



Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document

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Wisconsin Veterinary Diagnostic Laboratory

Standard Operating Procedure

1 Introduction

This protocol is to establish general guidelines for handling controls that are derived from animal tissue submitted to WVDL. Histology special stains and TSE IHC utilize internally produced controls from previously diagnosed cases. This procedure will describe the sources of material, handling, testing and validation for positive controls used in these tests.

2 Specimen submission

2.1 Type

Formalin fixed tissues that exhibit a characteristic that is distinguishable when a certain procedure is performed on it.

2.2 Special requirements for collection – N/A

2.3 Handling conditions

Formalin fixed tissue must be handled in a chemical safety hood.

2.4 Criteria for rejection of sample

Tissue, blocks or slides that do not possess the desired characteristics for a procedure are not acceptable.

3 Materials

3.1 Equipment & Instrumentation

- A. Slide storage box

3.2 Reagents & Media

- A. 10% Neutral Buffered Formalin - LabChem

3.3 Supplies

- A. Tissue cassettes
- B. Microscope slides

4 Safety Management

4.1 Required Safety Training:

- Chemical fume hood operation-Mandatory use when working with formalin fixed tissue
- 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
- Foot: Lab dedicated shoes or disposable shoe covers in TSE lab

4.3 Hazard Communication

A. Chemical: See MSDS

10% Neutral Buffered Formalin (**SDS-198**)

- o Health Hazards - Acute- 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.

- Health Hazards - Chronic- Ingestion (swallowing) Fatal or may cause blindness.

B. Biological: Biosafety Level 2 for Histology Biosafety Level 2 plus for TSE

4.4 Disposal of waste

See PNECWASTEDISP, PPATHRETAIN and PSTORAGEPRP.

5 Preparation for procedure

5.1 Equipment and instrumentation preparation – N/A

5.2 Reagents and media preparation – N/A

5.3 Standards/controls preparation

Control slides will be cut to the desired thickness and mounted on the slide according to the corresponding stain being performed.

5.4 Specimen preparation

Diagnostic slides will be cut to the desired thickness and mounted on the slide type according to the corresponding procedure being performed.

6 Performance of procedure

6.1 Histology Special Stains

A. Control Selection

- a. Pathologists suggest good controls based on their diagnostic cases.
- b. Their suggestions will be documented via the “Control Tissue Recommendation” on the pathologist screen in the Histology database (<H:\Path\Shortcut to Histology.accde>).
- c. Use the procedure listed in 6.1. Cut one section and affix to a blue slide that is labeled with the corresponding control block ID and stain with the desired procedure.
- d. A pathologist will interpret the quality of the staining and determine fitness for use as a control. Document in the Histology database.
- e. If this slide is deemed acceptable, then cut the number of slides determined in 6.1.E.
- f. If this slide is deemed unacceptable, then troubleshoot the procedure to determine if a non-conformance caused the outcome. Follow ANONCONFORM to document if necessary. If a non-conformance is not found, then do not use that block.

B. Tissue Trimming

- a. Retrieve the jar using the [fixed tissue storage log](#).
- b. Trim in the appropriate number of cassettes containing the tissue of interest according to PTRIMMINGPR.

- c. Label cassettes with the control type abbreviation <dash> letter (starting with A). E.g. the first control block for B&H and Giemsa would be Gram-A.
- d. Document completion of the task in the [Histology database](#).

C. Processing

- a. Process according to PPROCESSVIP5.

D. Embedding

- a. According to PEMBEDDING.

E. Sectioning

- a. Label blue slides according to the control block ID: the quantity is based upon the average number stained per year.
- b. Serial sections will be cut with the first tissue on slide #1 until the desired number is obtained. E.g. Gram-A-001 through Gram-A-100.
- c. Slides will be stored in clearly labeled slide boxes.
- d. Document in the [Histology database](#).

F. Validation Staining to acquire a stain set

- a. Stain the first and last slide of the control set, following one of the approved staining methods, to identify the target. E.g. Use the PRHODANINEST or PRUBEACUST method to identify the presence of copper in the control tissue. Document in the [Histology database](#).
- b. Pathologist will view the slides and determine if the staining is acceptable or not acceptable and document in the [Histology database](#).
- c. If both slides are acceptable and documentation is complete the remainder of the slides can be used as validated special stain controls.
- d. If only the first slide is acceptable, then the Histology technician will grossly examine the slide set for evidence of where the target area of the tissue has been exhausted. That slide will be stained and given to the Pathologist again for examination. If the slide is deemed acceptable then that will be the last slide of the validation set and subsequent slides will be discarded. If the slide is still not acceptable, then repeat.
- e. If only the last slide is acceptable, then the Histology technician will grossly examine the slide set for evidence of where the target area of the tissue is present. That slide will be stained and given to the Pathologist again for examination. If the slide is deemed acceptable then that will be the first slide of the validation set and the prior slides will be discarded. If the slide is still not acceptable, then repeat.
- f. If neither of the controls is acceptable, then consult with the supervisor to determine the root cause and troubleshoot. If no resolution is found then another control tissue or block will need to be validated.

- g. Document the troubleshooting in the [Histology database](#).

G. Routine staining with a valid stain set

- a. A special stain is requested by the pathologist through the Histology database.
- b. Histology technician pulls the corresponding diagnostic block.
- c. An appropriate control slide is pulled and its identity is documented in the Histology Database.
- d. Slides are printed for the diagnostic cases with the following info:
 - i. Accession Number
 - ii. Block Number
 - iii. Pathologist
 - iv. Stain Type
 - v. Corresponding control number
- e. Diagnostic slides are cut at the thickness and stained as stated in the corresponding SOP.

H. Retention

- a. Control blocks are retained in cassette drawers based on their status and stored according to PPATHRETAIN.
 - i. **Under Review** - Blocks from cases that were suggested by a Pathologist that are being stained by the desired procedure for Pathologist review to be deemed acceptable or not acceptable.
 - ii. **Approved** – Blocks deemed acceptable by a pathologist and may be used for control slide sets.
 - iii. **Archived** – Blocks that have been exhausted.
- b. The corresponding diagnostic blocks are filed according to date received in the histology lab.
- c. Control slides will be filed alpha-numerically by control type in slide drawers. (PPATHRETAIN)
- d. Diagnostic slides will be filled numerically by accession number.

6.2 TSE IHC

A. Control Selection

- a. Pathologists suggest good controls based on diagnostic cases.
- b. Their suggestion will be documented via “Control blocks” located in the [TSE database](#).

B. Tissue Trimming

- a. Locate the corresponding jar from fixed tissue storage and retrieve.
- b. Trim in the appropriate number of cassettes containing the tissue of interest. See PTRIMPRPIHC.
- c. Label cassettes with the designated control letter.

OR

- d. Locate the previously embedded tissue block.
 - e. Melt the wax to retrieve the tissue of choice and re-embed according to PEMBEDDINGPRP into a pink cassette labeled with the control letter.
- C. Processing
- a. Process according to PPROCESSPRPVIP5.
- D. Embedding
- a. According to PEMBEDDINGPRP.
- E. Sectioning (PSECTIONINGPRP)
- a. Label charged pink slides according to the control block ID.
 - b. Serial sections will be cut starting with the first tissue on slide #1 until the tissue in the block is exhausted. E.g. Z-001 through Z-200.
 - c. Slides will be stored in clearly labeled slide boxes.
 - d. Documentation will be in the [TSE Database](#) .
- F. Validation Staining
- a. Stain the first and last slide of the control set according to PSTAINVPRPIHC. Document in the TSE database.
 - b. Pathologist will view the slides and determine if the staining is acceptable or not acceptable and document in the [TSE database](#).
 - c. If both slides are acceptable and documentation is complete the remainder of the slides can be used as validated controls.
 - d. If only the first slide is acceptable, then the slide set will be grossly examined for evidence of where the target area of the tissue has been exhausted. That slide will be stained and given to the pathologist again for examination. If the slide is deemed acceptable then that will be the last slide of the validation set and subsequent slides will be discarded. If the slide is still not acceptable, then repeat.
 - e. If only the last slide is acceptable, then the slide set will be grossly examined for evidence of where the target area of the tissue is present. That slide will be stained and given to the Pathologist again for examination. If the slide is deemed acceptable then that will be the first slide of the validation set and the prior slides will be discarded. If the slide is still not acceptable, then repeat.
 - f. If neither of the controls is acceptable, then consult with the supervisor to determine the root cause and troubleshoot. If no resolution is found then another control tissue or block will need to be validated.
 - g. Documentation of the troubleshooting will be maintained in the [TSE database](#).
- G. Routine staining with a valid stain set
- a. The appropriate control slide is pulled and its identity is documented in the TSE Database prior to staining.

b. Staining is completed according to PSTAINIVPRPIHC.

H. Retention

- a. Control blocks are retained in cassette drawers based on their status and stored according to PPATHRETAIN.
 - i. **Under Review** - Blocks from cases that were suggested by a Pathologist that are being stained by the desired procedure for Pathologist review to be deemed acceptable or not acceptable.
 - ii. **Approved** – Blocks deemed acceptable by a pathologist and may be used for control slide sets.
 - iii. **Archived** – Blocks that have been exhausted following control.
- b. Diagnostic blocks are filed numerically.
- c. Control slides will be filed alpha-numerically in slide drawers.
- d. Diagnostic slides will be filled numerically by accession number.

7 Interpretation of results

A. Histology Special Stains

- a. Pathologist reviews the control slide prior to the diagnostic slide.
- b. Pathologist or designee documents whether the control slide is valid “Yes or No”, their initials and any additional comments in the Histology database.
- c. Pathologist will return slides to the histology lab for long term storage.

B. TSE IHC

- a. Pathologist reviews the control slide prior to the diagnostic slide.
- b. Pathologist documents whether the control slide is “valid” or “not valid”, and any additional comments in the TSE Database.
- c. Pathologist will return slides to the TSE lab for long term storage.

8 Report of results– N/A

9 Procedure notes– N/A

9.1 Details and helpful hints– N/A

9.2 Limitations of procedure– N/A

10 References

- A. AAVLD Pathology Committee, Anatomic Pathology Quality Assurance Guidelines, 2009

11 Summary of Current Revisions

- A. Section 6: Minor grammar changes.

12 Supplemental Information– N/A

- 12.1 Quick Procedure Reference
- 12.2 Flow Diagram
- 12.3 Manufacturer’s Information