools or implements used for general work should be returned to their appropriate place. Please follow instructions carefully and if in doubt - **ASK!**

3.7 Diversol

The general disinfectant used in the laboratory is “Diversol” and is available in the laboratory during specific practical sessions.

4.0 CLEAN UP PROCEDURE FOR BIOHAZARD SPILLS

'Spill Kits' containing necessary personal protective equipment are located in the histology laboratory. 'Spill Kits' are checked and maintained by the Technical Officer, access to the 'Spill Kit' is to be free from obstruction at all times. If the kit is used, please notify the Technical Officer so stocks can be replenished.

4.1 PROCEDURE

- Discontinue work in the immediate area.
- If hands have been contaminated, first wash hands with soap and water.
- Remove and replace any contaminated protective clothing.
- Put on Gloves
- Lay down absorbent material wetted with disinfectant over spill and allow it to sit for 10 minutes.
- After 10 minutes, mop up spill and place the contaminated materials into autoclavable bag.
- Wipe over general area again with paper towel dampened with disinfectant.
- Remove gloves and wash hands.

In the event of a major biohazard spill, staff are instructed to evacuate the histology facility and notify security at once.

5.0 PERSONAL PROTECTIVE EQUIPMENT and PROCEDURES

5.1 Gowns

Students are provided with gowns for use in the Histology Laboratory. These gowns **must not** be removed from the gowning room adjacent to the Histology laboratory. These gowns are laundered regularly by the School of Human Life Sciences.

5.2 Safety Glasses and Eye Protection

All students and staff are required to have "safety glasses" with them for all practical sessions, and are to use them when procedures are undertaken that involve significant risk of splashing with infectious or corrosive liquids e.g. during staining procedures.

5.3 Gloves

For routine work in the histology laboratory, gloves are not considered essential.

Gloves should be worn under the following circumstances;

- When mopping up a spill.
- When performing procedures where there is a high risk of contaminating hands e.g. staining procedures.
- If open cuts or skin conditions are present, that increases the risk of infection from accidental contamination.
- When instructed by the demonstrator.

5.4 Hand washing

Hands must always be washed before leaving the laboratory.

5.5 High Risk Individuals /Antenatal Considerations

Any student that has a medical condition that they feel might be compromised by exposure to reagents or chemicals in the laboratory is encouraged to discuss the matter with the demonstrator or Technical Officer.

5.6 Solvents

If solvents are used during practical sessions, the exhaust fan **must** be switched on. When using these solvents consult the appropriate Safety Data Sheet (SDS) (previously called Material Safety Data Sheets or MSDS) before commencing work. SDSs for all chemicals and reagents used within the histology laboratory are available both within the laboratory and in the preparation room (C222).

5.7 Staining procedures

Whenever doing staining procedures ensure that protective gowns, gloves and safety glasses are worn. When 'bringing sections to water' or 'dehydrating, clearing and mounting' always ensure that the exhaust system is turned on. Ensure you have read the SDS that relates to the

particular stain that you are using and you are aware of any possible hazards. SDSs are available during practical sessions that use stains.

5.8.1 Accident or Incident Report

All accidents and major spills should be documented on an “Incident Report Form”. Official reporting of incidents is to be done on-line. The form is available on-line at the Workplace Health and Safety site: <http://www.human-resources.utas.edu.au/health-and-safety>.

6.0 EQUIPMENT

6.1 Exhaust/Extraction Unit (Stainless Steel Bench)

All procedures involving solvents are to be carried out on this bench. The extraction fan will be turned on by the demonstrator, lecturer or technical staff. Please ask if it is not turned on.

6.2 Bunsen Burners

If Bunsen Burners are to be used in a practical sessions they will be set up by the Technical Officer using a small gas bottle. **Only histology staff are to do this.** When using the Bunsen burners be careful not to burn yourself. Be aware of burning back by noting a hollow burning sound and/or absence of a blue cone of unburned gas. Ensure that all burners are turned off at the end of the practical and gas bottles are secured.

6.3 Shandon Duplex Automatic Processors

Consist of a series of graded ethanol solutions, xylene and paraffin wax heated to 61°C. When using these machines ensure that all requirements for personal protective equipment are observed. Safe Operating Procedures are included as an appendix in this manual and are placed with the equipment when it is used for practical sessions. Ensure the exhaust fan is switched on when machines operational.

6.4 Microtomes

Rotary microtomes are used during practical sessions for cutting tissue sections. These are heavy pieces of equipment, which must be lifted or moved with care and strictly as instructed by the demonstrator. The knives used in these microtomes are very sharp, and must be used with extreme care by students and staff. Disposable blades for these knives are to be handled and disposed of strictly as instructed by the demonstrator.

NOTE - Microtomes with knives in place are not to be left unattended at any time.

The wheel of each microtome must be in the locked position any time the microtome is not being used for cutting sections. No person suspected of being under the influence of alcohol or drugs will be permitted to use this equipment. Any such person will be required to leave the practical session immediately.

6.5 Embedding Centres

Embedding Centres contain paraffin wax at 61°C and both a hot plate at about 70°C and a cold plate at -4° C. When using, ensure that you do not burn your fingers. Ensure that all personal protective equipment requirements are met and that you have familiarised yourself with the safe operating procedures for this piece of equipment. Gowns and eyeglasses must be worn when actually embedding.

APPENDIX

PROCEDURE – ROTARY MICROTOME

1. **Do not move Microtomes**, they are placed out by staff when required. They are extremely heavy and awkward to manoeuvre.
2. The handle-wheel on the Microtome should be in the locked position.
3. Take the microtome knife holder from its box and ensure that the disposable blade has been removed.
4. Using the disposable blade dispenser attach a new blade to the holder and position. Turn screws to tighten blade into place.
5. Disposable microtome blades are covered with oil that prevents them from rusting while in storage. You must remove this oil before cutting sections.
6. Using a tissue and a small amount of Xylene gently wipe the exposed area of the disposable blade. Make sure you wipe away from the edge of the blade to avoid cutting yourself.
7. Fix the disposable blade and handle into position in the microtome. Adjust the angle and inclination of the knife blade using the adjustment knobs.
8. Adjust the section width – usually about 5µm.
9. Get your paraffin block (it is best to keep them on a layer of ice close to the microtome) and insert into tissue clamp. Push the lever forward and place the block into clamp and release.
10. Manually advance the block forward towards the microtome blade.
11. As the microtome handle is rotated, the chuck will move up and down and advance forward with each revolution.
12. When ready release the microtome handle-wheel and slowly start to cut block.
13. The first few cuts will trim the block so you have an even surface and expose the embedded tissue.
14. Once the block has been trimmed it is a good idea to place it back onto the ice for a few minutes then re-attach to the tissue clamp.
15. Start cutting the block and ribbons should begin to form.
16. If your ribbons are not forming adequately, check the faults and remedies checklist provided with the Microtomy lecture notes.
17. When the ribbons are of the desired quality, move them to the water bath using forceps and/or brushes. The water bath temperature should be set about 10°C less than the melting point of the wax.
18. When you have finished cutting ensure that you lock the microtome handle-wheel into place.
19. Remove the microtome knife or disposable blade handle when finished cutting.
20. If using a disposable blade loosen screws and use the blade dispenser to remove the used blade.
21. Never store blade holders with disposable blades still attached.
22. Never leave the microtome with a knife or disposable blade attached, and ensure that the rotary wheel is in the locked position when not in use.

PROCEDURE – SHANDON DUPLEX PROCESSOR

1. With this type of tissue processor, the tissues are contained within a basket and are transferred through a series of stations arranged in a circular carousel plan. Fluid agitation is by vertical oscillation. Processing schedules are by notched pins.
2. Always ensure that the exhaust fan is working when the processor is being used.
3. Familiarise yourself with the SDSs for all the chemicals contained in the processor. Located in C222.
4. Ensure that you know what processing times are needed for the specimen and sections you wish to cut.
5. Add fixatives, clearing and drying agents to the processor. Make sure you work on the exhaust bench when completing this part of the procedure.
6. Set the correct immersion times for each station using the notched pins. Each pin is equivalent to 15 minutes.
7. Whether using one or two baskets it is imperative you start your specimen in the first fixative agent and that the cycle will finish with the specimen in the wax bath.
8. Place the specimen/s into the basket and connect top lid.
9. Switch on processor and start cycle.
10. If the processor is to run through its cycle while there are no staff present ensure that you have the times correct and that the process will finish where you desire.
11. If you are present during the processing cycle, it is a good idea to check that the processor is functioning properly.
12. Damage can be done to tissue samples if the processor loses power. Several things can happen in this case. The sample can be left in a drying agent for too long and dry out. The sample may be moved back into the clearing agents after being in the wax. Specimens can be left in the fixative for too long or not long enough, which can have adverse affects when it comes to cutting sections from the block.
13. Read and understand the processor-operating manual if you are to be responsible for instructing others in the use of the processor.
14. When the processor has completed its cycle, be sure to turn off the power to the unit. If the unit is to be stored for long periods without use empty all the stations of their contents only leaving the wax baths.

PROCEDURE - SHANDON HISTO CENTRE

1. Ensure the paraffin tank is full of paraffin and the stainless steel paraffin holding pan is half full of paraffin. Turn the switch to the **ON** position.
2. Set the temperature in the paraffin tank to the desired operating temperature, using the Push to Set Switch and the Set Point Control knob. Press and hold the Push to Set Switch and observe the changing display while adjusting the Set Point Control for the desired temperature. Once the desired set point shows on the display release the Push to Set Switch.
3. The paraffin tank temperature can be checked by removing the cover and placing a calibrated total immersion thermometer in the centre. Replace the cover and leave for one hour. The temperature should be within $\mp 0.5^{\circ}\text{C}$ of any setting.
4. If the paraffin is not melting in the stainless steel holding pan, adjust the temperature using the Temperature Adjustment Knob.
5. When the paraffin has melted, dispense paraffin into the clean waste pan and pour into cassette working area. If you prefer to partially immerse cassettes in paraffin, the waste valve must be closed to retain paraffin.
6. Keep forceps in the wells.
7. Turn cold plate switch to **ON** – Allow 30 minutes for the cold plate to reach optimum operating temperature.
8. Turn on the orientation platform lights and adjust hotplate temperature control to setting appropriate for molds being used. Typical operating temperatures are 60-70°C.
9. Place the processed tissue cassettes in the stainless steel holding pan in molten paraffin.
10. Remove a tissue cassette from the molten paraffin bath. Remove the cover and place in the cassette working area of the warming tank. A layer of paraffin in this well will prevent the specimen from solidifying.
11. Select from the pre-warmer area a base mold and place mold on the hot plate and fill to your desired level.
12. Remove the specimen from the tissue capsule or cassette and place in the base mold. Orient your specimen as required.
13. Slide the mold onto the cold plate section and position with the forceps if necessary. Wait a few seconds for the paraffin to change from clear to translucent appearance.
14. Do not allow a skin to form on the surface of the paraffin. Skin formation may cause the block to break in the microtome chuck at that point.
15. Slide molds onto the channeled section of the hot plate. Place your cassette on top of the base mold and fill. Fill mold to top if cassette is not used.
16. Slide the mold to the back of the cold plate for rapid chilling.
17. On completion of daily embedding, turn the cold plate off. The paraffin tank and warming tank are designed for continuous operation. Place covers over the base molds pre-warming area and holding-pan compartment. Insert forceps through port in holding-pan cover.

Operational Checks and Precautions

The following information is designed to assure optimum and safe operation of the Shandon Histo Centre Tissue Embedding Centre.

1. Periodically check the temperature of the paraffin in the paraffin tank with a calibrated thermometer, and compare the reading with the digital display. The two readings should be within $\pm 0.5^{\circ}\text{C}$ at any setting.
2. It is recommended that the cassette working area paraffin be drained daily to avoid cross-contamination. In addition, the forceps warming well should be cleaned daily or whenever necessary to avoid cross-contamination.
3. Do not place the Histo Centre directly under an air conditioner vent, as this can form a skin on the paraffin.
4. Do not block the airflow at the rear of the Histo Centre.
5. The Histo Centre is sealed in critical areas to prevent spilled paraffin from leaking inside the unit. A splash plate located at the rear of the centre deflects spilled paraffin, preventing it from clogging the condenser. Copious spillage of paraffin should be inspected thoroughly to assure paraffin did not leak inside the unit. In the event of an internal leakage, the unit should be disassembled and cleaned thoroughly.

DO NOT USE XYLENE FOR CLEANING OF THE UNIT.

Refer to Histo Centre Manual for proper cleaning requirements.

