



VA Pittsburgh Healthcare System Animal Research Facility: Operating Procedures

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Part I: Introduction

Standard Operating Procedures for Animal Research Facility Operated by the VA Pittsburgh Healthcare System

This VA Medical Center Animal Research Facility Standard Operating Procedure (SOP) is a reference for investigators, IACUC members, and staff. This SOP details the policies and procedures related to the care and use of laboratory animals within the Animal Research Facility.

Ethical Principles Governing the use of Animals in Research

Animal subjects contribute immeasurably to advancements in medical science. Most research and testing involving human patients is based on the results of animal experimentation. To provide hope for veterans suffering from diseases that currently lack cures or effective treatments, the VA actively supports the use of animals in research, teaching, and testing. However, the use of animals in VA research is a privilege granted with the understanding and expectation that such research is conducted according to the highest ethical and legal standards.

The Regulatory Mandates for Animal Experimentation

All animal care, husbandry, and animal research practices at VA animal facilities must be in accordance with applicable laws, regulations, and policy. The basic principles governing animal research in VA are found in the United States (U.S.) Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, which include the following imperatives:

- a. Animal experiments are undertaken only after due consideration of their relevance for human or animal health and the advancement of biological knowledge.
- b. The fewest number of animals needed to achieve scientific objectives is to be used.
- c. The least sentient species that will permit the attainment of research objectives is to be used.
- d. The least painful or distressful procedures needed to meet research objectives are to be used, and all reasonable measures to minimize pain and distress should be utilized.
- e. When planning and conducting studies, the principles of replacement, reduction, and refinement need to always be considered.
- f. Procedures that would be considered painful in a human need to be considered to be painful in an animal.
- g. The best possible living conditions need to be maintained for animals kept for research, training, or testing purposes. Animal care needs to be supervised by a veterinarian experienced in laboratory animal medicine. Housing needs to ensure that the general health of animals is safeguarded and that undue stress is avoided, with appropriate attention paid to environmental factors such as temperature, ventilation, and humidity.

h. Personnel need to have appropriate qualifications, training, and experience when conducting procedures on animals. Opportunities for hands-on training need to be provided as needed.

All animal research must comply with the Health Research Extension Act (codified at 42 U.S.C. Section 289d) and the Public Health Services (PHS) Policy. The PHS Policy includes the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (prepared by the Interagency Research Animal Committee), The Guide for the Care and Use of Laboratory Animals (prepared by the National Research Council); henceforth called the Guide and the Report of the AVMA Guidelines on Euthanasia. NOTE: Compliance with PHS Policy is mandated by VA policy, whether or not PHS funds are accepted by an individual VA facility. All animal research must be covered by a PHS Assurance. By law, all animal research must comply with the Animal Welfare Act [codified at 7 U.S.C. Sections 2131-2159, the USDA AWAR (Animal Welfare Act Regulations and Standards), Title 9 Code of Federal Regulations (CFR) Parts 1-4, and 42 CFR 73, Possession, Use, and Transfer of Select Agents and Toxins]. All VA animal research involving infectious or recombinant agents must also comply with guidelines found in the latest editions of the Centers for Disease Control and Prevention (CDC)-National Institutes of Health (NIH) publication entitled "Biosafety in Biomedical and Microbiological Laboratories" and the NIH publication entitled "NIH Guidelines for Research Involving Recombinant DNA Molecules."

Any research conducted within the VAPHS Animal Research Facility must be reviewed and approved by the VAPHS Institutional Animal Care and Use Committee (IACUC) and notification issued by the VAPHS Associate Chief of Staff for Research and Development (ACOS/R&D) before the project can begin. Please refer to the VAPHS IACUC SOPs for additional information regarding this process (Appendix A).

Definition of Animal Subject and Research

Animal research refers to any use of laboratory animals in research, testing, or training. The term "**animal**" is defined as any live vertebrate animal used or intended for use in research, research training, experimentation, or biological testing, or for a related purpose (see PHS Policy on Humane Care and Use of Animals, Sec. III). For the purpose of compliance with the Animal Welfare Act Regulations an animal is defined as any live or dead cat or dog, non-human primate, guinea pig, hamster, rabbit, or any other warm-blooded animal which is being used, or is intended for use in research, teaching, testing, or experimentation. The term excludes birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, and horses not used for research purposes and other farm animals, such as, but not limited to livestock or poultry, used or intended for use as food or fiber, or livestock or poultry used or intended for use in improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

Part II: Maintenance and Sanitation Procedures

A. General Maintenance and Sanitation Requirements

Note: Signs regarding contamination control policies/procedures are to be posted throughout the Animal Research Facility. The Animal Research Facility supervisor is responsible for this signage. See Appendix B for an example.

1. Mop buckets - After each use, (by room) are to be washed out with a mixture of hot water and Hypochlor, and then rinsed with hot water. Once a week the mop buckets and ringers are sanitized by hand with Quatricide and rinsed with hot water.

2. Mops - A clean mop head must be used for each animal room and for rooms that don't house animals. The same mop head cannot be used for each room if it has not been washed and dried using a washing machine and dryer.

3. Brooms and Dustpans – In order to prevent contamination, each room must have its own broom and dustpan.

4. Work Clothing and Shoes - All animal care personnel are required to wear scrubs while working in the Animal Facility. Prior to leaving the building, personnel are required to change into street apparel. The scrubs are to be laundered (using both the washer and dryer) at the Animal Research Facility. Personnel must have a separate pair of shoes specific for work.

5. Bedding - No bedding is to be accepted in bags that are or have been wet, broken or torn. Sawdust is unacceptable and forbidden as bedding for the animals.

6. Feed - Upon arrival, all bags of feed are to be inspected immediately for milling dates. These dates must be within 30 days prior to delivery. The feed must be used within the timeframes specified below:

- Monkey/Guinea pig chow - Must be used within 90 days of the milling date.
- Rat/Mouse/Rabbit/Dog chows - Must be used within 180 days of the milling date.

Feed in bags which are or have been wet, broken or torn cannot be accepted or used.

7. Soiled bedding removal- All soiled shoeboxes and cage pans are to be removed from the animal rooms and transferred to the dirty side of the cage washing area. The bedding is to be removed (scraping and dumping) and the shoeboxes and pans are to be sanitized in the cage washer. Soiled bedding is not to be removed and replaced with clean bedding unless shoeboxes are sanitized first. The frequency with which bedding is changed is outlined below:

Item	Frequency
Shoe box cages	Once per week*
Stainless Steel lids	Once per week*

Cage pans	Three times per week*
Cage racks and shelves	Bi-weekly*

* Frequency may increase depending on the condition of the animals (e.g., diabetic, breeding) and scraping procedures may be altered according to according to reason for quarantine.

Normal quarantine boxes are scraped in the cage washer room on separate days than regular rooms. Boxes in rooms that are under quarantine because of a known pathogen are scraped inside the animal room before removing.

B. Area-Specific Cleaning Procedures

1. Rooms GA 126 and GA 129:

- A. Shelves are to be wiped off once a week or more if needed. Everything on shelves is to be kept folded neatly.
- B. Plastic bags are to be kept folded neatly.
- C. Sink in room 26 is to be scoured once a week.
- D. Floors are to be swept and mopped once a week or more if needed.

2. Dead Animal Freezers and Disposal:

- A. Keep freezers clean inside and out at all times.
- B. Animal carcasses and tissues are sealed in leak proof plastic bag which are located next to the freezer in hallway 6D. They are bagged and placed in the freezer by the PI's tech and or animal care staff. They are kept in the freezer until an approximate weight of 22 pounds is met. They are then placed in a red Biohazard Waste bag by the animal care staff, then placed in Biohazard boxes not to exceed 50 pounds. Facility maintenance picks up on Monday, Wednesday and Fridays.

3. Stair Cases:

- A. Sweep and mop stair cases by rooms 18 and 5 once a month or more if needed.
- B. Wipe offhand railings also.

4. Hallways:

- A. Floors are to be swept and mopped everyday and as needed.

- B. Walls and ceiling are to be sanitized every three months using Quatricide.
- C. Accessories mounted on walls must be dusted twice a week and sanitized when doing walls.
- D. Overhead vents are to be dusted weekly and cleaned when doing walls.

5 Employee Lunch Room:

- A. Keep room clean at all times.
- B. Table, microwave, refrigerator, and cabinet must be free of food and drink spills.
- C. Sweep and mop floor once a week or more if needed.
- D. Air vents, door and doorframe are to be wiped off once a week.
- E. Lunchroom door should be kept closed at all times.

6. Ladies Locker Room 1A107:

The ladies locker room should be kept clean at all times. The following procedures should be conducted as necessary using Butcher's Disinfectant Spray, Quatricide, or Hypochlor.

Wipe off toilet, sinks, and counter top.

Wipe off mirrors.

Wipe off shower stall and toilet stall.

Wipe off lockers inside and out.

Empty trash containers.

Fill paper towel dispenser.

Wipe off air vents, door, and doorframe.

Sweep and mop floor.

7. Surgery Area:

Areas are checked daily for cleanliness. If an area has been used, the following is done:

A. Pre-op and Post-op Room:

1. Full sharps containers are disposed of, hazardous waste bags are emptied. Put a new sharps container in place.
2. Clean counter tops and all equipment using Quatricide. Floors are swept and mopped with Quatricide or Hypochlor.
3. Doors are to be wiped off with Quatricide.
4. Check surgical supplies and replace/order as needed.
5. Areas are sanitized every three months including ceilings and attachments.

B. Operating Rooms and Scrub Area

1. Before Surgery
 - a. All equipment should be checked to ensure proper working condition.
2. After Surgery
 - a. All flat surfaces are cleaned using Quatricide.
 - b. Sharps containers are disposed of if full and a new one installed and hazardous waste bags are disposed of.
 - c. All moveable equipment not needed is cleaned with Quatricide and removed from the room.
 - d. Floors are swept, and then mopped with Quatricide or Hypochlor.
 - e. Doors are cleaned with Quatricide.
 - f. Scrub sink is scoured with cleanser then polished with Stainless Steel Polish.
 - g. Rooms are sanitized every three months using Quatricide.

8. Procedure Room, Autoclave Room and Necropsy Room

- A. Check rooms daily for usage.
- B. If room has been used, check for full sharps (dispose of full containers and replace it with a new one) containers, and hazardous waste (dispose of used bags and replace them with new bags). Clean counter tops and equipment with Quatricide. Sweep and mop floors.
- C. Doors are to be wiped off.
- D. Room is to be Sanitized every three months using Quatricide.

9. Cage Washer Areas*:

A. Clean Side:

1. Floors are to be swept and mopped daily and as needed.
2. All equipment should be placed on shelves neatly, according to size.
3. All shelves are to be wiped off as needed and sanitized every 3 months.
4. All utility carts are to be sanitized daily.
5. Room is to be sanitized every three months using Quatricide.
6. Stainless steel on cage washer is to be polished with stainless steel polish once a week.
7. Floor drain is to be checked and cleaned daily.
8. Door and window is to be cleaned daily with glass cleaner.

B. Dirty Side

1. Floors are to be swept and mopped daily and as needed for all spills.
2. All equipment should be cleaned as used and replaced neatly.
3. Rubbish barrels are to be emptied and cleaned as needed and sanitized daily.
4. Room is to be sanitized every three months using Quatricide.
5. Stainless steel cage washer and sink (inside and out) is to be polished daily using stainless steel polish and cleaned and polished as needed.
6. Floor drain is to be checked and cleaned (swept and mopped) daily.
7. Doors are to be cleaned daily with Butcher's Disinfectant Spray, Quatricide, or Hypochlor.
8. The cage washer is to be checked and cleaned weekly, if necessary. The outside of the cage washer doors are to be cleaned and polished with stainless steel polish.

*** Safety glasses and/or face shield and ear protectors must be worn at all times.**

C. Laundry at the VA Pittsburgh Medical Center Animal Facility

1. Mop heads and rags

- A. Set dials on washing machine to high water level and hot water setting.
- B. Use one-half cup to three-fourth's cup of Tide detergent and five oz. of Clorox bleach.
- C. Allow water to fill half way before adding mops and rags.
- D. After completion of all cycles and machine turns off, reset for rinse and spin cycle.
- E. Mops and rags are then put in dryer for fifty to sixty minutes, depending on size of load.

2. Scrubs, lab coats and wraps (drapes)

- A. Set dials on washing machine to high water level and hot water settings.
- B. Use one-half cup to three-fourth's cup of Tide detergent.
- C. Allow water to fill half way before adding clothes.
- D. After completion of all cycles and machine turns off, reset for rinse and spin cycle.
- E. Clothes are then put in dryer for thirty to fifty minutes depending on size of load.

D. Cleaning and Maintenance of Equipment

1. Disinfection of Animal Transport Cartons

Animals are delivered to the receiving area at the end of the corridor (32c between rooms GA101 and GA 140). They are taken into the cage wash room (GA101) where the cartons are wiped off with Hypochlor (Sodium Hypochlorite). The cartons are then transported to the designated room where the animals will be housed.

2. Cleaning out the Cage washer

Preventative Maintenance

Daily	Prior to the initial start-up of the washer, inspect the interior of the wash compartment.
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*Keep in mind that some parts of the washer may still be very warm from prior use.

Inspect all of the jets and remove any debris from them by poking a small wire into the jet orifice. If debris is considerable, remove pipe plug located on header manifold and run pump manually to flush out jets. Remove any debris or broken glass found on or under the pump screen. Check around the door seals and remove any debris if found.

- Weekly When the washer is not in operation and is cool, clean water spots and stains from the exterior of the cabinet with a stainless steel cleaner. WD-40 or a similar penetrate oil works very well for this. Check all water, air and steam fittings for sign of leakage and repair as required.
- Monthly If the unit shows significant scale build-up , clean the cabinet interior with 'Lime-a-way", "Uri-solve" or some other similar rescaling product. When using any of these products, wear goggles and gloves as a minimum and any other protective gear as required by your safety supervisor. After rescaling the cabinet, rinse it with copious amounts of fresh water.
- Annually Apply approximately ONE OUNCE of good quality high temperature grease to the drive cylinder grease fitting. DO NOT OVER GREASE!
- As Required From time to time, fuses, seals, and other repair items will need to be replaced. Members of a trained staff should only perform these procedures. If need be, contact Schlyer Machine at 716-696-3171 and they will advise and/or provide maintenance assistance

3. Changing Shoebox Type Caging

- A. Take a cart with clean bedding-filled boxes, wire lids, filter lids (if needed), a bucket of mild Hypochlor in warm water, a clean rag, and exam gloves into the animal room.
- B. Take one box off of the shelf at a time and place on your cart.
- C. Wipe the shelf area off where the dirty box was with your Hypochlor rag.
- D. Transfer the animals from the dirty to clean box, fill the feeder, put on the clean water bottle (if available), and return the box to the shelf.
- E. Put fresh food in the feeders once a week or more often if necessary. Remove a small amount of dirty bedding, to include some feces, from each dirty cage and place this material into an empty (no bedding material) clean cage; this cage will be used for the sentinel rats.

- F. Repeat this procedure until the boxes are completed.
- G. Cage card holders must be changed once a month or more if necessary.
- H. Wipe off the tops of the shelves, the wheels, and any other empty spaces with your Hypochlor solution.
- I. Stack the dirty boxes and lids on a cart and take them to the dirty side of the cage washer room for scraping.
- J. Finish the room as instructed in your Husbandry Practices information.
- K. All procedures are subject to change according to the needs of the facility.

Part III. Animal Care

Animal Care

Rats

1. First thing every day, check cages to note the condition of the Animals, such as; deaths, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total lack of water and/or food consumption. Report any of these problems immediately to the Supervisor and/or investigator.
2. The bedding in the trays is to be at least once per week or more often as needed. Dirty bedding is removed on the dirty side of the cage washer.
3. The water bottles are to be checked every day and add with fresh water as needed. Check all cages for 'DO NOT WATER" labels. Be sure to return each bottle to its original cage.
4. The feed is to be checked every day and refilled if necessary. Check all cages for "DO NOT FEED" labels.
5. The water bottles are to be sanitized once a week.
6. The suspended wire rat cages are to be sanitized every two weeks or more often if needed. The log stating the dates of cage and room sanitation.
7. Room is to be sanitized every three months.
8. The floor is to be swept and mopped every day.
9. The feed barrel is to be wiped off every day. No loose feed is to be kept in barrel.
10. The plastic bags in the feed barrels are to be kept in good condition.
11. Air vent is to be wiped off every day.
12. Each rat cage must have an identification card with the following information: species, protocol number, investigator's name, department, date received, strain, sex, weight, date of birth, source and number of animals per cage.
13. Only items that are essential to the animal care of that room are stored in the animal room.
14. The floor drains are to be checked every day and flushed out if necessary.
15. The doors are to be wiped off every day.
16. The inventory is to be taken Monday, Wednesday, and Friday or more often if needed.

Cage Size and Weight Limits For Rats

Space allocations for a 19"x 10.5"x8" (143 sq.in. floor) Polycarbonate shoebox, as specified in the Guide for the Care and Use of Laboratory Animals are as follows:

- 1 Rat over 500g
- 2 Rats up to 500g each
- 3 Rats up to 400g each *
- 4 Rats up to 300g each *
- 5 Rats up to 200g each *

* The VAPHS Animal Care Facility recommends no more than 3 rats at 250g each per box. This does not apply to mothers with babies or to weanlings under 100g each.

Animal Care Nude and SCID Mice

1. Upon entering the mouse room, all personnel must wear a surgical mask, clean lab coat or gown, shoe covers and sterile surgical gloves when mice are handled,
2. The cages are to be changed and sanitized 1 time a week or more often if needed. Sanitation includes boxes and lids. Bedding is removed on the dirty side of cage washer.
3. The feed is to be checked every day and refilled when necessary. Check all cages for "DO NOT FEED" and "DO NOT WATER" labels.
4. The water bottles are to be checked every day. If necessary a new bottle of sterile water is given.
5. The shelves are to be wiped off every day.
6. The floors are to be swept and mopped every day.
7. The air vents are to be wiped off every day.
8. The doors are to be wiped off every day.
9. Each cage must have an identification card with the following information: species, protocol number, investigator's name, department, date received, strain, sex, weight, date of birth, sources and number of animals per cage.
10. The shelves are to be sanitized every two weeks or more if needed.
11. Room is to be sanitized every three months
12. The dates that the rooms and cage shelves are sanitized must be entered into the room log book..
13. The inventory is to be taken Monday, Wednesday, and Friday, or more often if needed.
14. All caging, food, water, water bottles and bedding is autoclaved.

Animal Care Mice

1. First thing every day, check cages to note the condition of the animals, such as; deaths, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total loss of water and/or food consumption. Report any of these problems immediately to the Supervisor and/or investigator.
2. The cages are to be changed and sanitized once a week or more often if needed. Sanitation includes boxes and lids. Bedding is removed on the dirty side of cage washer.
3. The feed is to be checked every day and refilled when necessary. Check all cages for "DO NOT FEED" and "DO NOT WATER" labels.
4. The water bottles are to be checked every day and filled with fresh water as needed. Be sure to return each bottle to its original cage.
5. The water bottles are to be sanitized once a week.
6. The shelves are to be wiped off every day.
7. The floors are to be swept and mopped every day.
8. The air vents are to be wiped off every day.
9. The doors are to be wiped off every day.
10. The feed barrel is to be wiped off every day.
11. The plastic bags in the feed barrels are to be kept in good condition.
12. Each cage must have an identification card with the following information: species, protocol number, investigator's name, department, date received, strain, sex, weight, date of birth, sources and number of animals per cage.
13. Nothing is to be kept on the shelves except the mouse cages.
14. The shelves are to be sanitized every two weeks or more if needed.
15. Room is to be sanitized every three months.
16. The dates that the rooms and cage shelves are sanitized must be entered into the room log book
17. The inventory is to be taken Monday, Wednesday, and Friday, or more often if needed.

Cage Size and Weight Limits For Mice

Space allocations for a 11 .5" x "7.5" x "5" (70 sq.in. floor) Polycarbonate shoebox, as specified in the Guide for the Care and Use of Laboratory Animals are as follows:

4 Mice over 25g
5 Mice up to 25g each *
7Mice up to 15g each*
10 Mice up to 10g each*

* The VAPHS Animal Care Facility recommends no more than 4 mice at any weight per box. This does not apply to mothers with babies.

Animal Care Rabbits

1. First thing every day, check cages to note the condition of the animals, such as deaths, caught feet, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total loss of water and/or food consumption. Report any of these problems immediately to the Supervisor and/or investigator.
2. The bedding in the trays is to be changed Monday, Wednesday and Friday. Trays are removed and replaced with sanitized trays (soiled bedding is removed on the dirty side of cage washer room).
3. The water bottles are to be checked every day and add fresh water as needed. Check all cages for 'DO NOT WATER" labels. Be sure to return each bottle to its original cage.
4. The feed is to be checked every day and refilled if necessary. Check all cages for "DO NOT FEED" labels.
5. The water bottles are to be sanitized once a week or more often if needed.
6. The rabbit cages are to be sanitized every two weeks or more if needed (no dirty empty cages should be stored in the animal room).
7. The floor is to be swept and mopped every day.
8. The feed barrel is to be wiped off every day. (No loose feed is to be kept in barrels).
9. The doors, including the vision panel are to be wiped off every day.
10. The air vent is to be wiped off every day.
11. The plastic bags in the feed barrels are to be kept in good condition.
12. The rabbits' ears are to have an ear tag for identification. These tags are to be checked every day, report to the Supervisor if any have fallen out.
13. Once a week, check the rabbits for ear mites, long incisor teeth, and long nails. Trim over grown incisors and nails as needed.
14. Each rabbit must have an identification card on its cage with the following information: ear tag number, protocol number, species, investigator's name, department, date received, weight, source and number of animals per cage.
15. The inventory is to be taken Monday, Wednesday, and Friday or more often if needed.
16. The dates that the rooms and cage shelves are sanitized must be entered into the room log book.
17. Nothing is to be stored in the room or placed on top of the cages.
18. The floor drains are to be checked every day and flushed with water if necessary.

***Trays for rabbit cages must be free of urine scale**

Cage Size and Weight Limits For Rabbits

1. Lab Products Rabbit Unit with 8 cages

<u>Cage Size</u>	<u>Allowable Rabbit Weight</u>
With divider - 4 sq. ft.	1 rabbit up to 11 lbs.
Without divider - 8 sq. ft.	1 rabbit over 11 lbs.

2. Allentown Rabbit Unit with 6 cages

<u>Cage Size</u>	<u>Allowable Rabbit Weight</u>
With divider - 4 sq. ft.	1 rabbit up to 11 lbs.
Without divider - 8 sq. ft.	1 rabbit over 11 lbs.

***Information obtained from the Guide for the Care and Use of Laboratory Animals -National Academy Press 1996.**

Animal Care Guinea Pigs

1. First thing every day, check cages to note the condition of the animals, such as; deaths, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total loss of water and/or food consumption. Report any of these problems immediately to the Supervisor and/or Investigator.
2. The cages are to be changed and sanitized three times a week or more often if needed. Sanitation includes boxes and feeders. Bedding is removed on the dirty side of the cage washer.
3. The feed is to be checked every day and feed added when necessary. Check all cages for “DO NOT FEED” and “DO NOT WATER” labels.
4. The water bottles are to be checked every day and fresh water added as needed. Be sure to return each bottle to its original cage.
5. The water bottles are to be sanitized once a week
6. The floors are to be swept and mopped every day.
7. The air vents are to be wiped off every day.
8. The doors are to be wiped off every day.
9. The outside of the feed barrel is to be wiped off every day.
10. The plastic bags in the feeding barrels are to be kept in good condition.
11. Each cage must have an identification card with the following information: species, protocol number, investigator’s name, and department, date received, strain, sex, weight, date of birth, sources and number of animals per cage.
12. The cage rack is to be sanitized every two weeks or more if needed.
13. The room is to be sanitized every three months.
14. The date that the room is sanitized must be entered into the room log book.
15. The inventory is to be taken Monday, Wednesday and Friday, or more often if needed.
16. Cleaning products used are Butcher’s Disinfectant Spray, Glass Cleaner, Quatricide, and Hypochlor.

Animal Care Pigs

1. First thing every day, check cages to note the condition of the animals, such as; deaths, wounds, abnormal secretions around the eyes or nose, respiratory distress, constipation or diarrhea, obvious swelling on the body, sluggishness, dull coat, and reduced or total loss of water and/or food consumption. Report any of these problems immediately to the manager, veterinarian technician and/or investigator.
2. The pigs are fed at approximately 7:00 am every day. Check all cages for 'Do not Feed" - 'Do not Water' labels.
3. The feed bowls are collected and sanitized every day. The automatic water system must be checked every day to ensure animals are able to get water.
4. The pig pens are to be hosed out twice a day or more often if needed. Be careful not to wet the pig.
5. The floors are to be hosed and mopped every day. The floor drains are to be flushed out every day.
6. The pig pens and room are to be sanitized every two weeks or more often if needed.
7. The doors are to be wiped off every day.
8. Make sure all the pigs are identified with cage cards with the following information: U.S.D.A. number, investigator's name, species, protocol number, department, date received, sex, weight, and source.
9. Make sure the identification card on the cages are always readable, if not, replace with new ones.
10. The feed barrel is to be wiped off every day.
11. The plastic bag inside of the feed barrel is to be kept in good condition.
12. The dates that the rooms and pens are sanitized must be entered into the room log book.
13. The inventory is to be taken Monday, Wednesday, and Friday or more often if needed.
14. The cleaning products used are Butcher's Disinfectant Spray, Glass Cleaner, Quatricide, and Hypochlor.

Part IV: Mouse Quarantine

All mice entering the VAPHS animal facility from facilities other than from the approved commercial vendors will be quarantined. When used in this policy, the term “imported mice” refers to mice that come from facilities other than the approved commercial suppliers.

Arranging for shipping mice:

After mice have been approved to be imported (see VAPHS Mouse Import Policy), with or without stipulations, the importing investigator must contact the animal facility supervisor to arrange for space and to set a time for bringing the mice into the facility.

Two types of imported mice

1. Importation of Breeding Mice: These mice are imported for the purpose of establishing a colony to produce research mice with a specific genome of interest.

2. Importation of Research Mice: These mice, whose genome makes them useful for specific studies, are imported to be the research subjects. They are not used to produce mice for studies.

I. Importation of Breeding Mice

Housing of imported breeding mice:

Imported mice will be held in the ventilated rack that will be operated in the negative air flow mode. The ventilated rack will be placed in a room near the dirty side of the cage washer. The air pressure in quarantine room will be negative to the corridor. Breeding of mice may be done while the mice are in quarantine at least to the extent necessary to maintain the stock.

Entering and leaving a quarantine room:

Only necessary animal care staff and necessary investigative staff will be permitted in quarantine rooms. Other mouse rooms are not to be entered in the same day after entering the quarantine room. Before entering the quarantine room, individuals are to don fresh shoe covers, gown or laboratory coat, and gloves. These items are to be removed just before leaving the quarantine room and placed in a receptacle provided for these items

Caging and cage changing:

Laminar Flow Units

Imported mice held in the laminar flow units will be held in filter-capped cages (microisolator cages). These cages will be autoclaved only when known immunoincompetent mice are imported. Only one cage of mice may be open at any time (obviously, the clean cage and the dirty cage must be open at one time). An empty clean cage will be open to receive dirty bedding when sentinel mice are to be exposed to dirty bedding. Operators will dip their gloved hands in Hypochlor solution before removing the filter cap from each cage. The smooth surface that the cages are placed on (the work surface) for changing will be wiped with Hypochlor before each cage is placed on it. Clean and dirty cages are to be stored on the work surface of the laminar flow units. When dirty cages are removed they are taken directly to the cage wash-room, emptied and cleaned in the cage washer, i.e., these dirty cages are not to be stored in the cage wash area. Dirty cages are to be placed on a solid bottom hand cart or other similar conveyance for removal from the quarantine room. The wheels of this truck/cart are sprayed with Hypochlor as it is rolled from the quarantine room. Of course, individuals moving cages from the quarantine room are to remove the protective items that they donned when entering the room (see Entering and leaving a quarantine room directly above).

Disinfection of quarantine rooms:

All room surfaces of the quarantine room(s) will be disinfected weekly with Hypochlor. The normal sanitations schedule for the facility will apply to quarantine rooms; this includes daily mopping of the rooms with Hypochlor (mops in the quarantine rooms will not be used elsewhere in the facility) and mopping of the corridors with Hypochlor late in the day.

Testing of imported mice for pathogens:

The mice to be tested, as outlined below, will be sent to the Univ. of Missouri testing facility and tested for a large number of mouse pathogens

All imported mice will be tested for the presence of pathogens using mice as described by one or more of the following systems:

1. The best mice to test for pathogens are the same mice shipped from the exporting facility. That is, mice produced by the same genotype as the mice to be used by the investigator so that they potentially have the same pathogens as the mice to be used. Two extra mice for testing for every 5 imported mice are needed. These mice can be tested two weeks after they arrive in the VAPHS facility. If the importing investigator wants, imported adult mice can be used for testing after they have weaned viable young; one adult for every two cages of mice imported with a minimum of two adults will need to be tested.
2. Pathogen-free sentinel mice that are housed in the same cages that house the imported mice are the second best mice for testing. In this case, three to four week old female sentinel mice that have a different hair coat color than the imported mice are used. One sentinel mouse is to be placed in each cage of imported mice. Sentinel mice that are observed to be pregnant, will be removed from the cage of imported mice to a clean cage shortly before delivering young. If these female sentinels deliver young before being sent for testing, the young are to be deeply anesthetized with CO₂ and then decapitated. These mice are tested five weeks after being placed in cages with the imported mice.
3. The least desirable but generally effective method of detecting pathogens in the imported mice is that of placing sentinel mice on dirty bedding from the imported mice. Pathogen-free sentinel mice are placed on bedding collected from all cages of imported mice every time the cages are changed/cleaned; the dirty bedding is placed in an empty clean cage (the more dirty bedding and feces the better). Three sentinel mice are to be housed in each cage and there is to be one cage of sentinel mice for each four cages of imported mice. These sentinel mice are tested after being exposed to dirty bedding for 6 weeks.

Release of mice from quarantine:

Mice will be released from quarantine by the Veterinary Medical Officer (VMO), if the tested mice are free of VA excluded mouse pathogens. The VMO will notify the animal facility supervisor, the program support clerk /R&D, and the importing investigator when mice can be released from quarantine. The animal facility supervisor will communicate with the investigator to arrange for the imported mice to be housed in the animal facility.

Handling of mice that cannot be released from quarantine:

Imported mice that are shown to have a pathogen or pathogens that are not already in the VAPHS animal facility mice will not be released from quarantine. If any pathogen is detected, the testing results will be validated and a course of action (treatment, eradication, rederivation, etc) will be made based on the agent(s) in question.

II. Importation of Research Mice

Only mice that are free of pathogens or agents that can be effectively treated or mice that have Mouse Norovirus only can be imported into the animal facility; In the case of pathogen identification, departure from quarantine for acute terminal use in investigator laboratories will be considered on a case by case basis based on the agent in question and only following a comprehensive strategy meeting to review transportation containment, disinfection and subsequent trafficking constraints.

1. Mice that are free of mouse pathogens and mice that are infected with Mouse Norovirus and/or Helicobacter sp. may be imported for research use and be quarantined in the ventilated rack. All conditions that apply to imported breeding mice apply to these mice. These quarantined mice will be tested for mouse pathogens the same as other imported mice.
2. When the testing of these mice for pathogens indicates that they are free of all mouse pathogens except for Mouse Norovirus or Helicobacter sp., the quarantined mice may be released from quarantine. Mice with Mouse Norovirus only may be placed in normal mouse rooms and housed and used as other mice in the facility. Mice that test positive for Helicobacter will be treated with medicated chow and retested to assure clearance.

Part V. Breeding Mice

Regarding Breeding Animals:

The Animal Care Staff needs to have white identification cards for each mouse. Along with the identification card, each female that is used for breeding, should have a Blue Breeding card. These cards shall follow the mouse from cage to cage. (1 white card for the male mouse), (2 cards for breeding females, 1 white card and 1 blue card)

On the Blue Breeding Card investigators/research staff must indicate the date that males and females are put into the same box for breeding. Additionally, the male and female's identification number must be on the card so that good and bad breeders can be tracked.

If the protocol's technician has not already done so, the Animal Care Staff will write in the date they notice that the female is showing on the breeding card.

It is the responsibility of the protocol's technician to know when pups are due and to be there to confirm date of birth if necessary. If, the Animal Care Staff discovers the pups in the box and the protocol's technician has not been in to confirm the date of birth the Animal care staff will indicate the date that the pups were noticed as date of birth (minus one to seven days depending on when the box was changed last).

This information is needed so that the technician has a record of the number of pregnancies, the length of the pregnancy, the number of live birth vs. the number of still births, the females that kill their pups, etc. The Animal Care Staff will monitor the females closely so as to prevent any undo pain or suffering for the mother or her pups.

Finally, the Animal Care Staff will mark the date, that the pups are to be weaned from their mothers, (pups may be left with the mother longer but the wean date will be the date the pups are added to the investigator's Inventory).

Info that is needed on the ID cards:

1. Investigator
2. Protocol #
3. Date received
4. Gender
5. Supplier
6. Strain
7. Date of birth
8. Identification

Part VI: Mouse and Rat Disease Surveillance Program

The following procedures are to be followed to assess for disease in mice and rats.

1. Purchase two 3-4 week old non-inbred animals (mice or rats as applies) from Taconic Farms each time sentinels are sent for testing.
2. Use two sentinels per cage and one cage of sentinels for every two racks.
3. At cage changing, place a small amount of dirty bedding from each monitored cage in a clean cage that was empty at the beginning of the cage changing.
4. After the cages on the rack have been changed, place the sentinel animals in the new cage that contains dirty bedding. A filter cap is placed on the sentinel cage.
5. Every four months submit one animal from each sentinel cage for testing. The second sentinel animal is to remain in the cage for 1 month after the two new animals are entered into the cage; this animal is then euthanized. This delay keeps the second sentinel available for testing until after the test results for the submitted animal have been received and reviewed, and the new sentinels will have been exposed to the old sentinel in case any pathogen was incubating when the sentinel was submitted.
6. Sentinels are tested for exposure to murine viruses, internal and external parasites, bacterial pathogens, and *Mycoplasma pulmonis*.
7. Sentinel cages are otherwise handled as other cages as describe in the cage changing procedure for filter capped cages to prevent the spread of any pathogen that they may have contracted.

Part VII: Anesthesia, Analgesia, and Euthanasia

It should always be considered that responses to drugs vary greatly within each species depending upon factors such as sex, age, weight, time of day (due to circadian rhythms), interaction with other drugs, exposure of animals to various environmental chemicals, etc. Therefore, care should always be taken to give drugs cautiously and to the desired effect before proceeding with any procedure which might cause pain.

In selecting an anesthetic for experimental animals, consideration must be given to requirements such as: 1) anesthetic duration; 2) recovery time; 3) degree of analgesia and muscular relaxation; 4) availability of equipment and personnel; and 5) the pharmacodynamics of the drugs on the organ system being studied.

Some of the anesthetic combinations in laboratory animals may have distinct advantages over the use of single agents by facilitating restraint or induction, improving muscular relaxation, increasing analgesics and easing recovery. However, the use of more than one drug makes accurate evaluation of the pharmacodynamic effects of the anesthetic combination more difficult and may necessarily be avoided for some studies.

Definitions and General Information:

Analgesic agents are those which provide relief from pain. They may be used as preanesthetics, in balanced anesthesia, or during post surgical recovery periods. Opioids (morphine) and NSAIDs are examples of analgesics. When systemic analgesics cannot be used, the use of local anesthetic agents should be considered, such as lidocaine, bupivacaine, etc.

Barbiturates are used primarily for the induction or maintenance of general anesthesia. They are commonly classified according to their duration of action. Pentobarbital (Nembutal) has a duration of 1-3 hours while thiopental (Surital) and thiopental (Pentothal) produce a comparatively short duration of anesthesia, 10-45 minutes. The total amount used to produce surgical planes of anesthesia will vary depending upon the animal's condition. The use of pre-anesthetic will vary depending upon the animal's condition, the use of pre-anesthetic drugs, etc. When administered intravenously, they should be given "to effect". The barbiturates are controlled drugs.

Controlled drugs are drugs with potential for abuse. Some of the drugs used for animal analgesia, anesthesia, euthanasia and tranquilization that fit this category are barbiturates and narcotics. The drugs are classified into five schedules. All controlled drugs have a "C" and the schedule printed as a Roman numeral on the label. The Department of Justice Drug Enforcement Administration (DEA) requires appropriate security and record management of these substances.

Local anesthetics can be used without additional medication for minor procedures; however, restraint of the animal is still necessary. For general surgery, local anesthesia may be used in conjunction with heavy sedation and analgesia. Local anesthetics with an extended effect may provide some post-operative analgesia. Spinal anesthesia may be an alternative to general anesthesia for intra-uterine surgery in pregnant animals. Toxic levels of local anesthetics can occur, especially when used to produce epidural analgesia. The total time of anesthesia depends upon the drug, concentration, rate of injection, volume of drug injected, initial level of anesthesia, age and condition of the animal, and whether or not a vasoconstrictor is added to the solution. Some of the more commonly used local anesthetic agents are bupivacaine, lidocaine, and tetracaine. Bupivacaine (Marcaine) has a duration of effect significantly longer than that of any other commonly used local anesthetic. The duration can

be extended with addition of epinephrine. Analgesia persists for a period after the return of sensation, thus reducing the need for strong analgesics. Injectable preparations of Lidocaine (Xylocaine) are commonly used for infiltration and nerve blocks resulting in local anesthesia. The volume injected should raise a small bubble. This preparation has a rapid rate of onset and lasts up to two hours when it contains epinephrine. Injectable preparations of Tetracaine (Pontocaine) also may be used for infiltration and nerve blocks; however, onset of anesthesia is slower than observed with lidocaine. Tetracaine is commonly used for epidural analgesia in sheep and may be selected for surgical procedures lasting 2-3 hours.

Paralytic agents can be used in conjunction with anesthetic agents to produce more complete muscle relaxation; however, all protocols using such agents must have the prior approval of the Institutional Animal Care and Use Committee. Investigators should personally observe any experiments in which these drugs are used and thoroughly train their personnel in appropriate use of these drugs. These agents produce a progressive paralysis which in high doses affects the respiratory muscles, thus they should only be used with extreme care. Oxygen and positive pressure ventilation should always be available when these drugs are used. These agents do not produce analgesia; therefore, no painful stimuli should be initiated without concurrent administration of analgesics or anesthetics. When paralyzing agents are used with anesthetics, it is imperative that reliable methods are available to distinguish between paralysis and anesthesia.

Narcotics are controlled drugs which provide analgesia and sedation. They can be used with other drugs for "balanced anesthesia". Specific antagonists are available.

Tranquilizers should be used to assist in easier animal handling during induction of anesthesia, to reduce the dose of general anesthetic; and to ease the recovery from general anesthesia. Acetylpromazine (Acepromazine) is a commonly used tranquilizer in veterinary practice. Tranquilizers may be used with other drugs for "balanced anesthesia"; however, they are not analgesics.

Euthanasia

Euthanasia is the act of inducing painless death. Criteria to be considered for a painless death are rapidly occurring unconsciousness and unconsciousness followed by cardiac or respiratory arrest. Although not an adequate criterion, observers may mistakenly relate any movement with consciousness and lack of movement with unconsciousness. Euthanasia techniques by which animals exhibit little or no movement are the most acceptable to most people.

Selection of the most appropriate method of euthanasia in any given situation depends upon the species involved, available means of animal control, skill of personnel, numbers of animals, economic factors and other considerations.

The Animal Welfare Act and the NIH Policy state that euthanasia methods selected should be in compliance with the recommendations of the American Veterinary Medical Association Panel on Euthanasia.

Recommended anesthetic, analgesic, and euthanasia methods for varying species are included on the proceeding pages. These recommendations are made based upon those provided by the American Veterinary Medical Association, as well as experience in animal use at this institution. Note: Additional guidance regarding anesthetic and analgesic use in rodents can be found in Appendix H.

Mice

Agent	Dose (mg/kg)	Route	Comments
I. Tranquilizers			
Telazol (Tiletamine HCL/Zolazepam HCL)	80 80-100	IP IM	For immobilization
Acetylpromazine (Acepromazine)	0.5	IM	
II. General Anesthetics			
Alpha Choralose	114	IP	Scientific Justification Needed
Ketamine (Ketaset, Vetalar)	50-100 50	IM/IP IV	Sleep produced in mice 2-3 minutes after injection of mixture Surgical anesthesia lasts about 80 minutes. Full recovery about 110 minutes
Mixtures with 1. Diazepam 2. Xylazine	1. 200 mg Ketamine with 5 mg Diazepam 2. 100 mg Ketamine with 5-15 mg Xylazine	IM/IP IM/IP	
Xylazine (Rompun)	5 13	IM IP	Post injection. Calm induction and recovery
Morphine	7-10	SC	Give 10 mg/kg ever 2-4 hours as needed
Pentobarbital (Nembutal)	40-90	IP	Dosage varies greatly;
	40-70	IV	There is narrow margin of safety

Mice (Continued)

Agent	Dose (mg/kg)	Route	Comments
III. Analgesics			
Buprenex (Buprenorphine)	0.05-0.1	SC	Every 12 Hours
Metacam (Meloxicam)	0.2	PO or SQ	Has a duration of action of 24-48 hours in most species; may be used for prolonged periods of time; also very effective when used in combination with opioids. Use once daily
Ketaprofen	5		
Butorphanol	1-3		Every 2-4 hours
IV. Miscellaneous			
Atropine	0.05	IM, SC	Give about 30 minutes prior to other agents.
V. Euthanasia			
A. Injectable			
Pentobarbital	150	IP, IV	Highly effective euthanasia agent when appropriately administered. Drug retained in tissue after death.

MICE (continued)

Agent	Dose (mg/kg)	Route	Comments
B. Inhalant			
Carbon Dioxide (70- 100%)			Safe, inexpensive and effective. Tissue changes associated with hypoxemia may be seen. Time required to produce death may be prolonged in immature and neonatal animals.
Isoflurane			Use a non-breathing system. Induction 2-3% Maintenance 0.25- 2%

MICE (continued)

Agent	Dose (mg/kg)	Route	Comments
VI. Physical			
Cervical Dislocation			Acceptable with prior sedation or anesthetization. Exceptions must be approved by IACUC.
Decapitation			Acceptable with prior sedation or anesthetization. If unable to sedate or anesthetize because chemical residue-free tissues are required, head should be immediately frozen in liquid nitrogen subsequent to severing. Exceptions must be approved by IACUC.
Exsanguinations			Acceptable when preceded by other methods that relieve anxiety, consciousness.

Rats

Agent	Dose (mg/kg)	Route	Comments
I. Tranquilizers			
Acetylpromazine (Acepromazine)	0.5	IM	
Diazepam (Valium)	3-5	IM/IP	
Ketamine (Ketaset, Vetalar)	50-100	IM/IP	
Telazol (Tiletamine HCL/ Zolazepam HCL)	20-60 40	IM IP	For chemical restraint For light Anesthesia
II. General Anesthetics			
A. Injectable			
Alpha Choralose	55	IP	Scientific justification needed. Terminal studies only.
Ketamine/Xylazine (Rompun) Mixture	Ketamine 40-75 mg Xylazine 5-15 mg	IM/IP	Make a mixture by adding 0.15 ml Xylazine (100mg/ml stock solution) to each ml of Ketamine (100 mg/ml stock solution). Maximal anesthetic effect is achieved within 15 minutes and lasts 15-30 minutes.
Ketamine Diazepam Mixture	Ketamine 40-60 mg Diazepam 5-10 mg	IP	

Rats (Continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthesia (continued)			
Telazol (Tiletamine HCL, Zolazepam HCL)	20-60 40	IM IP	For chemical restraint For light anesthesia
Thiopental (Pentothal)	20 40	IV IP	For chemical restraint
B. Inhalant			
Carbon Dioxide: O ₂ (1:1)			Anesthesia is induced within seconds in a chamber. Suitable for injection or cardiac puncture but do not try to maintain for periods in excess of 2 minutes. Safe, inexpensive and effective. Tissue changes associated with hypoxemia may be seen. Time required to produce death may be prolonged in immature and neonatal animals.

Rats (Continued)

Agent	Dose (mg/kg)	Route	Comments
Isoflurane	Induction 2-3% Maintenance 0.25% - 2%		Use a non-breathing system. Nonflammable and nonexplosive. Euthanasia easily performed in a closed container. May produce changes in parenchymatous organs.
C. Other			
Hypothermia			Effective for short-term procedures in new born pups. Reduce body temperature to 2 degrees C (not less than 1 degree C) by placing pups for 5-15 minutes in dry, filter paper lined test tubes immersed in an ice water bath. Resulting immobilization lasts 3-10 minutes at room temperature. For recovery, keeping in a cool place 10-30 minutes, then in a warm place for 20 minutes. Return to mothers only when spontaneous activity has been restored.

Rats (Continued)

Agent	Dose (mg/kg)	Route	Comments
III. Analgesics			
Buprenex (Buprenorphine)	0.05	SC or IV	Every 8-12 hours
	0.1 – 0.25	PO	Every 8-12 hours
Metacam (Meloxicam)	0.2	PO or SC	Once daily, has duration of action for 24- 48 hours. For musculoskeletal and mild visceral pain. Very Effective when used in conjunction with Opioids
Morphine	1.6 10 20	SC IM, SC IV	Give 10 mg/kg SC every 2-3 hours as needed.
Oxymorphone (Numorphan)	0.25- 0.5	SC,IM	Duration of Action 6-12 hours
Ketoprofen	5	SC	
IV. Miscellaneous			
Atropine	0.05	IM, SC	Give about 30 minutes prior to other agents to reduce salivary and bronchial secretions.

Rats (continued)

Agent	Dose (mg/kg)	Route	Comments
V. Euthanasia			
A. Inhalant			
Carbon Dioxide			Anesthesia is induced within seconds in a chamber. Suitable for injection or cardiac puncture but do not try to maintain for periods in excess of 2 minutes. Safe, inexpensive and effective. Tissue changes associated with hypoxemia may be seen. Time required to produce death may be prolonged in immature and neonatal animals.
Isoflurane	Induction 2-3% Maintenance 0.25% - 2%		Use a non-breathing system. Nonflammable and nonexplosive. Euthanasia easily performed in a closed container. May produce changes in parenchymatous organs.

Rats (continued)

Agent	Dose (mg/kg)	Route	Comments
B. Physical			
Cervical Dislocation			Suitable only in rats weighing less than 200 grams. Acceptable with prior sedation or anesthetization. Exceptions must be approved by IACUC.
Decapitation			Acceptable with prior sedation or anesthetization. If unable to sedate or anesthetize because chemical residue-free tissues are required, head should be immediately frozen in liquid nitrogen subsequent to severing. Exceptions must be approved by IACUC.
Exsanguinations			Acceptable when preceded by other methods that relieve anxiety, consciousness.

Guinea Pigs

Agent	Dose (mg/kg)	Route	Comments
I. Tranquilizers			
Diazepam (Valium)	2.5 5	IM, IP IP	Provides no analgesia. The 5 mg/kg IP dose produces complete tranquility and relaxation for abdominal surgery using spinal epidural analgesia.
Ketamine (Ketaset, Vetalar)	20-60	IM	
Acetylpromazine (Acepromazine)	0.5	IM	
II. General Anesthetics			
A. Injectable			
Chioral Hydrate- 10%	200 – 300	IP	Onset of narcosis in 3-5 minutes with peak effect maintained for 60- 120 minutes. Provides little analgesia and severely depresses the respiratory system at anesthetic doses. Must justify use.

Guinea Pigs (Continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthetics (continued)			
Thiopental (Pentothal)	55 20	IP IV	Not recommended for routine use. Sleep time is unduly prolonged.
B. Inhalant			
Carbon Dioxide: O ₂ (1:1)			Pipe into a face mask or induction chamber. Produces rapid narcosis with anesthesia lasting up to 2 minutes.
Isoflurane	Induction 2-3% Maintenance 0.25% - 2%		Use a non-breathing system.
III. Analgesics			
Buprenex (Buprenorphine HCl)	0.05	SC, IV	Every 8 -12 Hours
Metacam (Meloxicam)	0.2	PO or SQ SID	Has a duration of action of 24-48 hours in most species; may be used for prolonged periods of time; also very effective when used in combination with opioids.
Morphine	2-5	IM, SC	Administer 10 mg/kg or SC every 2-3 hours as needed

Guinea Pigs (Continued)

Agent	Dose (mg/kg)	Route	Comments
IV. Miscellaneous			
Atropine	0.05	IM, SC	Give about 30 minutes prior to other agents.
V. Euthanasia			
A. Injectable			
Pentobarbital	2-3 x anesthetic dose	IP, IV	Highly effective euthanasia agent when appropriately administered. Drug retained in tissue after death.
B. Inhalant			
Carbon Dioxide (70- 100%)			Safe, inexpensive and effective. Tissue changes associated with hypoxemia may be seen. Time required to produce death may be prolonged in immature and neonatal animals.
Isoflurane	Induction 2-3% Maintenance 0.25-2.0%		Nonflammable and nonexplosive. Easily performed in a closed container. May produce changes in parenchymatous organ.

Guinea Pigs (continued)

Agent	Dose (mg/kg)	Route	Comments
VI. Physical			
Exsanguinations			Acceptable when preceded by other methods that relieve anxiety, consciousness.

Rabbits

Agent	Dose (mg/kg)	Route	Comments
I. Tranquilizers			
Acetylpromazine (Acepromazine)	1.0	IM	Tranquilization occurs in 5-10 minutes and lasts 1-2 hours. Useful when given prior to methoxyflurane anesthesia.
Diazepam (Valium) Pre-Anesthetic	5-10 2-10	IM, IP IM	Provides good tranquilization and muscle relaxation but no analgesia. Onset of effect in 3-5 minutes; full recovery may require up to 12 hours.
Ketamine (Ketaset, Vetalar)	40- 50 15-20	IM IV	Duration approximately 15- 30 minutes. Suitable only for minor procedures. Poor analgesia and muscle relaxation when used alone.
Telazol (Tiletamine HCL, Zolazepam HCL)			Not recommended
II. General Anesthetics			
A. Injectable			
Alpha Chloralose- 1% solution	120 80-100	IV IV	Use only for "physiological" non-recovery preparations. Scientific justification needed.

Rabbits (continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthetics (Continued)			
Ketamine	20-60 15-20	IM IV	Heart rate, respiratory rate, and blood pressure are depressed. FSH is suppressed. (See entry under tranquilizers).
Mixture of Ketamine and Diazepam	Ketamine 60 Diazepam 5-10	IM	
Ketamine and Xylanzine (Rompum)	25-50 5-10	IM	Duration of surgical anesthesia (20- 30 minutes) is dose-dependent). Analgesia adequate for intra-abdominal procedures.
Pentobarbital	20-50	IV	Narrow margin of safety. Administer dilute (1-3%) slowly to effect. More concentrated solutions or intra-arterial injections can cause vessel damage and can lead to occlusion and necrosis. Duration of anesthesia is 30-60 minutes, but complete recovery may require 1-10 hours. Peak effect of the IP dosage reached in 5-7 minutes; light surgical anesthesia lasts about 120 minutes and complete recovery may take 12- 20 hours.

Guinea Pigs (Continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthetics (continued)			
Thiamylal (Surital)	22-54	IV	Dilute to 1% solution. Useful for induction of gas anesthesia and short-term procedures. Intra-arterial administration may lead to vessel damage and tissue slough.
Thiopental (Pentothal)	12- 50	IV	Duration of anesthesia is 5-20 minutes with full recovery within 15 minutes. Pedal reflex is unreliable for monitoring anesthetic depth. Intra-arterial administration may lead to vessel damage and tissue slough.
B. Inhalant			
Isoflurane	Induction 2-3% Maintenance 0.25% - 2%		Use a non-breathing system.

Swine

Agent	Dose (mg/kg)	Route	Comments
I. Tranquilizers			
Acetylpromazine (Acepromazine)	0.11- 0.22 0.03-0.1	IM, SC IV	Total dose for any pig should not exceed 15 mg.
Diazepam (Valium)	5.5- 8.5 1-2	IM IM, IV	Will develop recumbency within 10 minutes. Max dose will reduce pentobarbital dose by 50%
Ketamine (Ketaset, Vetalar) AND Diazepam	10 2	IM IM	Pigs are usually recumbent in 5 minutes and the peak effect is reached in 10 minutes.
II. General Anesthetics			
A. Injectable			
Ketamine	20 4	IM IV	Analgesia is adequate for minor surgical procedures lasting 10-20 minutes; however, muscle relaxation is poor and it is best to use with other agents. Administration of Diazepam at 1-2 mg/kg IM 3 minutes prior to Ketamine will eliminate excitement phase observed in some pigs.

Swine (continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthetics (Continued)			
Ketamine and Xylanzine (Rompum) AND Thiopental (Pentothal) 0.25%	20 2 30 drops/min	IM IM IV	Use for pigs weighing 20-45 kg. Fast animals 24 hours. Pretreat with Atropine to prevent fatal cardiac arrhythmias. Inject Ketamine and Xylazine simultaneously. After anesthesia induced, maintain with IV Thiopental.
Pentobarbital	24	IV	Wide margin of safety in pigs weighing 10-22.5 kg. For swine weighing < 45 kg, give 20-30 mg/kg IV). Inject slowly to effect. Reported duration of action ranges from 10- 15 minutes to 60-90 minutes.
Thiamylal (Surital)	6.6-11 12-15 26	IV IV IV	

Swine (Continued)

Agent	Dose (mg/kg)	Route	Comments
II. General Anesthetics			
B. Inhalant			
Isoflurane	To effect		Recommended for young or sick pigs, rapid recovery.
III. Analgesics			
Morphine	0.2-0.9	IM	Has more CNS stimulant than depressant effects but useful as premedication for barbiturate anesthesia at this dose.
Phenylbutazone	4-8	PO	Not a potent visceral analgesic
Deramaxx (Deracoximb)		PO	For DOGS ONLY
Metacam (Meloxicam)	0.4 mg/kg	IM	
Buprenex (Buprenorphine)	0.005 – 0.01	IM	Every 12 hours
IV. Miscellaneous			
Atropine	0.07- 0.09	IM	
Doxopram (Dopram-V)	5-10	IV	
V. Euthanasia			
A. Injectable			
Pentobarbital	2-3 x anesthetic dose	IV	Highly effective euthanasia agent when appropriately administered. Drug retained in tissues after death.

IX. Procedures for Use of Euthanasia Gases

USING EUTHANASIA GASES IN THE VA PITTSBURGH MEDICAL CENTER ANIMAL RESEARCH FACILITY

General procedure for using the core Operating Room GA147:

1. Make sure you have all necessary equipment and supplies.
2. Place a fresh Absorbent Pad down on the work area to contain your surgical waste.
3. Turn the exhaust switch (EF-4 Control) ON (on the wall beside the sink) Red light will be ON when the switch is turned ON.
4. Close the door to maintain the room with appropriate negative pressure and air flow.

Components of the Euthanasia System are as follows:

1. The Carbon Dioxide gas cylinder is along the wall behind the door.
2. The Top Valve of the cylinder must be turned counter clockwise (on) and clockwise (off).
3. DO NOT adjust the regulator valve on the gas cylinder.
4. The wall pipe from the gas cylinder delivers Carbon Dioxide into the Euthanasia chamber.

To Use:

1. Line the chamber floor with an Absorbent Pad.
2. Turn on the scavenging system.
3. Place animals inside the chamber. Secure both latches.
4. Turn on the Carbon Dioxide cylinder by the top tank valve ONLY.
5. Turn on the valve to the Carbon Dioxide line on the wall behind the chamber to release the gas into the chamber. Give the flow valve setting to deliver the CO₂ at 20% of the chamber volume per minute.
6. Turn off the Carbon Dioxide cylinder by the top tank valve ONLY.
7. Remove deceased animals. Very young mice and rats should be decapitated after respiration ceases as it is difficult to check for a heart beat.
8. Turn off the valve of the Carbon Dioxide line to the chamber and let line bleed out.
9. Leave scavenging system on to evacuate the gas from the chamber.

Clean-Up:

1. Remove the Absorbent Pad(s) and clean up extensively!! Wipe all work surfaces, euthanasia chamber, etc., with Butcher's Bright Spray Disinfectant
2. Turn the scavenging exhaust switch OFF.
3. Lock the door when you leave.

**IF YOU HAVE ANY QUESTIONS, CONTACT ANIMAL FACILITY SUPERVISOR,
PAM MATEY, x60-6107.**

X. Procedures for Use of Anesthetic Gases

**USING ANESTHETIC GASES IN THE
V.A. PITTSBURGH MEDICAL CENTER ANIMAL RESEARCH FACILITY**

General procedure for using the core Operating Room in GA 147

1. Make sure you have all the necessary surgical equipment and supplies for that day's procedures (such as balance, surgical instruments, lamp, gauze, needles, saline, etc.)
2. Place a fresh Absorbent Pad down on the work area to contain your surgical messes.
3. Turn the exhaust switch (ER-I Control) ON (on the wall behind the set-up area); Red light will be ON when the switch is turned ON.
4. Close the Door to maintain the room with appropriate positive pressure and air flow.
5. Ensure the Blue Hose (Scavenging System) used to remove the Waste Anesthetic Gases (WAG) is connected to the animal chamber and the surgery table.

Components of the Anesthesia system:

1. The two compressed gas cylinders(N_2O and O_2) are along the wall.
2. The TOP valves must be turned counterclockwise (on) and clockwise (off).
3. DO NOT adjust the regulator valves.
4. The black hose from the NITROUS OXIDE cylinder regulator delivers N_2O and the clear line delivers OXYGEN (O_2). When using both gases, set the two flow meters to deliver the desired percentage of both gases.
5. These lines are connected to the air mixer (far right side as you face it), where the gases are mixed and go directly to the vaporizer,
6. The VAPORIZER is the machine that adds the anesthetic (isoflurane) to the carrier gas(es).
7. At the top of the vaporizer are the two hoses that supply the O_2 and N_2O to the vaporizer. Check and make sure these hoses are connected to the supply tanks and are plugged into the vaporizer.
8. The VAPORIZER contains Isoflurane; check the level in the machine and add accordingly. A glass funnel might assist in pouring it into the chamber.
9. The vaporizer settings for isoflurane are 0.25 to 4.0% + to effect for rats and mice.
10. The vaporizer has an exit hose with a plastic connector to which you can attach the anesthesia chamber, anesthesia cone, or other apparatuses that deliver the anesthetic mixture to the animal.
11. As noted above, the Blue Hose must be connected for scavenging the WAG.

To Use:

1. Turn on both compressed gas cylinders by their top tank valves ONLY.
2. Turn on the workstation supply lines (Handset above the vaporizer) for the compressed gas flow.
3. Adjust the gas mixer proportions: set the blue knob for N₂O and the green knob for O₂.
4. Turn the vaporizer ON. Assure the outlet hose is in the anesthetizing chamber and allow to equilibrate.
5. Put the animal into the anesthesia chamber and monitor its breathing.
6. Remove the anesthetized animal from chamber, and attach the outlet hose clip to the operating apparatus.
7. Place the animal on the operating table with nose positioned in the nose cone Or other anesthetic deliver apparatus / Or attach to ventilator, intubate animal and then attach to endotracheal tube
8. Conduct your surgery.

WHEN PROCEDURES ARE COMPLETE:

1. Return the vaporizer setting back to zero.
2. Turn off the compressed gas cylinders along the wall (turn ONLY the valves on the top of the cylinders clockwise).
3. Turn off the work station supply lines.
4. The vaporizer gases should be bled off
5. The flow meter knobs are turned clockwise, causing the indicator balls to fall to the bottom, zero pressure.
6. Turn the exhaust switch OFF.

CLEAN-UP

1. Remove absorbent pad(s) and clean up extensively!
2. Wipe all work surfaces, euthanasia chamber, etc., with Butcher's Bright Disinfectant Spray following instructions on the can.
3. Turn the scavenging exhaust switch OFF.
4. Lock the door when you leave.

**IF YOU HAVE ANY QUESTIONS, CONTACT ANIMAL FACILITY SUPERVISOR,
PAM MATEY, x60-6107.**

USING LIQUID (ISOFLURANE) for ANESTHESIA in the V.A. PITTSBURGH MEDICAL CENTER ANIMAL RESEARCH FACILITY

The following rooms are equipped with chemical hoods appropriate for use with inhalant anesthetic gases. GA119, GA146, GA147. Instructions for each area follow:

Room GA147 - the small, bench top hood.

1. Make sure you have all the necessary surgical equipment and supplies for that day's procedures (such as balance, surgical instruments, lamp, gauze, needles, saline, etc.)
2. Place a fresh Absorbent Pad down on the work area to contain your surgical wastes.
3. Turn the exhaust switch ON (EF-4 Control); Red light will be ON when the switch is turned ON.
4. Close the door to maintain the required negative pressure in the room.
5. Place your anesthesia jar containing absorbent material (cotton, gauze) in the hood. Keep lid/cover on as much as possible to minimize your potential exposure.
6. A 2-4% concentration of isoflurane gas can be achieved in the jar by applying isoflurane at 0.2 ml/1000ml of jar volume to the absorbent material (preferably below a false floor in the jar)
7. Anesthesia of mice can be anticipated after approximately 57 seconds exposure.
8. At the conclusion of your procedure(s) the jar and absorbent material are left under the hood with the exhaust ON to permit the vapors to disperse to the outside air.
9. Return your stock bottle of isoflurane to its storage area.
10. Turn off exhaust (EF-4 Control).
11. Remove absorbent pad(s) and clean up extensively!
12. Lock the door when you leave.

**IF YOU HAVE ANY QUESTIONS, CONTACT ANIMAL FACILITY SUPERVISOR,
PAM MATEY, x60-6107.**

XI: Procedures for Using Glass Sterilizer Beads

F*S*T
FINE SCIENCE TOOLS

INSTRUCTIONS FOR
Using a Glass-bead Sterilize
FST No. 18000-45, FST No. 18000-50

Fill sterilizer well with glass beads provided, to within 3 mm below rim.

Insert electrical plug into wall socket. Do NOT cover well with lid. Switch on unit. It will take 15 to 20 minutes to reach operating temperature of approximately 250° C.

Carefully insert the working part of **clean** surgical instruments into well after unit has reached operating temperature and leave for 10 to 20 seconds. Do not leave longer as instruments becomes too hot to handle. Sterilization is on contact with glass beads, so tubes (canulae) cannot effectively be sterilized.

Unit can be left in the “ON” position all day, and is safe for use in laminar flow cabinets.

Only when unit is switched off and cool, cover with lid to reduce dust accumulation. Do not cover with lid when unit is hot. Should unit overheat and trip safety switch, let it cool off completely, then turn it over and push in red button visible inside the unit as viewed through one of the cooling holes.

The glass beads should be cleaned routinely and replaced in a dry state. Put dirty beads in a mesh bag with warm soap and water to clean. Rinse thoroughly and do not replace until completely dry.

APPENDICES

Appendix A: VAPHS IACUC SOP

The VAPHS Institutional Animal Care and Uses Committee Standard Operating Procedures may be accessed by clicking [here](#) or going to the VAPHS Research Office Website http://www.vaphs.research.med.va.gov/revamp/animal_research.htm.

Appendix B: Contamination Control Procedures

Example Signage

ANIMAL RESEARCH FACILITY CONTAMINATION CONTROL POLICIES

TO ALL PERSONS ENTERING THE ANIMAL FACILITY:

You must put on new shoe covers every time you enter the facility from the outside of the building.

Before entering any animal room, lab, or operating room, you must put on shoe covers-a yellow isolation gown-and exam gloves. The gloves are necessary if you will be touching any animals, their cages, water bottles, any equipment that animals may come in contact with, or feed. These items are provided for you at several locations in the hallways of the facility.

Some animal rooms may also require surgical face masks. These rooms will have the appropriate signage on their doors.

These simple procedures are very effective in controlling the spread of infectious agents throughout your animal colonies.

Thank you for your cooperation,
Pam Matey-Animal Facility Supervisor

Appendix C: VAPHS Waste Anesthetic Gases and Vapors Exposure Control Policy

The VAPHS Waste Anesthetic Gases and Vapors Exposure Control Policy may be accessed [here](#) or by going to the VAPHS Research Office website (http://www.vaphs.research.med.va.gov/revamp/animal_research.htm)

Appendix D: Badge Monitoring: User Instructions

Steve Baker will provide monitoring badges for employees who are exposed to Waste Anesthesia Gas. All employees who are working with Waste Anesthesia Gas in rooms GA119, GA115, and GA147 will be required to wear a monitoring badge. The badge is used to monitor a single procedure or time frame not to exceed 8 hours. For your convenience the badges will be located on the door of GA119 in a basket along with these instructions. Any questions, please contact the Industrial Hygienist Steve Baker at 412-360-3704.

Instructions for use of the badges and required documentation:

1. To prevent damage to the resealable seam, cut an opening at the top of the white zip loc package, above the notched area.
2. Remove the monitoring badge from the white zip loc package and then remove from the clear resealable plastic bag. Save both the outer white zip loc package and the clear resealable plastic bag.
3. Record the Date, the Start time of your procedure, and the Name of the Technician who is actually doing the procedure on the badge and on the outer white zip loc package.
4. Clip the badge near to your breathing zone (collar or lapel). Make sure you have the white, blank surface facing away from your body so it is exposed to the gas. Make sure the white side of the badge is exposed to the gas throughout the entire procedure.
5. When your procedure has been completed, remove the badge from your breathing zone and record the Stop Time on the badge and on the outer white zip loc package. Also somewhere on the outer white zip loc package record the room number where you did your procedure, number of animals used, the type of anesthetic gas you used and a general description of your procedure. Examples of procedures would include:
 - Clipping and shaving the mice
 - Any surgical procedures
 - Euthanasia of the specimen
6. Place the exposed monitoring badge into the resealable plastic bag and then into the white outer zip loc package and seal. Make sure the seal is tight.
7. After completion of procedure and all documentation, take the sealed bag to the office of Stephen C. Baker, Industrial Hygienist in Building 1, Room AN 222 or contact him at 412-360-3704 for other arrangements.

Appendix E: VAPHS Animal Exposure Preventative Medicine Program

The Animal Exposure Preventative Medicine Program (AEPMP) may be accessed by clicking [here or going to the VAPHS Research Website at http://www.vaphs.research.med.va.gov/revamp/animal_research.htm](http://www.vaphs.research.med.va.gov/revamp/animal_research.htm).

Appendix F: Employee Injury

All employee work-related injuries are handled in accordance with VAPHS [Medical Center Memorandum HR-023](#), Compensation for Work Injuries. This document can also be accessed by going to [https://vaww.visn4.portal.va.gov/pittsburgh/home/KC/Documents/HR-023 Compensation For Work Injuries.doc](https://vaww.visn4.portal.va.gov/pittsburgh/home/KC/Documents/HR-023%20Compensation%20For%20Work%20Injuries.doc)

Appendix G: Guidelines on Multiple Major Survival Surgeries

VA Pittsburgh Healthcare System IACUC Guidelines on Multiple Major Survival Surgeries

Purpose

The purpose of these guidelines is to give guidance to investigators who find it necessary to perform multiple major survival surgeries in animals in order to fulfill the research objectives, for humane purposes, to conserve scarce animal resources, or for the animal's well being. Necessary veterinary procedures may result in multiple major surgeries in an animal, but do not require justification.

Background

Both the USDA Regulations and the Guide for the Care and Use of Laboratory Animals address this issue in terms similar to those used above in the 'Purpose' of this document. "Major surgery" is defined as surgery that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological function. A second major surgery in which the animal is euthanatized without recovering from anesthesia is not considered to be multiple major survival surgery.

Guidelines

Multiple major surgeries on the same animal are discouraged. If it is proposed, the PI must justify its use either for scientific reasons, or the other reasons listed above under "Purpose". Multiple major surgery cannot be justified by cost savings alone. It is the obligation of the Subcommittee on Animal Research to evaluate any justification of multiple major survival surgery, to communicate their determination to the PI, and to further consider any disagreement(s) that exist between the PI and the subcommittee.

(Approved 9/19/02)

Appendix H: Guidelines for Anesthetics and Analgesics for Use in Rodents

VA Pittsburgh Healthcare System IACUC Guidelines Recommended for Anesthetics and Analgesics for use in Rodents

The following drugs and combinations of drugs are recommended by the Pittsburgh VAMC Subcommittee on Animal Research (SAR). All drugs proposed to be used in animals must be described in the ACORP and approved by the SAR. Drugs of these categories and combinations of them that are not listed in this document can be used when approved by the SAR.

Anesthetics

Inhalants

Inhalants are preferred because of the ease of controlling depth of anesthesia and the brevity of recovery. However, the animal's vital signs must be monitored to avoid an overdose or light anesthesia. Reliable vaporizers must be used for the effective delivery of volatile anesthetics; oxygen or a mixture of oxygen and nitrous oxide are the carrier gases of choice, The combination of oxygen and nitrous oxide can be used at 30%/70% or 50%/50% respectively. Humans must not be exposed to the vapors of inhalant anesthetics.

- Isoflurane: Induction is best done in a chamber using 4% isoflurane. Most rodents can usually be maintained in surgical anesthesia using 1 to 3% isoflurane, but it is used at +1- to effect.

Injectables

Injectable anesthetics are more difficult to titrate than inhalants and usually have a longer recovery period. However, they have the advantage of ease of administration and the lack of concern of human exposure.

1. Ketamine: By itself, ketamine should be used for restraint and for minor procedures as it usually does not produce deep anesthesia and causes muscular rigidity. However, in the following combinations, it can be used for major surgical procedures in rodents:

A. Ketamine and Xylazine (respectively)

Rat: 40-80mg/kg TM and 5-10 mg/kg IM

Mouse: 80-120mg/kg TM and 5-10 mg/kg IM

B. Ketamine, Xylazine, and Acepromazine (respectively)

Mouse: 30 mg/kg IM, 6 mg/kg TM, and 1mg/kg TM

2. Sodium Pentobarbital: The former "gold standard" of veterinary anesthesia, pentobarbital, can still be used but the lack of good analgesia is a drawback.

Rat: 40-50 mg/kg IP to effect

Mouse: 40-70 mg/kg IP to effect. Recommend giving ketoprofen 5 mg/kg SC for supplemental analgesia; other analgesics can be used, but some cause additional respiratory and cardiac depression.

3. Avertin: No longer available commercially but can be formulated as follows: 2.5 gm. 2,2,2-tribromoethanol in 5.0 ml of 2-methyl-2-butanol (tertiary amyl alcohol) and raise temperature to 50 degrees C; add this mixture to 200 ml of sterile distilled water and stir to dissolve. This gives a 1.25% solution. Avertin must be protected from light and stored at 4 degrees C; discard if the pH falls below 5.0 as it becomes toxic.

Mouse: 0.2ml of the above solution/10 gram BW, IP

Analgesics

It has been shown that animals, including humans, recover faster from surgery when postsurgical analgesics are used. Analgesics are most effective if they are used in effect before the pain starts. Analgesics should be used postsurgically, except for very minor procedures, and when other painful situations are expected unless there is a scientific reason for not using them.

1. Ketoprofen:
Rat: 5.0mg/kg, SC, Q 12hrs
Mouse: 5.0 mg/kg, SC, Q 12 hrs

2. Buprenorphine:
Rat: 0.01 to 0.05 mg/kg, SC, Q 8-12 hrs
Mouse: 0.05 to 0.1 mg/kg, SC or IV, Q 12 hrs

3. Butorphenol:
Rat: 2.0 mg/kg, SC, Q 4 hrs
Mouse: 1 to 5 mg/kg, SC, Q 4 hrs

4. Morphine:
Rat: 2.5 mg/kg, SC, Q 2-4 hrs
Mouse: 2.5 mg/kg, SC, Q 2-4 hrs

Appendix I: Animal Research Facility Disaster Plan

The Animal Research Facility Disaster Plan can be accessed [here](#) or by going here http://www.vaphs.research.med.va.gov/revamp/animal_research.htm.

Appendix J: Preparation Instructions for Clidox-S

STANDARD OPERATING PROCEDURE FOR PREPARING STERILANT DILUTION OF CLIDOX-S

1. Determine the amount of mixed product that will be needed for a period not to exceed 24 hours.
2. Triple rinse the holding container with tap water.
3. Measure out one (1) part of CLIDOX-S base, add to holding container.
4. Measure out five (5) parts TAP WATER, add to base in holding container.
5. Measure out one (1) part of CLIDOX-S activator, add to base-water mixture in holding container.
6. Wait 10 - 15 minutes before using.
7. Label container with the following information:
Clidox-S Sterilant dilution
Today's date
8. Proceed with fogging instructions (or mopping instructions).