Use of Robarts Imaging Suites: Biosafety Requirements for In Vivo and In Vitro Work

Approved: July 2012
Western University Biohazards Subcommittee
Supersedes: February 2009
Original Approved February 13, 2008

1.0 Introduction and Scope:

Imaging Facilities at Robarts are used for *in vitro* and *in vivo* work by researchers throughout London affiliated with Western University. The objective of this document is to ensure that this research meets the standards set by the latest versions of the Public Health Agency of Canada Laboratory Biosafety Guidelines, the Containment Standards for Veterinary Facilities by the Canadian Food Inspection Agency and, where animals are involved, the Canadian Council for Animal Care (CCAC). This work must also follow the Biosafety Guidelines and Procedures Manual.

The goal of this document is to ensure that in vitro and in vivo experiments meet all applicable guidelines and regulations and are done within the proper containment to protect the work, the animals, the facilities, and the faculty, staff, and students who perform the work.

- This document applies to the 3T MRI, 9.4T Imaging Suite (MRI), Human High Field MRI Laboratory (3T & 7T), and Preclinical Imaging Suite (MicroCT, Ultrasound, SPECT CT) imaging facilities and includes procedures for transport to the facility. With respect to the primate facilities (including the 9.4T MRI suite), upon arrival at the facility the approved facility Standard Operating Procedures (SOPs) take effect.
- This document applies only to containment Level 1 (CL1), containment Level 2 (CL2) or containment Level 2 with Level 3 operations (CL2+). Research requiring Level 3containment must contact the Biosafety Officer at biosafety@uwo.ca. Level 2 research involving live non-human primates must follow the SOPs for the Center for the Brain and Mind.

1.1 General Safety Precautions for In vivo and In vitro Imaging

- All personnel operating the imaging equipment (9.4T MRI, 3T MRI, 7T MRI, MicroCT, SPECT CT, and Ultrasound) must be trained by Facility Manager or designate.

- All personnel handling animals must have the required Animal Care and Veterinary Services (ACVS) training.
- All animal work must be outlined in an approved animal use protocol.
- All personnel using the Imaging facilities must be trained and follow the SOPs in place for each facility.
- Supervisors must ensure that people using the Imaging facilities have the appropriate health and safety training for the work being performed, per Health and Safety Training requirements.
- Personnel using each Imaging facility must wear the appropriate personal protective equipment. For more information, see the Laboratory Health and Safety Manual, or contact the Lab Safety Coordinator.
- Disposal of waste, including hazardous chemical waste, biomedical waste, animal waste and carcasses, must follow the Hazardous Materials and Waste Management Handbook.
- Work carried out must meet the requirements of the Biosafety Guidelines and Procedures Manual.
- Personnel should complete their Hazard Communication Form (Western login required) and have the appropriate medical surveillance. For information, please see Workplace Health.
- In case of an emergency, such as medical or fire, personnel follow the SOPs in place for the facility accessible on-line or in the Robarts Health and Safety Office:
    - Preclinical Imaging Suite: SOP 900 – Emergency Procedures
    - 9.4T MRI Facility: SOP 300 – Standard Operating Procedure: Emergency Fire Procedures
    - 3T MRI Facility: SOP 3T 215, 210, and 205 – Standard Operating Procedures for Emergency Quench, Fire Code Blue
    - Human High Field MRI Lab: SOP 220, 230, and 210 – Emergency Fire, Emergency Quench, Emergency Code Blue

Where there is an emergency involving human and animal wellbeing, human health and safety is the priority.

- The Principal Investigator must have an approved, current Biological Agents Registry Form on file with the Biosafety Office which reflects the research being done. For more information, visit the Biosafety website.
- The Biosafety Officer(s) in association with the Director, Animal Care and Veterinary Services and the Biohazards Subcommittee determine the containment level required for the work being performed.

1.2 Transportation of Animals

1.2.1 Transportation of Level 1 Rodents

Level 1 rodents are those not exposed to a Containment Level 2 (CL2) or higher CL agent via ingestion, inhalation, injection, or absorption and are not known to carry a Level 2 zoonotic agents. Level 1 rodents may be transported to the Robarts imaging facilities and within the Robarts building using standard cages. Level 1 rodents may be transported to the University or within the University buildings in standard cages. During transportation, these cages should be covered with a blanket (or equivalent).

1.2.2 Transportation of Level 2 Rodents

Level 2 rodents are those which have been exposed to a CL2 agent. Level 2 rodents must be transported in a covered HEPA-filtered cage or an apparatus. The cages or apparatus must be approved by the Director, ACVS and the Biosafety Officer(s) for Robarts. The transportation of Level 2 animals by road, rail, water or air must also follow the appropriate transportation of dangerous goods regulations.

1.2.3 Transportation of Non Human Primates (NHP)

Transportation of non human primates is governed by a separate set of SOPs that have been approved by ACVS, members of the Centre for Brian and Mind, and the Biosafety Officers for Robarts. These SOPs are available in the Brain and Mind Facility or the Robarts Health and Safety office and are to be followed for the transportation of primates (NHP) to and from the primate (NHP) quarters and the MRI suites.

2.0 Introduction to Rodent and Non Human Primate (NHP) Imaging Research

Animal projects must be approved by the Animal Use Subcommittee of the University Council on Animal Care. Animals are housed in areas approved by Animal Care and Veterinary Services (ACVS) and the Canadian Council on Animal Care (CCAC). Animals are transported to the facility in cages on carts.

2.1 Imaging Involving Level 1 Rodents

- Level 1 rodent work involves rodents that have not been exposed to a Level 2 (or higher CL) agent via ingestion, inhalation, injection or absorption and that are not known to carry a Level 2 (or higher CL) zoonotic agent. An example of a Level 1 rodent is an animal procured from a commercial supplier or one injected with a murine pathogen free cell line approved by Biosafety at Level 1.

2.1.1 Safety Precautions

- Follow the SOPs for the decontamination of samples entering the facility and the clean-up of animal excrement, including surface disinfection. Disinfectants must be approved by the Biosafety Officer or in the SOP and must be effective and safe to use on the equipment. The SOPs are available on-line or in the Robarts Health and Safety Office:

    - Third Floor Preclinical Imaging Suite: SOP 500 – Cleaning and Decontamination
    - First Floor 9.4T MRI Facility: SOP 415 – Cleaning and Disinfection – Level 1 & 2 Experiments
    - Second Floor 3T MRI Facility: SOP 400 – Standard Operating
    - Procedure for MRI Decontamination
    - First Floor Human High Field MRI Lab: SOP 415 – Cleaning and Decontamination – Level 1 & 2 Experiments

- Gloves and other personal protective equipment must be changed if they have been in contact with animal wastes.
- Procedures such as injections, surgery, anesthesia, and euthanasia can be done on the open bench. Scavenging devices must be used in association with anesthesia or euthanasia with a gaseous agent. If a hazardous chemical or radioactive material is involved, this may require the use of a fume hood elsewhere and additional precautions/approvals.
- The animal may be placed in the coil or bed on the open bench.
- In case of a veterinary emergency, life-saving procedures can be done on the open bench.

2.2 Imaging Involving Level 2 Rodents

- Level 2 Rodent work involves animals that have been exposed to a Level agent via ingestion, inhalation, injection or absorption or carry a level 2 zoonotic agent. Examples of Level 2 pathogens include:

    - Viral vectors such as adenovirus and retroviruses
    - Human cell lines such as HEK293, which carries an activated human oncogene, or non-human primate cell lines such as cos-7, because they may carry viruses capable of infecting humans
    - Microorganisms such as bacteria, parasites, etc.
    - Biological toxins such as pertussis aflatoxin and cholera toxin.

Contact the Biosafety Officer at biosafety@uwo.ca for the containment level of the project. For more information, please visit the Biosafety website.

2.2.1 Safety Precautions

2.2.1.1 Projects Involving Level 2 Cell Lines, Primary Cells and Biological Toxins

Projects involving animals that are exposed to Level 2 cell lines or Level 2 primary cells, which do not contain viruses and/or are not shedding biohazardous agents, must follow the measures listed in Section 2.1.1. In addition to this:

- Level 2 agents must be handled in a certified biological/ HEPA filtered safety cabinet
- Animals must be transported to the facility in cages or carts
- Animals must be housed in an approved ACVS Level 2 housing facility

Projects involving Level 2 viral vectors, viruses, microorganisms or cells that are transformed using vectors require an additional level of safety unless it can be shown that the agent(s) are no longer present and/or being shed. For more information, please see the Viral Vector Policy.

2.2.1.2 Projects Involving Other Level 2 Agents

For these Level 2 projects, there are additional Safety Precautions to those in Section 2.1.1.

- Level 2 agents must be handled in a certified Class 2 biological safety cabinet. Animals that have been exposed to a Level 2 agent must be kept in an approved HEPA-filtered cage or apparatus during the duration of the experiment, including housing, transportation, imaging and during veterinary life saving measures.

- Personnel using an approved HEPA-filtered cage or apparatus must have a plastic container with them. In case of failure or leakage of the cage or apparatus, the cage or apparatus (with the animal inside) is put in the plastic container. The container can only be opened in a biological safety cabinet.

- Animals exposed to a Level 2 agent must be housed in a certified ACVS approved Level 2 housing facility.

2.2.1.3 Preclinical Imaging Suite and Second Floor 3T MRI Facilities

Personnel can transport the animals in a HEPA-filtered cage to the imaging facility. The cage must be opened in the certified biological safety cabinet to perform procedures such as injections, anesthesia and veterinary life saving measures. The animal is placed in a HEPA-filtered apparatus for imaging in the biological safety cabinet. After imaging, the rodent is transported to a biological safety cabinet in an approved Level 2 housing facility. The apparatus is never opened except in a biological safety cabinet.

The apparatus must be certified by a certified contractor such as HEPA Filters Inc. The apparatus must be approved by the Biosafety Officers for Robarts and Animal Care and Veterinary Services. The apparatus must maintain Level 2 containment, and requires safety features such as HEPA filtration, O-rings, threaded ends.

HEPA-filtered cages must be approved by the Biosafety Officers for Robarts and by Animal Care and Veterinary Services.

Waste is collected from the certified biological safety cabinet in bags. The bag is closed in the biological safety cabinet and disposed of by the research personnel. Carcasses are disposed of by research personnel. Waste is disposed of per the Hazardous Materials and Waste Management Handbook.

2.2.1.4 9.4T MRI Facility and Human High Field MRI Laboratory (3T & 7T)

2.2.1.4.1 Approach #1

This facility does not contain a biological safety cabinet. Procedures must be done in a certified biological safety cabinet in an approved Level 2 facility elsewhere.

Animals must be placed in an approved HEPA-filtered imaging apparatus in a certified biological safety cabinet in an approved Level 2 laboratory. Animals are transported to the facility and imaged in this apparatus. The apparatus is never opened except in a biological safety cabinet.

Waste is collected in autoclaveable bags and disposed of by the research personnel. Carcasses are also disposed of by research personnel. Waste is disposed of per the Hazardous Materials Management Handbook.

The apparatus must be certified by a certified contractor such as HEPA Filters Inc. The apparatus must be approved by the Biosafety Officers for Robarts and Animal Care and Veterinary

Services. The apparatus must maintain Level 2 containment, and requires safety features such as HEPA filtration, O-rings, threaded ends.

2.2.1.4.2 Approach #2

In some cases, approach #1 is impractical; approach #2 can then be used for Level 2 rodents. This is based on a case-by-case risk assessment and is approved by the Biosafety Officers for Robarts and Animal Care and Veterinary Services.

When the rodents have been previously exposed to a Level 2 agent, they are brought to the MRI facilities for imaging using an approved HEPA-filtered transport cage on a cart and placed in the appropriate imaging insert coils.

Approach #2 for MRI and fiber optic imaging of Level 2 animals in the MRI suites is based on designing and constructing the whole lab to be under Level 2 containment. This means that the air entering and leaving the MRI suites is HEPA-filtered. Entrance is through a controlled air lock and the room is under negative air pressure to the adjacent corridor. Personnel must wear the appropriate personal protective equipment as mandated by the MRI Facility's SOP 210-01. This includes the wearing of a fit-tested N95 respirator when working with Level 2 animals as a certified biological safety cabinet is not available. Protective clothing must be removed before leaving the MRI facilities as stated in SOP 210. Decontamination procedures for the suites are outlined in the Facility's SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 for the Center for Brain and Mind. Personnel must be specially trained to work in the MRI Level 2 containment suites.

Waste is collected in autoclaveable bags and disposed of by the research personnel. Carcasses are also disposed of by research personnel. Waste is disposed of per the Hazardous Materials Management Handbook.

2.3 Imaging Involving Non-Human Primates

Approach #2 for MRI and fiber optic imaging of Level 2 animals in the MRI suites is based on designing and constructing the whole lab to be under Level 2 containment. This means that the air coming in and leaving the MRI suites is HEPA-filtered. Entrance is through a controlled air lock and the room is under negative air pressure to

the adjacent corridor. Personnel must wear the appropriate personnel protective equipment as mandated by the MRI Facility's SOP 210-01. This includes the wearing of a fit-tested N95 respirator when working with Level 2 animals as a certified biological safety cabinet is not available. Protective clothing must be removed before leaving the MRI facilities as stated in SOP 210. Decontamination procedures for the MRI suites are outlined in the Facility SOP 415 and the MRI Suite Decontamination procedures for the suites are outlined in the Facility's SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 and other Center for Brain and Mind Rhesus Facility Standard Operating Procedures. Personnel must be specially trained to work in the MRI Level 2 containment suites.

3.0 Introduction to *In vitro* Research Involving Imaging

Samples are prepared for imaging in an approved biosafety laboratory. Samples are brought to the imaging facility in sealed leak- and shatter-proof containers. Samples are put in a coil or a bed and/or HEPA-filtered apparatus for imaging purposes.

3.1 Imaging Involving Fixed Samples

Level 2 or Level 2plus samples fixed with chemicals such as formalin or comparable agent are no longer considered biohazardous. These samples can be imaged as Level 1 samples. If samples need to be opened, they should be opened in a chemical fume hood.

3.2 Imaging Involving Level 1 *In Vitro* Work

Samples must be transported to the facility in sealed leak- and shatter-proof containers. Containers must be wiped off with a disinfectant before they leave the laboratory and per the SOPs for the facility. Work with these samples can be done on the open bench, providing that no hazardous chemicals are involved. If hazardous chemicals or radioactive materials are involved, work must be done in a fume hood elsewhere and additional precautions/approvals are required.

3.3 Imaging Involving Level 2 *In vitro* Work

For Level 2 projects, there are additional safety precautions to those in 3.1. Samples must be worked with using a certified biological safety cabinet.

3.3.1 Preclinical Imaging Suite and 3T MRI Facilities

If required, samples can be opened under the certified biological safety cabinet provided.

3.3.2 9.4T MRI Facility and Human High Field MRI Laboratory (3T & 7T)

There are no biological safety cabinets in these facilities. Samples must be prepared in a biological safety cabinet in an approved Level 2 laboratory elsewhere. Sealed leak- and shatter-proof containers are not to be opened in the facilities. The sample is kept closed during transportation and imaging of the samples.

4.0 Imaging Involving Work at Level 2 plus Level 3 Operations

The researcher must have an approved, current Biological Agents Registry Form on file with the Biosafety Office which reflects the research being done. For more information, visit the Biosafety webpage.

Certain projects, such as some research involving lentiviral-based vectors, require Level 2 plus Level 3 operations. For Level 2 plus Level 3 projects there are additional safety precautions. All work must be carried out in a certified biological safety cabinet.

4.1 Imaging

- Use portable autoclave to decontaminate waste prior to leaving the imaging facility. Follow the “SOP for the Sanyo Portable Autoclave”.
- Injections must be done in the approved Level 2 plus 3 laboratory or the Level 3 facility on DSB, 6th floor.
- Animals transported on a cart to or within Robarts for imaging must be in a HEPA-filtered cage unit approved by Biosafety and ACVS.
- The cages can be removed from the transport cart and placed in a certified biological safety cabinet. Animals must be placed in an approved HEPA-filtered imaging apparatus (see section 2.2.1.1) in a biological safety cabinet in an approved Level 2 plus 3 laboratory. Animals are transported to the facility and imaged in this apparatus. The apparatus is never opened except in a biological safety cabinet.
- After scanning, all reusable material (i.e. forceps) must be decontaminated in a Wescodyne solution in a biological safety cabinet. The Wescodyne working solution has: 40% H_2O, 40% ethanol and 20% Wescodyne. It can be prepared in advance.
- Submerse all the reusable instruments (surgical) in the labelled Wescodyne solution for 2 hours.
- Rinse the instruments after 2 hours with H_20 and let dry.
- After drying, pack in autoclave bags and sterilize in the portable autoclave (this is done to ensure successful sterilization).

- The procedures for disinfection of contaminated animal cages and bedding must be completed. Bedding must be emptied into a biohazard bag inside of the biosafety cabinet. The bedding must be then double bagged and sealed inside a biological safety cabinet. The bag must be wiped with a disinfectant before it is removed from the biological safety cabinet for disposal per the Hazardous Materials Management Handbook.

- Inside the hood, to the empty cage add Wescodyne solution and swirl to ensure contact of all surfaces. Wipe the cage lid with Wescodyne as well and ensure contact for 2 hours (either leave the cage in a dunk tank for 2 hours or put the wet cage into an autoclave bag and leave in the hood for 2 hours). Drain the Wescodyne and return the cages and lids for washing and packing to be autoclaved. Follow the procedures for the facility where the cages came from (ACVS or Robarts barrier facility).

- All sharps must be disposed of in a sharps container within the biosafety cabinet. The container must be wiped on the outside with the Wescodyne solution. The containers are then sent to the incinerator.

- All waste must be labelled appropriately before it is taken for disposal.

- After the scan the rodent/animal must be returned to the biological safety cabinet before it is removed from the HEPA-filtered apparatus and then it can be returned to its cage.

- Disposable personal protective equipment, such as gloves, must be put in an autoclaveable biohazard bag leaving the room.

- Wescodyne solution can be treated as hazardous waste after use per the Hazardous Materials and Waste Management Handbook.