



Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document

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<i>Number</i>	PSECTIONING
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Wisconsin Veterinary Diagnostic Laboratory

Standard Operating Procedure

1 Introduction

Sectioning of fixed tissue by microtomy is a necessary step in histopathology. After the tissue has been embedded in paraffin (PEMBEDDING), sections are taken and placed on slides so they can be appropriately stained. Sectioning can be difficult and requires practice and patience.

2 Specimen submission

2.1 Type – Paraffin embedded block.

2.2 Special requirements for collection - NA

2.3 Handling conditions

2.4 Criteria for rejection of sample

If a tissue is improperly embedded (i.e., the tissue does not all lie at the face of the block), or if tissue or a large amount of paraffin breaks out of the block during sectioning, it must be re-embedded.

3 Materials

3.1 Equipment & Instrumentation

1. Leica microtome RM2255
2. Leica Microtome RM2235
3. Leica Microtome
4. Water bath 40-47°C
5. Freezer
6. Tissue pick
7. Forceps
8. Paint brush
9. Histo-cool tray
10. Leica slide printer

3.2 Reagents & Media

1. RO/DIH₂O
2. PARA/GARD cleaner

3.3 Supplies

1. Kimwipe
2. WypAlls
3. Statlab Statmark black permanent slide markers
4. Fisherbrand sharps container – 1 gallon
5. Surgipath Colorfrost slides – blue, white, pink, green and peach
6. Fisherbrand Superfrost/Plus or Leica APEX slide-yellow

4 Safety Management

4.1 Required Safety Training:

- Biosafety 104: Building Biosafety into Your Research - Safe Use of Sharps

4.2 Required personal protective equipment (PPE):

- Minimum: Lab coat, Safety glasses and closed toe shoes– upon entry of lab
Gloves: Nitrile
 Chemical resistant – when immersing fingers in Xylene

4.3 Hazard Communication

A. Chemical: See MSDS

Xylene (MSDS-574)

- Flammable liquid and vapor. Possible cancer hazard. May cause cancer based on animal data. Harmful if absorbed through skin or if inhaled. Causes eye, skin, and respiratory tract irritation. Inhalation may cause central nervous system effects. Aspiration hazard if swallowed - can enter lungs and cause damage.

B. Biological: Biosafety Level 2

C. Physical: Microtomes have moving parts and an extremely sharp blade.

D. Electrical: NA

E. Sharps: Use caution with the blade as they are extremely sharp.

F. Ergonomics: Use good posture and allow hands to rest as needed.

4.4 Waste Disposal

- Kim-wipes, paraffin shavings and other waste generated during this procedure must be disposed in a biohazard bag and incinerated.
- Used water from the water bath and HistoCool can be dumped down the drain.
- Used blades must be discarded in a sharps container.
- Broken slides must be discarded in a broken glass box that is lined with a plastic bag.

5 Preparation for procedure

5.1 Equipment and instrumentation preparation

- Prior to sectioning, fill the water bath with RO/DI H₂O, turn on and set temperature to 40- 47°C to allow water to warm.
- Remove a Histo-cool tray from the freezer and add RO/DI H₂O
- Slides can be pre-labeled with a black permanent slide marker or slide printer
- The standard thickness for general histological purposes is 5 µm. Check the SOP of the stain that will be performed on the tissue to verify thickness. If necessary, adjust the thickness. This can be accomplished by turning the control knob on the front of the microtome.
- If "thick and thin" sections occur, the blade angle can be adjusted with the lever on the stage, to the left-hand side of the blade. The optimum blade angle is 2.5 degrees, but can vary.
- The adjustment levers which are located on the top of the chuck on the microtome can be used to adjust the block in vertical and horizontal planes.

5.2 Reagents and media preparation - NA

5.3 Standards/controls preparation

5.4 Specimen preparation

- The blocks containing tissue to be sectioned are stored at room temperature on the shelving unit near the microtomes prior to sectioning.

6 Performance of procedure

Three different types of microtomes are currently in use in the Histopathology lab. Model RM2255 is semi-automated. Models RM2125 and RM2235 are not automated.

6.1 Slide color coding

- Use Surgipath Colorfrost slides:
 - Pink – Biopsy
 - White – Madison H&E
 - Green or Lavender – Barron H&E
 - Blue – Special Stain -- Positive Controls
 - Peach – NWHC H&E
- Use Fisherbrand Superfrost/Plus slides:
 - Yellow – as needed for special stains

6.2 Label slides manually

1. Obtain appropriate accession number from the submission form.
2. Obtain appropriate color and type of slide
3. Use a StatLab Statmark Pen
4. Write the accession number on the top of the frosted portion of each slide, (ie.M09-12345-1).

6.3 Label slides with Leica Slide Printer

1. Load slides with frosted portion facing up into the Leica slide printer.
2. Double click “aaa.Leica_Shortcut_Files” on the desktop.
3. Double click “Leica HistoPAL™ Clinical” icon to open printer software.
4. Double click “Print Slides” icon to open the data entry form.
5. Select the appropriate Prefix (M13-, B13-, POS CT) from the drop down menu.
6. Type in the accession number, (ie. 12345).
7. When printing ascending accession number, enter an integer in the corresponding “Quantity” field to determine the amount of accession to be printed.
8. Enter data into the “1st Position” field and select the “A” or “N” button for Alpha or Numeric.
 - a. Enter the number of auto-incremented slides needed in the “quantity” field.
9. Enter data into the “2nd Position” field and select the “A” or “N” button for Alpha or numeric.
 - a. Enter the number of auto-incremented slides needed in the quantity field.
10. Select the appropriate stain code from the “Line 2” drop down menu or manually enter other information as needed.

11. Select the appropriate Pathologist initials from the "Pathologist" field or manually enter other initials as needed.
12. Select the appropriate magazine from the Cassette Magazine field or select the manual feed function.
13. Enter the number of identical duplicate slides needed in the "Duplicates" field.
14. Click the "Slides" button to print slides.
15. Transport the printed slides to the microtome workstation shelving unit.

6.4 Trim/Face Off (Leica Microtome RM2125, RM2235)

1. Obtain a tray with blocks from the shelving unit and place them next to the microtome.
2. Place one block into the microtome using the block locking lever on top of the specimen holder.
3. Make sure the cartridge corners are snapped into place.
4. Unlock the blade clamp lever on the stage and slide a new blade in.
5. Lock the blade clamp lever.
6. When the first side of the blade gets dull, unlock the blade clamp lever, and move the fresh side of the blade under the block. The blade shall be changed after approximately five blocks, but this number can vary.
7. Dispose of used blade in a sharps container.
8. Rotate large handwheel so chuck is above blade holder.
9. Rotate large handwheel one quarter turn towards operator.
10. Unlock the base holder lever.
11. Push base holder forward until the blade holder touches the block.
12. Lock the base holder lever.
13. Rotate handwheel up to 12 o'clock position.
14. Use small hand wheel to advance and retract the block.
15. Set the cutting thickness to 20-30 μ m.
16. Turn handwheel with full clockwise rotations until all tissue in the block is fully represented.
17. Place the block on the Histo-Cool tray.

6.5 Sectioning without Automation (Leica Microtome RM2125, RM2235)

1. Remove block from Histo-Cool tray and place into the microtome using the block locking lever on top of the specimen holder.
2. Make sure the cartridge corners are snapped into place.
3. Set cutting thickness appropriately. (See 5.1)
4. Unlock large hand wheel and rotate clockwise to advance specimen towards knife.
5. Section ribbons will form by cutting into the block.
6. Lock large hand wheel.
7. Using forceps, a tissue pick or your fingers grab each end of the ribbon to transport and float sections in 40-47 °C degree water bath.
8. Select a section that is free of bubbles and wrinkles.

9. Obtain the labeled slide that matches the block.
10. Insert slide into water bath and gently lift under selected section.
11. Place section on the middle of the slide and raise the slide out of the water bath.
12. Remove excess tissue from slide.
13. Place slide into a staining rack.
14. Float a Kimwipe over the water bath to remove excess sections and paraffin.
15. When staining rack is full or sectioning is complete then the slides are ready to be deparaffinized.

6.6 Sectioning with Automation (Leica Microtome RM2255)

1. Follow procedures 6.2-6.5 along with the following steps.
2. Turn Leica RM2255 on or off by switching the power to the 1 (on) or 0 (off) position. Switch is located on back of microtome. A beep indicates microtome is on.
3. Use the Separate Control Panel to operate the microtome.
4. The microtome can be operated with full automation or semi-automation.
5. Select automatic mode by pushing cut mode button until the LED light indicates CONT for continuous motion of large hand wheel. (Additional modes are discussed in the Instruction Manual (REF 10.4), sections (5.1-5.9)
6. Adjust knob to select sectioning speed. 1 is the slowest setting and 10 is the fastest setting. Usual sectioning speeds are between 8 and 10.
7. Push TRIM/SECT button to select a TRIM of facing μm depth; push it again to select a SECT or sectioning μm depth.
8. Switch between TRIM and SECT as needed to face blocks off and to section ribbons.
9. Use – and + buttons to adjust μm depth up or down. Suggested settings are 30 μm for TRIM and 5 μm for SECT.
10. Use the coarse feed buttons to move the specimen towards and away from the knife. Double arrows indicate fast movement. Single arrows indicate slow movement.
11. To engage automation, unlock large hand wheel. Push Run/Stop plus Enable buttons simultaneously. Be sure there is clearance between the knife and the block before engaging. The block should advance slowly. The operator may increase speed of automation as needed.
12. To stop automation push Run/Stop or Enable buttons.
13. See Leica RM2255 Rotary Microtome Instruction Manual for more program settings and maintenance (REF 10.4).

6.7 Clean up

1. Remove and dispose of blade, or place the blade safety guard over the blade.
2. Empty water bath into sink.
3. Remove shavings from microtome by vacuum or paint brush.
4. Seal blocks by quickly touching them to a hotplate.

5. Put blocks in the block filling tray. The blocks are sorted by test type (Barron, Biopsy, Madison) and then filed numerically by date in appropriate storage trays.
6. Clean microtome and workstation with paraguard solution and Wyp-all.

7 Interpretation of results

1. A "good section" on a slide is one that contains no bubbles, wrinkles or knife lines.
2. An "acceptable section" is one that contains a few small bubbles or small wrinkles
3. A "poor section" contains large bubbles, wrinkles, or knife lines in the tissue that will make it difficult to read under a microscope and shall be resectioned.
4. The section shall fully represent the embedded tissue.
5. "Chatter" or folds in the tissue are not acceptable.
6. Changing the blade frequently will prevent poor sections.

8 Report of results - NA

9 Procedure notes

9.1 Details and helpful hints

1. If a tissue section has not been on a slide too long, it can be removed from the slide and re-oriented by floating it back into the waterbath and then catching it again with the slide. A pick and/or forceps can be used to try to tease wrinkles out of the section.
2. The specimen holder and knife holder can be cleaned of paraffin build up by physical or chemical means.
 - a. Physical
 - i. Remove the holders from the microtome and place in a 60 degree incubator until the paraffin has liquefied.
 - ii. Spray holder with Parapel and wipe with a Wypall.
 - b. Chemical
 - i. Spray holder with Parapel and wipe with a Wypall.
3. BRAIN TISSUE must be picked up by a slide relatively soon after being floated on the waterbath, since the brain tissue may come apart with heat and hydration.

9.2 Limitations of procedure

1. There can be bubbles or wrinkles in a section that are virtually impossible to eliminate. If the tissue remaining in the block is getting low, it may be necessary to take a section with bubbles or wrinkles.

10 References

1. Luna, Lee G., editor. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. McGraw-Hill: USA 1968;1-258.
2. Leica Microsystems.. Nussloch GmbH . Instruction Manual Leica RM2125 Rotary Microtome; V2.1 English – 03/2006 (MAN-P-3).

3. Leica Microsystems.. Nussloch GmbH . Instruction Manual Leica RM2235 Rotary Microtome; V1.2 English – 02/2007 (MAN-P-7).
4. Leica Microsystems.. Nussloch GmbH . Instruction Manual Leica RM2255 Rotary Microtome; V1.3 English – 02/2005 (MAN-P-8).

11 Summary of Current Revisions

1. Sections 5.5.1, 6.6.4.6, 7.3, 7.4, 9.9.1.1, and 9.2.1: changed all indirect wording.

12 Supplemental Information

12.1 Quick Procedure Reference

Sectioning

1. Fill waterbath with RO/DIH₂O and turn on.
2. Remove Histo-Cool tray from freezer and add RO/DIH₂O.
3. Label slides.
4. Place blocks on shelving unit.
5. Face off blocks.
6. Place blocks on Histo-Cool tray.
7. Section blocks.
8. Place section on slide.
9. Place slide in staining rack.
10. Seal blocks.
11. Clean up work station.

12.2 Flow Diagram - NA

12.3 Manufacturer's Information

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