



Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document

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| <i>Number</i> | PTRIMMINGPR |
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Wisconsin Veterinary Diagnostic Laboratory

Standard Operating Procedure

1 Introduction

Tissue trimming involves slicing fixed tissue uniformly to fit into cassettes prior to tissue processing (PPROCESSVIP5). After trimming, the tissue is processed and infiltrated with liquid paraffin before sectioning (PSECTIONING).

2 Specimen submission

2.1 Type

1. Tissues fixed in 10% neutral buffered formalin.
2. Tissues fixed in 70% ethanol.
3. Eye samples may be placed in Davidson's fixative for 24 hours and then transferred to 10% neutral buffered formalin for long term storage.

2.2 Special requirements for collection

1. Tissue samples are received from the Wisconsin Veterinary Diagnostic Laboratory (WVDL) Sample Receiving already logged into UVIS (AANSPTLOGIN) and will have a WVDL accession number. A portion of tissues arrive fixed in formalin, others arrive fresh and are fixed on site.

2.3 Handling conditions

1. Formalin must be stored at room temperature – do not freeze.

2.4 Criteria for rejection of sample

1. Tissue that is inadequately fixed.
2. There must be enough tissue to proceed with trimming.

3 Materials

3.1 Equipment & Instrumentation

1. Laminar Flow Workstation
2. Sakura Tissue-Tek Stainless steel cassette basket with cover
3. Plastic storage container
4. Leica cassette printer IP-C
5. Grossing blade
6. Forceps
7. Cutting board
8. Formalin waste container
9. Strainer
10. Stainless steel cassette lids
11. Pencil

3.2 Reagents & Media

1. Acetic Acid, Glacial
2. Ethanol, 100%
3. Formaldehyde, 37%
4. Neutral buffered formalin, 10%

3.3 Supplies

1. WypAlls

2. Surgipath high-profile microtome blades
3. Surgipath embedding cassettes – white, yellow, green, pink
4. Surgipath microbiopsy cassettes– white, yellow, green, pink
5. Biopsy sponges
6. Sharps container
7. Biohazard bags and container

4 Safety Management

4.1 Required Safety Training:

- Chemical fume hood operation - mandatory when working with Xylene
- Hazardous chemical use and disposal training

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

-  **Acetic Acid, Glacial** (MSDS-27)
 - DANGER! CORROSIVE. COMBUSTIBLE LIQUID AND VAPOR.
 - Causes severe burns to eyes, skin, and respiratory tract.
 - Harmful or fatal if swallowed.
 - Harmful if inhaled. May be harmful if absorbed through the skin.
 - Keep away from heat, sparks, and open flame.
-  **Ethanol 100%** (MSDS-261)
 - Flammable liquid and vapor
-  **Formaldehyde** (MSDS-282)
 - Flammable liquid and vapor. Cancer hazard. Poison, may be fatal or cause blindness if swallowed. Cannot be made non-poisonous. Toxic by inhalation, in contact with skin and if swallowed. Causes burns by all exposure routes. Vapor harmful. May cause an allergic skin reaction. May cause central nervous system effects.
-  **Neutral Buffered Formalin, 10%** (MSDS-198)
 - Strongly irritating to skin, eyes, respiratory tract, and mucous membranes. Poison. May cause allergic reactions. May cause permanent eye damage. Harmful if inhaled. Harmful if absorbed through skin, causes general tissue damage. Causes eye burns.
 - Ingestion fatal or may cause blindness.

B. Biological: Biosafety Level 2

C. Sharps: In order to prevent injury, colored tape is available to apply to the dull side of the microtome blade as a reference. Be sure to properly orient the blade each time you press down with the blade.

4.4 Disposal of waste

See PFORMALINREDUCT, PPATHRETAIN and PNECWASTEDISP

5 Preparation for procedure

5.1 Equipment and instrumentation preparation

Place a stainless steel cassette basket inside a plastic storage container and add enough formalin to completely cover the stainless steel cassette basket. Open the histology database (H:\Path\Shortcut to Histology.accde).

5.2 Reagents and media preparation

- 10% neutral buffered formalin
- Davidson's Fixative:
 - Two parts 37% formaldehyde
 - Three parts 100% ETOH
 - Three parts tap water
 - One part glacial acetic acid

5.3 Standards/controls preparation - N/A

5.4 Specimen preparation

Tissues must have fixed in 10% formalin for at least 24 hours. Neurologic tissue such as brain or spinal cord may take longer. Tissue should be trimmed as indicated by the pathologist or when completely fixed.

6 Performance of procedure

6.1 Cassette color coding

- Use Surgipath Cassettes:
 - Pink – Biopsy
 - White – Madison H&E
 - Green – Barron H&E
 - Yellow – Special Stain -- Positive Controls
 - Orange – NWHC H&E

6.2 Label cassettes manually

1. Obtain appropriate accession number from the pathology submission form.
2. Obtain appropriate color and type of cassette
3. Use a #2 pencil
4. Write the accession number on the front of the angled portion of each cassette, (ie.M09-12345).
5. No further numbering required for cases that have only 1 cassette.
6. For cases that have more than one cassette add a “-“ followed by incremental numbering, (-1, -2, -3)
7. For cases that have more than one animal, the letter “A” is added to the first animal, the letter “B” is added to the second animal and so on.
8. For cases that have both multiple animals and cassettes will be numbered with the animal designation (A), followed by the cassette number (1 to 5).

6.3 Label cassettes with Leica Cassette Printer

1. Obtain appropriate accession number from the pathology submission form.
2. Load cassettes with angled portion facing up into the Leica cassette printer magazines.
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 (M011-, B11-, Gram) from the drop down menu.
6. Type in the accession number if applicable, (i.e. 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. For cases that have more than one cassette:
 - a. Enter the number that you would like the labeling to begin within the "1st Position" field.
 - b. Enter the number of auto-incremented cassettes needed in the "quantity" field.
 - c. Select the "N" button for numeric printing.
9. For cases that have more than one animal:
 - a. Enter the letter that you would like the labeling to begin within the "1st Position" field.
 - b. Enter the number of auto-incremented cassettes needed in the "quantity" field.
 - c. Select the "A" button for alphabetic printing.
10. For cases that have both multiple animals and cassettes:
 - a. Enter the letter that you would like the labeling to begin within the "1st Position" field.
 - b. Enter the number of auto-incremented cassettes needed in the "quantity" field.
 - c. Select the "A" button for alphabetic printing.
 - d. Enter the number that you would like the labeling to begin within the into the "2nd Position" field.
 - e. Enter the number of auto-incremented cassettes needed in the quantity field.
 - f. Select the "N" button for numeric printing.
11. Select the appropriate magazine from the Cassette Magazine field or select the manual feed function.
12. Enter the number of identical duplicate cassettes needed in the "Duplicates" field.
13. Click the "Cassettes" button to print cassettes.
14. Transport the printed cassettes to the trim workstation.
15. Additional information is needed when printing positive control cassettes.
 - a. Select the appropriate Prefix from the drop down menu.

1. **AFB**

- a. Fites – Acid Fast Bacteria Fite’s Method (PFITESST)
- b. Acid Fast Bacteria Ziehl Neelsen Method (PZNACIDFASTST)
2. **ALC**
 - a. Alcian Blue pH=2.5 (PALCIANBLST)
 - b. Alcian Blue pH=2.5 with PAS (PALCNBLPASST)
3. **AMYL**
 - a. Congo Red Benholds Method (PCONGOREDST)
 - b. Sulfated Alcian Blue (PSABST)
4. **Bili**
 - a. Bilirubin Hall’s Method (PHALLBILIST)
5. **BSS**
 - a. Bielschowsky’s Silver Stain (PBSSST)
6. **Ca**
 - a. Calcium VonKossa’s Method (PVKOSSACAST)
7. **Cu**
 - a. Copper Rhodanine Method (PRHODANINEST)
 - b. Copper Howell’s Rubeanic Method (PRUBEANICST)
8. **EL**
 - a. Verhoeff/Van Gieson Elastic (PELASTICST)
9. **Fe**
 - a. Perl’s Iron (PPERLSIRONST)
10. **FM**
 - a. Fontana-Masson (PFONT-MASSONST)
11. **GMS**
 - a. Grocott’s Methenamine Silver (PGROCOTTFUNGIST)
12. **Gram**
 - a. Brown and Hopps Modification (PBROWNHOPPSST)
 - b. Giemsa (PGIEMSAST)
13. **GRF**
 - a. Gomori’s Stain for Reticular Fibers (PGORETICST)
14. **GTC**
 - a. Gomori’s One Step Tri-chrome (PTRICHORMEST)
15. **HBFP**
 - a. Hematoxylin / Basic Fuchsin / Picric Acid (PHBFPST)
16. **LUNA**
 - a. Luna’s Method (PLUNAST)
17. **LFB**
 - a. Luxol Fast Blue (PLUXOLFBST)
 - b. Luxol Fast Blue w/Cresyl Violet (PLUXOLFBST)
18. **MUCI**

- a. Sigma-Aldrich ACCUSTAIN Mucicarmine Method (PMUCIST)
- 19. **MS**
 - a. Revised Modified Steiner (PSTEINREVMODST)
- 20. **OKJ**
 - a. Okajima Method (POKAJIMAST)
- 21. **PAS**
 - a. Period Acid Schiff (PPASST)
- 22. **PTAH**
 - a. Phosphotungstic Acid Hematoxylin (PPTAHST)
- 23. **PVK**
 - a. Pierce-Vanderkamp (PPVKST)
- 24. **TB**
 - a. Toluidine Blue (PTOLUIDINEST)

6.4 Trim tissue (general)

1. Retrieve sample jar from the externally vented cabinet that is designated for short term storage.
2. Match the WVDL accession ID from the primary container to the pathology submission form, which is stored electronically in the folders labeled by month and year, on "Archive on `wvdlfs`"
3. Strain the tissue and capture the fixative.
4. Examine each tissue for abnormalities such as lesions, edemas, discolorations, etc.
5. Using a blade, slice a representation of each piece of tissue in the jar. Include any abnormalities.
6. Tissues should not be greater than the thickness of the cassettes or larger than the cassette itself.
7. Log each accession into the Histology Database (H:\Path\Shortcut to Histology.accde), under the Necropsy tab, via the Trim Sheet option. The Histology Daily Task Summary (FM-P-21) can also be used during trimming to record information prior to entry into the Database.
 - a) Log in each accession with the WVDL Received Date, Accession, Test Type (i.e. Madison Diagnostic), Species, and Pathologist (case coordinator).
 - b) In the Trim Info portion, enter the number of cassettes, tissues, any pertinent comments (i.e. If a pathologist has marked any tissues, then indicate in which cassette the tissues are contained), trim initials, and the trim date (auto-populates today's date).
 - c) In the Fixed Tissue Storage portion, enter the bin number the samples will be stored in, the storage type (i.e. Regular, Long-Term Hold, etc.). The 60 day disposal date will automatically populate based on the trim date.
 - d) Add-ons

- i) Accessions with add-ons are treated as another entry in the histology database. To document, go to the Necropsy tab, and Add-ons option in the histology database.
 - ii) Enter the accession which has add-ons in the pop up box.
 - iii) On a new line, enter the number of add-on cassettes, tissues, put "Add-ons" in the comments box along with any additional comments (i.e. the start number of the add-on cassette for printing purposes), the trim initials, and the trim date.
8. Place tissue in a properly labeled cassette and apply lid.
 9. Place cassette(s) in the stainless steel basket inside of a container filled with the appropriate fixative.
 10. Return the remaining tissues and fixative to the original jar and store.
 11. Store full bins in the externally vented cabinets in room 1204.
 12. Transfer the container holding the cassettes to the Histology lab.

6.5 Trim brain tissue

1. If brain is not designated as a "neuro" case, take sections outlined in blue and yellow from Figure 1 and put in 3-6 cassettes depending on the size of the brain.
2. If path form or jar is noted as "neuro or neurological", the whole brain will be bread loafed and laid out to look for any obvious lesions. If any questions or concerns arise, call the case pathologist. If no lesions are grossly found, only include sections outlined in the red boxes from Figure 2 (4-7 cassettes).
3. Fetal brains need a maximum of two cassettes.

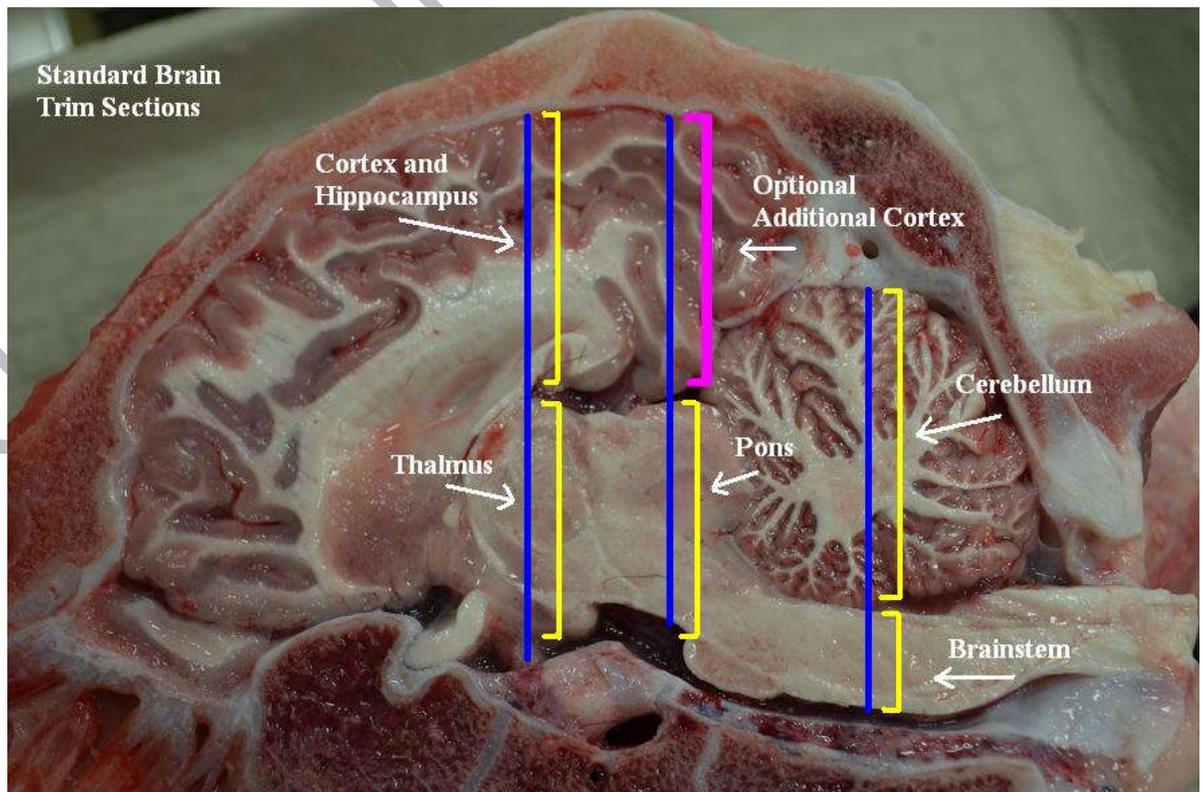
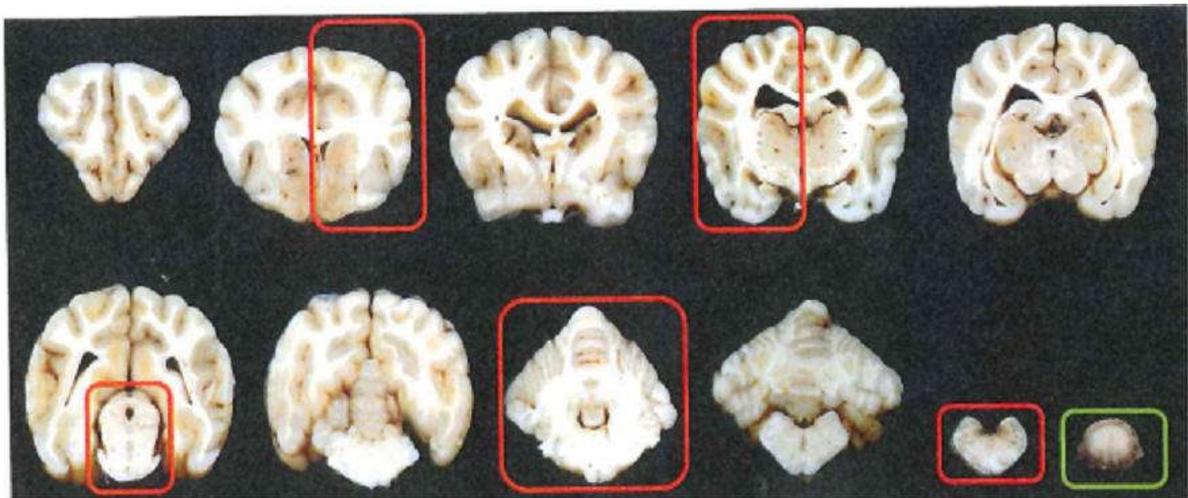


Figure 1: Photograph taken by Delwyn Keane, WVDL, March 2014.



Brain sections for neurologic cases. Breadloaf, lay out and look for obvious lesions. Take sections as shown in red boxes unless otherwise noted.

Figure 2: Image taken from reference 10.3

7 Interpretation of results - N/A

8 Report of results - N/A

9 Procedure notes

9.1 Details and helpful hints

- A good cassette is one in which all tissues fit uniformly.
- Tissues may touch each other, but must not overlap
- Tissue should be smoothly trimmed, devoid of knife marks, and representative of the tissue submitted.
- Tissue that is not properly fixed will have a pink/red hue and should be allowed to properly fix prior to processing.
- Use a sharp blade on autolytic and fetal tissue.
- Be extremely careful when trimming, the blades used are very sharp.
- When you are not sure what or how to trim a particular tissue, ask the pathologist assigned to the case for advice.
- Keep the number of tissues in the cassettes to a reasonable amount.

9.2 Limitations of procedure

- Tissue must be properly fixed prior to trimming.
- Tissue must fit into the desired cassette.

10 References

1. Vandeveld Marc, Higgins Robert, Oevermann Anna Chapter 1 General Neuropathology. In: Lang Johann, Wiesner Eric, editors. *Veterinary*

Neuropathology Essentials of Theory and Practice. 1st edition. Wiley-Blackwell: West Sussex, UK 2012; page 12-13.

11 Summary of Current Revisions

1. Section 5.5.1: Added opening the histology database
2. Section 6.6.4: Changed wording to incorporate new histology database entry of trim information and disposal information. Also incorporated Add-on entry in the histology database.

12 Supplemental Information

12.1 Quick Procedure Reference

1. Retrieve sample jar from designated area.
2. Match the WVDL accession ID from the primary container to the WVDL accession ID on the WVDL histology submission form.
3. Strain the tissue and capture the formalin
4. Examine each tissue for abnormalities such as lesions, discolorations etc.
5. Using blade, slice a good representation of each piece of tissue in the jar. Include any of the above abnormalities.
6. Log each accession into the Histology Database under the Necropsy tab, via the Trim Sheet option.
7. Label each cassette with automatic printer or pencil.
8. Count the number of cassettes and tissues, and document.
9. Place tissue in a cassette and apply lid.
10. Place tissues in formalin container.

12.2 Flow Diagram – N/A

12.3 Manufacturer's Information - N/A