

APPENDIX A
BEST MANAGEMENT PRACTICES FOR HANDLING OF
POTENTIALLY CWD PRION-CONTAMINATED WASTE

Appendix A contains Best Management Practices (BMPs) which have been developed by the Colorado Division of Wildlife for the Foothills Wildlife Health Laboratory (WHL) as part of the laboratory Standard Operating Procedures (SOPs). The BMPs contained in Appendix A of this permit have been excerpted from the larger SOP document and are enforceable requirements under this Permit. Page numbers along the left margin refer to the page number in the WHL SOP document where this requirement is found.

The following Standard Operating Procedures (SOPs) are compliant with the SCS/NIH Biosafety Level 2 section of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines. The SOPs require that the SOP document is available to all personnel working in the WHL, and that laboratory personnel read, understand and comply with relevant sections of these SOPs when working in the Wildlife Health Laboratories (WHL). According to the SOPs, compliance with biosafe practices is the mandatory responsibility of each person using the WHL facilities.

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A. NECROPSY LABORATORY

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2. Entry and Exit Procedures

b. Entry

- i. The following protective clothing should be worn while performing necropsies on carcasses or extraction of tissue samples:
 - Coveralls
 - Plastic disposable apron
 - Rubber boots or disposable plastic boots
 - Latex/nitrile gloves
- ii. The following protective clothing should be worn when handling containerized tissue samples:
 - Lab coat or coveralls
 - Rubber boots or disposable plastic boots
 - Latex/nitrile gloves

c. Exit

i. Boots

- 1) Rubber boots are to be rinsed free of any organic material, and the entire surface to be scrubbed with 5% LpH while standing in 5% LpH boot bath before exiting lab.
- 2) Rubber Boots are to be removed after exiting and placed in designated storage area where they will remain in wet contact with 5% LpH for 30 minutes.
- 3) Disposable plastic booties are to be removed at point of exit and disposed of in appropriate waste container within the lab.

ii. Clothing

- 1) Plastic apron is to be removed before exiting and disposed of in appropriate waste container within lab.
- 2) Coveralls are to be removed after exiting and hung in designated storage area if clean, or placed in container for washing if necessary.

iii. Hands

- 1) After removing apron and scrubbing boots, gloves are to be removed before exiting and disposed of in appropriate waste container within lab.

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3. Decontamination Procedures

b. Instruments

- i. All instruments used in the lab for necropsy or tissue dissection need to be decontaminated. This includes
 - Scalpel handles
 - Scalpel blades
 - Thumb forceps
 - Tissue scissors
 - Grapefruit knives
 - Knives
 - Cleavers
 - Pliers
 - Cutting boards
 - Hand saw

ii. Instrument Decontamination Procedure

- 1) Contaminated instruments are to be placed in a 5% LpH solution immediately after use to soak for >30 minutes.
- 2) Blades are removed from scalpel handles and placed in sharps container.
- 3) Instruments are then placed in an Inspector's Choice solution for >5 minutes, scrubbed to remove all tissue debris, and rinsed in lab sink. All water from the sink empties through a double layer of mesh covering the drains.
- 4) Instruments are then rinsed thoroughly with water and placed into 5% LpH solution again for >30 minutes.

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A. NECROPSY LABORATORY

c. Surfaces

- i. Necropsy laboratory surfaces used regularly include
 - necropsy tables
 - floor (including cooler and freezer)
 - walls (including cooler and freezer)
 - shelves
 - carts and plastic bins
 - cement dropoff pad & walkway
 - trailers & trucks beds

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- ii. **Surface Decontamination Procedures**

- 2) Contaminated surfaces are to be coated entirely with 5% LpH solution using a pressurized hand sprayer or airchem machine and left to decontaminate for >30 minutes of wet contact time. Personnel are recommended to leave the laboratory during this time to avoid inhaling irritating fumes.

d. Drains

- i. Laboratory drains, each covered with wire mesh screen, include:
 - 3 sink drains, covered with 2 layers of wire mesh
 - floor trench drain on south wall of lab, covered with 1.5 mm mesh
 - floor trench drain in center of cement pad, covered with 1.5 mm mesh

- ii. **Drain Decontamination Procedure**

- 1) **Sink Drains**

- a) Hose is used to spray inside of sink and push all debris into drain screen.
- b) Screens containing debris are removed and replaced with clean, decontaminated screens on a weekly basis, or more often if needed.
- c) Used screens are then cleaned out and all debris is deposited in appropriate tissue waste container.
- d) Used screens are placed in 5% LpH solution for >30 minutes then rinsed.
- e) Screens can be re-used after cleaning and decontamination.

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- 2) **Trench drains**

- a) Water and debris on floor/cement pad is squeegeed into trench drain.
- b) Debris is collected from the wire mesh covering the drains and deposited in appropriate waste container.
- c) Trench drain is then sprayed with 5% LpH and left to decontaminate for >30 minutes wet contact time before allowed to dry.

e. Spills

- i. **Biological spills** include blood or tissue on the floor or dissecting tables, or other laboratory surfaces.

- 1) **Biological Spill Decontamination Procedure**

- a) Affected area should be decontaminated as a surface according to Part A, Section 3.c.ii of the SOP
- b) Spill area can then be cleaned with the rest of the laboratory surfaces according to Part A, Section 3.c.ii of the SOP.

- ii. **Chemical spills** include LpH, formalin, or other chemicals that occur on persons or clothing.

- 1) **Chemical Spill Decontamination Procedure**

- a) Chemical spills should be prevented from entering any floor or sink drains, or any other conduit to the septic system.
- b) Wipe up spill with appropriate absorbent material and dispose of materials in accordance with Federal, State, and local regulations and requirements.

A. NECROPSY LABORATORY

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4. Maintenance

b. Daily Maintenance

- i. Daily cleaning and decontamination is the responsibility of personnel working in the facility.
- ii. The necropsy laboratory is to be cleaned and decontaminated after use as described in Part A, Section 3 of the SOP.
- iii. Temperature and water usage logs (Appendix III) are to be updated on a daily basis according to lab use.
- iv. Chemicals are to be dated upon purchase, and recorded in chemical usage log (Appendix III) when opened.
- v. All waste containers are to be emptied on a daily basis, and replaced with clean garbage bags. Waste is to be disposed of daily according to Part A, Section 7.e of the SOP.
- vi. The interior of the walk-in cooler and freezer will be cleaned and decontaminated on a weekly basis, or more often if necessary.
- vii. Necessary repairs to the necropsy facility, including freezer, cooler, drain mesh, or shelving will be addressed immediately and repaired on an as-needed basis.

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c. Weekly Maintenance

- i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:
 - Disposable gloves, aprons, and boots
 - Paper towels
 - Garbage bags
 - Ziploc bags
 - Whirlpacs (sample bags)
 - Inspector's Choice Detergent
 - LpH
- ii. Screens covering trench drains and sink drains will be inspected and decontaminated on a weekly basis.
- iii. Footbath and instrument bath containing 5% LpH solution will be changed at the start of each week.

d. Monthly Maintenance

- i. Water usage log, found in Appendix III, is to be totaled and updated at the beginning of each month.
- ii. Chemical usage logs, found in Appendix III, are to be updated and totaled on a monthly basis for the following chemicals
 - LpH
 - Inspector's Choice Detergent
 - Form-C (formalin)
 - Bleach (Sodium Hypochlorite)

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5. Handling of Formalin and Formalin-Fixed Tissues

b. General Formalin Handling Guidelines

- i. Always use adequate ventilation when handling formalin to avoid direct inhalation:
 - Turn ceiling vent on
 - Open windows and overhead doors
 - Set up portable fan
- ii. Coveralls, eye protection, Nitrile/latex Gloves, and rubber boots always be used when handling formalin.
- iii. Always dilute formalin according to manufacturer's recommendations on label.

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- iv. Formalin spills must be handled as a chemical spill according to 3.e.ii of Part A.
 - c. **Storage of Formalin**
 - i. Concentrated formalin is to be stored in the manufacturer's 1-gallon container on a designated shelf within the lab.
 - ii. Diluted formalin is to be stored in a closed 5-gallon carboy on accessible shelf in lab.
 - iii. Used formalin is to be stored in a sealed carboy or 1-gallon container until disposed.
 - iv. Instruments used to cut in tissues must be decontaminated according to Part A , Section 3.b.ii of the SOP.
 - e. Formalin material safety data sheet (MSDS) must be available to laboratory personnel.

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6. Sample Extraction Procedures for CWD Surveillance

- c. **General CWD Sampling Guidelines**
 - iv. Instruments that are to be re-used for more than one animal must be rinsed in 5% LpH.
 - v. After sampling instruments and surfaces are to be decontaminated according to Part A, Section 3 of the SOP.
- d. **Head Sample Extraction Procedures**
 - i. Begin by placing the head on a layer of lab matting atop the necropsy table ...
- e. **Carcass sample extraction procedures**
 - iv. Handle any leakage of body fluids onto the floor as a biological spill according to Part A, Section 3.e.1.

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7. Waste Disposal

c. Tissue Disposal

- i. **CWD Tissues**
 - 2) Any laboratory surfaces contacted by potential CWD suspect tissues must be decontaminated according to Part A, Section 3.c of the SOP.
 - 3) Small bits of tissue (greater than 1 gm) are to be collected and placed in designated tissue waste containers (large red garbage cans) for disposal according to Part A Section 7.b.2.
 - 4) Body parts and large pieces of tissue are to be collected and placed in designated tissue waste containers or trailer for disposal according to Part A Section 7.b.2.

B. MOLECULAR BIOLOGY LABORATORY

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1. General Guidelines

- iv. Laboratory SOP must be available within laboratory to personnel on duty.
- v. MSDS for every chemical used in laboratory must be available to personnel on duty.
- vi. Always remove protective clothing and wash hands before exiting lab.

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c. Biosafety Cabinet (BSC)

- i. All manipulation of tissues that may potentially cause splattering or aerosolization must be performed in the BSC.
- iii. The working surface of the BSC is to be covered with absorbent, plastic-lined lab matting, with plastic side down. The lab matting must be changed at the end of the day, between tissue types, or more frequently if spilled on or contaminated.

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2. Entry and Exit Procedures

b. Entry

- i. The following protective clothing should be worn at all times in the molecular biology laboratory:
 - lab coat
 - latex/nitrile gloves
- ii. A current logbook must be posted near entry to the lab containing a record of all spills or personnel exposure requiring medical attention. A permanent record is necessary due to the long incubation time of TSEs.

c. Exit

- i. Lab coat, eye protection, and gloves are not to be worn outside the laboratory, and must be removed before exiting.

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3. Decontamination Procedures

b. Instruments

- i. All instruments used for tissue preparation or assays must be decontaminated. This includes:
 - Thumb forceps
 - Scalpel handles
 - Cutting boards
 - Blender
- ii. **Instrument decontamination procedures**
 - 1) Contaminated instruments are to be placed in a 5% LpH bath immediately after use to soak for >30 minutes.

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c. Equipment

- i. All equipment used for tissue preparation or assays must be decontaminated. This includes:
 - Pipettes
 - Scales
 - Incubators
 - Electrophoresis boxes
 - Blotter
 - Vortexes
 - Centrifuges

B. MOLECULAR BIOLOGY LABORATORY

ii. Equipment decontamination procedure

- 1) All items that may have become contaminated must be wiped down after use with a 5% LpH solution with >30 minutes of wet contact time before allowed to dry.
- 2) After each use, electrophoresis boxes should be rinsed with 5% LpH solution and left for >30 minutes of wet contact time, then rinsed with water.

d. Surfaces

i. Molecular biology laboratory surfaces include:

- Biosafety cabinet (BSC)
- Countertops
- Refrigerator
- Floor

ii. The BSC, countertops, and refrigerator surfaces must be decontaminated on a daily basis when the lab is in use.

iii. The floor must be decontaminated when spills or other contamination occurs.

iv. Surface decontamination procedure

- 1) The biosafety cabinet must be wiped down on the inside with a 5% LpH solution and, after >30 minutes wet contact time, left to dry. Surfaces may be wiped with water after 30 minutes if a film remains. Glass is to be wiped with a 70% isopropyl alcohol solution.
- 2) Countertops are to be decontaminated in a manner proven to inactivate prions.
- 3) Refrigerator doors are to be wiped with a 5% LpH solution that is to remain wet on the surface for >30 minutes.
- 4) Floor is to be decontaminated with 5% LpH only in occurrence of a spill. See Part B, Section 3.f of the Molecular Biology lab SOP for cleanup of spill.

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e. Fluids

i. All fluids used in CWD tissue processing or assays must be decontaminated. This includes:

- Used buffers
- Pipette contaminated liquids

ii. Fluid decontamination procedure

- 1) Small volumes (<50 ml) of contaminated liquids are to be decontaminated in a manner proven to inactivate prions. For example:
 - Place fluids in a 50 ml disposable plastic tube (w/ cap) containing ~10 ml concentrated LpH for >30 minutes, then dispose of in biohazard waste container.
- 2) Large volumes (>50 ml) of contaminated liquids are to be decontaminated in a manner proven to inactivate prions. For example:
 - Add concentrated LpH to fluids to a minimum of 5% total fluid, and let sit for >30 minutes in covered container. Fluids may then be washed down the drain with running water.

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f. Spills

i. Spills Decontamination procedures

- 1) Affected area should be wetted immediately using 5% LpH from a squirt bottle; enough LpH should be used to adequately cover the entire spill.
- 2) Spill area should then be covered with paper towels and left for >30 minutes of wet contact time.

B. MOLECULAR BIOLOGY LABORATORY

- 3) If spill occurs in the biosafety cabinet, the fan should run during the 30-minute period.
 - 4) Contaminated materials are to be placed directly into biohazard waste container.
 - 5) After spill has been removed, the entire area or in such cases, the entire biosafety cabinet, must be wiped down with 5% LpH and, after >30 minutes of wet contact time, allowed to dry.
- iv. Spills that occur on persons or clothing should be handled as an emergency according to Part E, Section B of the SOP.

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4. Maintenance

b. Daily Maintenance

- i. Daily cleaning and decontamination is the responsibility of personnel working in the laboratory.
- ii. The laboratory is to be cleaned and decontaminated after use as described in Part B, Section 3 of the SOP.
- v. Chemical usage must be noted on log every time a new liter of bleach is opened, or liter of LpH solution is made, according to Part H of the SOP.

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c. Weekly Maintenance

- i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:
 - Latex/Nitrile gloves
 - Paper towels
 - Biohazard bags
 - Bleach (sodium hypochlorite)
 - LpH

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5. Handling of Tissues

b. CWD Tissues

- i. Both fresh and frozen tissues must be contained in a sample cup or bag until processed.
- ii. Frozen tissues should be kept in freezer until processing, and returned to freezer immediately after processing to maintain integrity of tissue.
- v. Any procedures that could potentially aerosolize or splatter tissues, such as grinding or blending, must be performed in the biosafety cabinet.

C. DNA/PCR LABORATORY

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1 General Guidelines

- vi. Laboratory SOP must be available to personnel on duty.
- vii. MSDS for every chemical used in laboratory must be available on lab shelf to personnel on duty.

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2. Entry and Exit Procedures

b. Entry

- i. Gloves should be worn at all times when working in the DNA/PCR lab.
- ii. A lab coat and eye protection are recommended when working in the DNA/PCR lab.

c. Exit

- i. Lab coat, eye protection, and gloves are not to be worn outside the laboratory, and must be removed before exiting.

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3. Decontamination Procedures

b. Decontamination Solvents

- i. LpH, or another agent proven effective at the inactivation of prions, used according to label instructions, and in the concentration and wet exposure time verified to inactivate prions.
- ii. Eliminase must be used to decontaminate any instruments, equipment, surfaces, or fluids that come into contact with DNA or RNA.

c. Instruments & Equipment

- i. Instruments and equipments used in the DNA laboratory include:
 - pipettes
 - Disposable pipettes
 - Scales
 - Incubators
 - Vortexes
 - Centrifuges
 - Thermal cycler
 - UV camera
 - Agarose gel electrophoresis machine
 - Light microscope

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- ii. All instruments and equipment used in the DNA lab must be decontaminated or disposed of according to use.
- iii. Decontamination procedures
 - 1) Disposable instruments used in the DNA lab are to be placed in a biohazard waste container to be autoclaved.
 - 2) Non-disposable instruments are to be decontaminated using appropriate solvent according to Part C, Section 3.b of the SOP.

d. Surfaces

- i. DNA laboratory surfaces include:
 - Biosafety cabinet (BSC)
 - Countertops
 - Refrigerator & freezer
 - Floor
- ii. **Surface Decontamination Procedure**
 - 1) Biosafety cabinet is to be wiped on the inside with appropriate solvent according to Part C, Section 3.b of the SOP.

C. DNA/PCR LABORATORY

- 2) Countertops are to be wiped with appropriate solvent according to Part C, Section 3.b of the SOP.
- 3) Refrigerator freezer doors are to be wiped with appropriate solvent according to Part C, Section 3.b of the SOP.
- 4) Floor is to be decontaminated with appropriate solvent only in occurrence of a spill. See Part C, Section 3.f of the DNA laboratory SOP for cleanup of spills.

Page 40 **e. Fluids**

- i. Fluids and their containers must be properly disposed of. This includes:
 - Buffers
 - Blood
 - Serum tubes
 - EDTA tubes
 - DNA vials
 - Syringes
- ii. **Fluid Decontamination Procedures**
 - 1) All containers holding biological fluids (blood, urine, etc.) must be placed in a biohazard waste container to be autoclaved.
 - 2) CWD-contaminated fluids must be treated according to Part B, Section 3.e of the SOP.
 - 3) Non-biological fluids may be poured down the sink with running water. Note: Bleach should NOT be poured down the sink unless heavily diluted with running water, as it can affect the septic system.

f. Spills

- i. **Spill Cleanup Procedure**
 - 1) Personnel must always wear gloves when cleaning up a spill.
 - 2) Affected area should be covered immediately with paper towels, absorbed, and dried. Spill area should then be treated with LpH, or another agent proven effective at the inactivation of prions, used according to label instructions, and in the concentration and wet exposure time verified to inactivate prions and left to dry.
 - 3) If spill occurs in the biosafety cabinet, the fan should run during cleanup.
 - 4) Materials used during cleanup are to be placed directly into biohazard waste container.
- ii. Spills that occur on persons or clothing should be handled as an emergency according to Part E of the SOP.
- iii. All spills involving potential exposure must be recorded in laboratory logbook, including the date, time, agents of exposure, and names of personnel exposed.

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4. Laboratory Maintenance

b. Daily Maintenance

- i. Daily cleaning is the responsibility of personnel working in the laboratory.
- ii. The laboratory is to be cleaned after use as described in Part C, Section 3 of the SOP.

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c. Weekly Maintenance

- i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:
 - Latex/Nitrile gloves
 - Paper towels
 - Biohazard bags
 - Bleach (sodium hypochlorite)

C. DNA/PCR LABORATORY

- Eliminate

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5. Handling and Storage of Biological Samples

b. Handling of Biological Samples

- ii. All biological samples must be handled wearing latex/nitrile gloves.
- iii. Surface should be covered with lab matting before handling samples.
- iv. Tubes containing blood and/or blood products are to remain capped during transport and centrifugation.
- v. Used tubes that are either empty or still contain blood products are to be placed in biohazard waste container.

c. Storage of Biological Samples

- i. All biological samples are to be properly labeled with contents, sample # and date before storage in the refrigerator or freezer.
- ii. CWD samples may be stored in the -70oF freezer or refrigerator located in the DNA lab and should be double packaged. CWD samples MUST NOT be removed from their packaging within the DNA laboratory; they must be taken into the molecular biology laboratory.

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6. Laboratory Waste Disposal

b. Biological samples

- i. Any excess, leftover, or processed biological samples and their containers are to be placed in a biohazard waste container.

E. EMERGENCIES AND EXPOSURES OF PERSONNEL TO HAZARDOUS MATERIAL

b. Exposures of Personnel to Hazardous material

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iii. Spills

- 1) Spills must be contained, cleaned up and decontaminated immediately to avoid unnecessary exposure to laboratory personnel.
 - If spill occurs on personnel, immediately remove contaminated clothing according to Part E, Section b.iii.5 and follow the SOP below.
 - For spills that occur in the necropsy laboratory, please refer to Part A, Section 3.e of the SOP.
 - For spills that occur in the microbiology laboratory, please refer to Part B, Section 3.f of the SOP.

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5) Contaminated Garments

- a) If protective clothing becomes contaminated during a procedure or spill, immediately remove garment and squirt contaminated area with 5% LpH.
- b) Place garment in biohazard waste container, don new garments as necessary, and continue spill decontamination procedures.