STATE OF COLORADO

Bill Owens, Governor DEPARTMENT OF NATURAL RESOURCES

DIVISION OF WILDLIFE

AN EQUAL OPPORTUNITY EMPLOYER

Bruce McCloskey, Director 6060 Broadway Denver, Colorado 80216 Telephone: (303) 297-1192

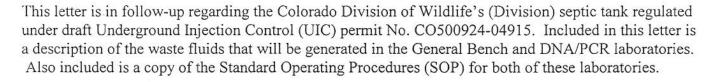


November 6, 2006

TO: Valois Shea
U.S. Environmental Protection Agency
Region 8, 8P-W-GW
999 18th Street, Suite 300
Denver, CO 80202

RE: Underground Injection Control (UIC)
Draft Area Permit No. CO500924-04915
Colorado Division of Wildlife
Wildlife Health Laboratory
4330 LaPorte Avenue
Fort Collins, CO 80521

Dear Ms. Shea,



The General Bench laboratory is used for processing of biological samples that are collected during field, management and research projects. These biological samples include blood, feces, swab and tissue samples taken from a variety of wildlife species. Processing includes centrifugation, splitting and repackaging of samples or tissues, and freezing of samples.

The General Bench laboratory is also used for running diagnostic tests or assays. These include such tests as fecal analysis, enzyme linked immunosorbent assay (ELISA), rapid plate agglutination (RPA), cytology and microscopic examinations of fresh tissues or smears.

The DNA/PCR laboratory is used to extract DNA from biological samples that are collected during field, management and research projects. These procedures generate aliquots of DNA for archiving and genetic testing. The DNA/PCR laboratory is also used to run polymerase chain reaction (PCR) assays for various diseases (West Nile virus). Other assays that are run in the DNA/PCR laboratory include agarose and electrophoresis gels.



The waste fluid from both of these laboratories is limited to routine hand washing, general instrument and equipment clean-up. Please refer to the enclosed SOP for more detailed descriptions of activities that occur in each of the laboratories. Decontamination methods are outlined in the SOP for each laboratory. You will note that any contaminated materials or fluids are disposed of via solid waste methods or transferred to the necropsy laboratory for disposal.

The only reagents or fluids that enter the waste stream from the two laboratories are occasional buffer solutions from commercial assay kits. These solutions were detailed in our meeting of March 29, 2005 and have not changed. The volume of solution that enters the waste stream varies over time but is limited to less than 30 ml at a time.

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Please feel free to contact me if you have any questions regarding this communication.

Sincerely,

Laurie A. Baeten, DVM Wildlife Health Group

Colorado Division of Wildlife



B. General Bench Laboratory

1. Title-General Guidelines

a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of general guidelines, including use of the biosafety cabinet, to be followed at all times. All laboratory activities are managed by the Wildlife Health Laboratory Working Group Leader. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Guidelines

- i. Access to the laboratory is restricted to authorized personnel only.
- ii. No food or drink is permitted within the laboratory.
- iii. GB laboratory instruments MAY NOT be used in the DNA/PCR laboratory.
- iv. A BL2 biohazard sign must be posted on entry door to lab, indicating the agents in use, laboratory working group leader, and contact numbers.
- v. MSDS for every chemical used in laboratory must be available to personnel on duty. MSDS are located in Appendix VI of the SOP, as well as in the DNA/PCR laboratory.
- vi. Always remove protective clothing and wash hands before exiting lab.
- vii. Shut down biosafety cabinet and turn off lights before exiting lab at the end of the day.



c. Biosafety Cabinet (BSC)

- i. All manipulation of tissues that may potentially cause splattering or aerosolization must be performed in the BSC.
- ii. The blower and light are to be turned on prior to beginning any work in the BSC.
- iii. The working surface of the BSC is to be covered with absorbent lab matting. The lab matting must be changed at the end of the day, between tissue types, or more frequently if they become contaminated.
- iv. The BSC must be certified on an annual basis. BSC certification sticker is located on the hood.

d. Wildlife Health Laboratory Working Group Leader

• Dr. Laurie A. Baeten, DVM

Office: 970-472-4478

Cell: Pager:

Home:



B. General Bench Laboratory

2. Title- Entry and Exit Procedures

a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of the proper entry and exit procedures. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Entry

- i. The following protective clothing should be worn at all times in the GB laboratory:
 - Lab coat
 - Eye protection
 - Latex/nitrile gloves
 - Close-toed shoes

c. Exit

- i. Lab coat, eye protection, and gloves are not to be worn outside the GB laboratory, and must be removed before exiting.
- ii. Hands must be washed with anti-bacterial soap before exiting laboratory.



B. General Bench Laboratory

3. Title- Decontamination Procedures

a. Purpose:

To inform personnel using the WHL GB laboratory of the decontamination procedures for instruments, equipment, surfaces, fluids and spills. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Instruments

- i. All instruments used for tissue preparation or assays must be decontaminated. This includes:
 - Thumb forceps and scissors
 - Scalpel handles and blades
 - Cutting boards
 - Blender

ii. Instrument Decontamination Procedure

- 1) Blades are to be removed from scalpel handles using pliers. Blades are to be placed in approved sharps container.
- Instruments that are potentially contaminated with CWD tissues are to be placed in a 5%Environ LpH[®] bath immediately after use to soak for 30 minutes.
- 3) Instruments are to be placed in Inspectors Choice[®] bath for 5 minutes and scrubbed to remove tissue debris.
- 4) Instruments are then to be rinsed thoroughly with tap water and placed in drying rack.
- 5) Instruments are to be returned to appropriate storage location when dry.

c. Equipment

i. All equipment used for tissue preparation or assays must be decontaminated. This includes:

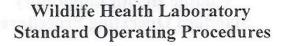


- Pipettors
- Scales
- Vortexes
- Centrifuges
- ii. Equipment Decontamination Procedure
 - 1) All items that may have become contaminated must be wiped down after use with a 10% bleach solution and left to dry. Use squirt bottles for dispensing bleach to avoid aerosolization.

d. Surfaces

- i. General bench 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 is to be wiped on the inside with a 10% bleach solution and 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 wiped with a 50% bleach (sodium hypochlorite) solution and left to dry.
 - 3) Refrigerator doors are to be wiped with a 10% bleach solution.
 - 4) Floor is to be decontaminated with 10% bleach in the occurrence of a spill. See section (B.3.f.) of the GB lab SOP for cleanup of spills.





e. Fluids

- i. All fluids used in CWD tissue processing or assays must be decontaminated. This includes:
 - Used buffers
 - Pipet contaminated liquids
- Fluid Decontamination Procedure
 - 1) Small volumes (30-40 ml) of contaminated liquids must be treated by one of the following procedures:
 - Place fluid in a 50 ml disposable plastic tube (w/cap) containing ~10 ml concentrated Environ LpH[®] for 30 minutes, then dispose of in biohazard waste container.
 - Place fluid in 50 ml disposable plastic tube (w/cap) containing ~25 ml concentrated bleach (sodium hypochlorite) for 1 hour and dispose of tube in biohazard waste container.
 - 2) Large volumes (>40 ml) of contaminated liquids must be treated by one of the following procedures:
 - Add concentrated Environ LpH[®] to fluid to a minimum of 5% of total fluid volume. Leave fluid for 30 minutes in covered container. Fluid may then be flushed down the drain in the WHL necropsy laboratory with a copious amount of water.
 - Add concentrated bleach (sodium hypochlorite) equal in volume to contaminated liquid, achieving a 50% bleach solution. Leave fluid for 1 hour in covered container. Fluid may then be flushed down the drain in the WHL necropsy laboratory with a copious amount of running water.



f. Spills

- ii. Spills Decontamination Procedure
 - Affected area should be wetted immediately using 10% bleach from a squirt bottle; enough bleach solution should be used to adequately cover the entire spill.
 - 2) Spill area should then be covered with paper towels and left for 30 minutes.
 - c) If spill occurs in the biosafety cabinet, the fan should run during the 30-minute period.
 - d) Contaminated paper towels are to be placed directly into biohazard waste container.
 - e) After spill has been removed, the entire area must be wiped down with 10% bleach and allowed to dry.
- iii. Spills that occur on persons or clothing should be handled as an emergency according to section (E.) of the SOP.
- iv. All spills requiring medical attention must be recorded in laboratory logbook, including the date, time, agents of exposure, and names of personnel exposed.



B. General Bench Laboratory

4. Title- Maintenance

a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of the proper daily, weekly, and annual maintenance procedures. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

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 section (B.3.) of the SOP.
- iii. Biohazard bags are to be checked daily and if full, removed and autoclaved at 121 °C for 1 hour. Autoclaved bags are to be packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.
- iv. Necessary repairs to the refrigerator, freezer, biosafety cabinet, or other laboratory equipment will be addressed immediately and repaired on an as-needed basis.
- v. The temperature log is to be updated on a daily basis according to lab use (Appendix III).
- vi. Chemical usage must be noted in log every time a logged chemical container is opened (Appendix III).

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)
- Deionized H₂0
- ii. Items needed for specific laboratory procedures may be added to the supply list.
- iii. Laboratory floor should be mopped with anti-bacterial cleaner (such as Lysol®) on a weekly basis.

d. Annual Maintenance

- i. Biosafety cabinet must be inspected and certified annually to maintain proper certification.
- ii. Certification is performed by: Technical Safety Services, Inc.



B. General Bench Laboratory

5. Title- Handling of Tissues

a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of the proper procedures for handling and preparing tissues. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Chronic wasting disease (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.
- iii. Tissues must be removed from sample bag/cup one at a time and returned to original sample bag/cup.
- iv. Cutting and weighing of CWD tissues may be done on an open countertop.
- v. Any procedure that could potentially aerosolize or splatter tissue, such as grinding or blending, must be performed in the biosafety cabinet.

c. Non-CWD Tissues

i. Other tissues should be handled with the same precaution as CWD tissues, unless otherwise directed by the laboratory working group leader.



B. General Bench Laboratory

- 6. Title- Assay Procedures
 - a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of the standard assays performed in the lab, and the proper protocols. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

- See Appendix V for specific protocols.
- a. ELISA This is an enzyme-linked immunosorbest assy (ELISA). Most common assay used in the general bench laboratory for diagnostic testing.
- **b.** RPA This is a rapid plate agglutination assay (RPA). Most common assay used in the general bench laboratory for diagnostic testing.



B. General Bench Laboratory

7. Title-Laboratory Waste Disposal

a. Purpose:

To inform personnel using the WHL General Bench (GB) laboratory of how to properly dispose of tissues, equipment, sharps, and other lab waste. Most of the work conducted in the WHL GB laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Tissues

- i. Any excess, leftover, or processed tissues in the laboratory must be treated with 5%Environ LpH solution for 30 minutes, and placed in a biohazard bag.
- All biohazard bags are autoclaved at 121 °C for 1 hour and packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.

c. Fluids

- i. Uncontaminated assay reagents or buffers may be disposed of in closed containers and placed into biohazard bags. Uncontaminated buffers may be flushed down the drain with supervisor approval.
- ii. Used decontamination fluids, including 5% Environ LpH and 50% bleach (sodium hypochlorite), are to be flushed down the trench drain in the WHL necropsy laboratory with copious amounts of water.

d. Equipment

i. Equipment and solid laboratory materials that may be disposed of after use in the laboratory includes:



- Pipette tips
- Tubes and lids
- Disposable pipettes
- Biopsy punches
- Assay plates
- Gloves and other disposable handling materials
- iii. Contaminated disposable equipment should be placed in biohazard bags.
- iv. All biohazard bags are autoclaved at 121 °C for 1 hour packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.

e. Sharps

- i. Sharps used in the laboratory include:
 - Disposable scalpels
 - Scalpel or razor blades
 - Hypodermic needles
 - Biopsy punches
- ii. Used scalpel blades are to be removed from blade handle using pliers and placed into an approved sharps container.
- iii. Used needles, razor blades and biopsy punches are to be placed into approved sharps containers.
- iv. Full sharps containers are to be filled with 10% bleach and allowed to soak overnight. The liquids are to be strained and decanted.
- v. The sharps containers are to be permanently sealed and then autoclaved at 121°C for 1 hour and packed into a cardboard box labeled as 'decontaminated sharps' and disposed of in the facility dumpster.



C. DNA/PCR Laboratory

1. Title- General Guidelines

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of general guidelines to be followed at all times. All laboratory activities are managed by the Wildlife Health Laboratory Working Group Leader. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Guidelines

- i. Access to the DNA/PCR lab is restricted to authorized personnel only.
- ii. No food or drink is permitted within the lab.
- iii. Always wash hands with anti-bacterial soap prior to exiting the DNA/PCR lab.
- iv. All CWD tissues except for blood must be processed in the general bench laboratory for processing.
- v. PCR/DNA lab instruments MAY NOT be used in the General Bench laboratory.
- vi. DNA/PCR Laboratory SOPs 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.

c. Wildlife Health Laboratory Working Group Leader

Dr. Laurie A. Baeten, DVM

Office: 970-472-4478
Pager:

Cell: Home:



C. DNA/PCR Laboratory

2. Title- Entry and Exit Procedures

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of the proper entry and exit procedures. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Entry

- 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 DNA/PCR laboratory, and must be removed before exiting.
- ii. Hands must be washed with anti-bacterial soap before exiting the DNA/PCR laboratory.



C. DNA/PCR Laboratory

3. Title- Decontamination Procedures

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of the decontamination procedures for instruments, equipment, surfaces, fluids and spills. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Decontamination Solvents

- 50% bleach (sodium hypochlorite) must be used to decontaminate any instruments, equipment, surfaces, or fluids that come into contact with CWD or infectious tissue or bodily fluids.
- 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 equipment used in the DNA/PCR laboratory include:
 - Pipettes
 - Disposable pipettes
 - Scales
 - Incubators
 - Vortexes
 - Centrifuges
 - Thermal cycler
 - UV Camera
 - · Agarose gel electrophoresis
- ii. All instruments and equipment used in the DNA/PCR laboratory must be decontaminated or disposed of according to use.



iii. Decontamination Procedures

- 1) Disposable instruments used in the DNA/PCR laboratory are to be placed in a biohazard waste container.
- 2) Non-disposable instruments are to be decontaminated using methods described in section (C.3.b.).
- 3) Sharps are to be disposed of in approved sharps container.

d. Surfaces

- i. DNA laboratory surfaces include:
 - Biosafety cabinet (hood)
 - Countertops
 - Refrigerator & freezer
 - Floor

ii. Surface Decontamination Procedure

- 1) Biosafety cabinet is to be wiped on the inside with appropriate solution listed in section (C.3.b.) of the SOP. Glass is to be wiped with a 70% isopropyl alcohol solution.
- 2) Countertops are to be wiped with appropriate solution listed in section (C.3.b.) of the SOP.
- 3) Refrigerator freezer doors are to be wiped with appropriate solution listed in section (C.3.b.) of the SOP.
- 4) Floor is to be decontaminated with 50% bleach in the occurrence of a spill. See section (B.3.f.) of the SOP for cleanup of spills.

e. Fluids

Fluids and their containers must be properly disposed of. This includes:



- Buffers
- Blood
- Serum tubes
- EDTA tubes
- DNA vials

ii. Fluid Procedures

- 1) All containers holding biological fluids (blood, urine, etc) must be placed in a biohazard waste container.
- 2) CWD-contaminated fluids must be treated in accordance to section (B.3.e.) of the SOP.
- 3) Approved assay reagents or buffers may be poured down the sink with running water.

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 wiped with 50% bleach, 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 section (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.



C. DNA/PCR Laboratory

4. Title- Laboratory Maintenance

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of the proper daily, weekly, and annual maintenance procedures. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

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 section (C.3.) of the SOP.
- iii. Full biohazard waste containers are to be autoclaved at 121 °C for 1 hour. Autoclaved bags are packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.
- iv. Necessary repairs to the refrigerator, freezer, biosafety cabinet, or other laboratory equipment will be addressed immediately and repaired on an as-needed basis.

c. Weekly Maintenance

- 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)
 - Eliminase
 - Viricide



- ii. Items needed for specific laboratory procedures may be added to the supply list.
- iii. Laboratory floor should be mopped with anti-bacterial cleaner on a weekly basis.

d. Annual Maintenance

- i. Biosafety cabinet must be inspected and approved annually to maintain proper certification.
- ii. Certification is performed by: Technical Safety Services, Inc.



C. DNA/PCR Laboratory

5. Title- Handling and Storage of Biological Samples

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of the proper procedures for handling and storage of tissues. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Handling of Biological Samples

- All biological samples must be handled wearing latex/nitrile gloves.
- ii. Surfaces should be covered with lab matting before handling samples.
- iii. Tubes containing blood and/or blood products are to remain capped during transport and centrifugation.
- iv. 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 number and date before storage in the refrigerators or freezers.



C. DNA/PCR Laboratory

6. Title-Laboratory Waste Disposal

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of how to properly dispose of tissues, equipment, sharps, and other lab waste. Most of the work conducted in the WHL DNA/PCR laboratory is related to ongoing field studies of wildlife populations and may involve infectious agents, some of which may potentially cause disease in humans. These guidelines are necessary to limit personnel exposure and transmission of infectious agents.

b. Biological Samples

- i. Any excess, leftover, or processed biological samples and their containers are to be placed in a biohazard waste container.
- ii. All biohazard bags are autoclaved at 121 °C for 1 hour and then packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.

f. Equipment

- i. Equipment that may be disposed of after use in the laboratory includes:
 - Pipette tips
 - Tubes, vials and lids
 - Disposable pipettes
 - Assay plates
- ii. Disposable equipment must be placed directly into a biohazard waste container.
- iii. All biohazard bags are autoclaved at 121 °C for 1 hour and then packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.



g. Sharps

- i. Sharps used in the laboratory include:
 - Scalpel or razor blades
 - Hypodermic needles
- ii. Sharps are to be placed in approved sharps container after use.
- iii. Full sharps containers are to be disposed of a listed in section (B.7.e) of the SOP.

h. Other lab waste

- Laboratory waste that may have come into contact with biological samples, such as soiled gloves or lab matting, must be placed in a biohazard waste container. All biohazard bags are autoclaved at 121°C for 1 hour and then packed into a cardboard box labeled as 'decontaminated materials' and disposed of in the facility dumpster.
- Other laboratory waste, such as packaging materials, may be placed into a regular waste container.



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