



USE OF ROBARTS IMAGING SUITES: BIOSAFETY REQUIREMENTS FOR *IN VIVO* AND *IN VITRO* WORK

Draft: Original Approved February 13, 2008 (University of Western Ontario Biosafety Committee)

1.0 Introduction and Scope:

- Imaging Facilities at Robarts are used for *in vitro* and *in vivo* work by researchers throughout London affiliated with the University of Western Ontario. The objective of this document is to ensure that this research meets the standards set by the latest versions of the Health 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 found at: www.uwo.ca/humanresources/biosafety. As guidelines and regulations continually change this document will be updated and revised to reflect those changes as we become aware of them.
- The goal of this document is to ensure that *in vitro* and *in vivo* experiments are done under the proper containment to protect the work, the animals, the facilities and the faculty, staff, students who perform the work. Containment may be accomplished by various approaches, such as by using an apparatus to contain the biohazardous agent(s) or through facility design and standard operating procedures.
- **Within the Robarts Research Institute there are two approaches to accomplish imaging when biohazards are involved for *in vitro* prepared samples, *ex vivo* prepared samples and live *in vivo* animal imaging:**

Option 1

Imaging of containment level 1, 2 or level 2 with level 3 operational requirements can be completed safely using the appropriate containment standards as outlined in the latest version of Health Canada Laboratory Biosafety Guidelines, the containment standards for Veterinary Facilities by the CFIA and where animals are involved the Canadian Council for Animal Care, Westerns Biosafety Guidelines and Procedures and this document. (see section 2.2.1.2.).

Option 2

Alternatively, where possible imaging can be conducted in specially constructed Biosafety Level 2 facilities where the facility and the exhaust air out of the facility are H.E.P.A. filtered and are constructed to meet all other biosafety level 2 requirements. Personnel **must** wear appropriate personal protective equipment, see section 2.2.1.2.1. The 9.4T MRI suite (contains the 9.4T MRI scanner and the fiber optic confocal microscope) and the new Brain and Mind Imaging suite currently under construction (will contain 3T and 7T MRI scanners) are the only two imaging facilities that meet the appropriate biosafety containment standards. Both these suites house animal preparation areas but do not have laminar flow hoods. Thus when live infectious recombinant vectors are to be used personal protective equipment must be worn including an N95 mask

- This document applies to the 3T MRI, 9.4T Imaging Suite (MRI), and Preclinical Imaging Suite (MicroCT, Ultrasound, SPECT CT) imaging facilities, including transport to the facility. With respect to the primate facilities (including the 9.4T MRI suite) upon arrival at the facility the approved facility SOPs take effect.
- This document applies to Level 1, Level 2 or Level 2 (plus Level 3 operations) only. Research requiring Level 3 containment must contact the biosafety office at biosafety@uwo.ca. Level 2 research involving live non-human primates must follow the Standard Operating Procedures (SOP's) 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, 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 training.
- All personnel using the facility must be trained and follow the Standard Operating Procedures (SOP's) in place for the facility.
- Supervisors must ensure that people using the facility have the appropriate health and safety training for the work being performed, per the Health and Safety Training found at:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/training/training_idx.htm
- Personnel using this facility must wear the appropriate personal protective equipment. For more information, see the Laboratory Safety Manual, www.uwo.ca/humanresources or contact the Lab Safety Coordinator.
- Disposal of waste, including hazardous chemical waste, biomedical waste and carcasses, must follow the Hazardous Material Management Handbook:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/enviromental_prog/enviromental_idx.htm

- Work carried out must meet the requirements of the Biosafety Guidelines and Procedures Manual found at: www.uwo.ca/humanresources/biosafety
- Personnel should complete their Position Hazard Communication Form and have the appropriate medical surveillance. For information, please see: <http://www.wph.uwo.ca/newposition.htm>.
- In case of an emergency, such as medical or fire, personnel follow the SOP's 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

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

- The researcher must have an approved, current Biohazardous Agents Registry Form on file with the biosafety office which reflects the research being done. For more information, see: www.uwo.ca/humanresources/biosafety.
- The biosafety officer(s) in association with the Director, Animal Care and Veterinary Services and the Biohazard 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 rodents not exposed to a Level 2 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.

1.2.2 Transportation of Level 2 Rodents

Level 2 rodents must be transported in a HEPA-filtered cages 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

Transportation of non human primates is governed by a separate set of SOP's that have been approved by ACVS, members of the Brain 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 to and from the primate quarters and the MRI suites.

2.0 Introduction to Rodent and Non-Human Primate Imaging Research

Animal projects must be approved by the Animal Use Subcommittee. **Animals** are housed in areas approved by Animal Care and Veterinary Services (ACVS) and CCAC. **Animals** are transported to the facility in cages on carts. **Animals** may undergo procedures, such as injections or anesthesia. Animals are placed inside a suitable coil, bed and/or approved HEPA-filtered apparatus. It is then scanned by a trained operator. Animals may be euthanized if necessary after the imaging is complete.

2.1 Imaging Involving Level 1 Rodents

- Level 1 Rodent work involves rodents that have not been exposed to a Level 2 agent via ingestion, inhalation, injection or absorption and that are not known to carry a Level 2 zoonotic agent. An example of a Level 1 rodent is an animal procured from a commercial supplier or rodents injected with a cell line classified by biosafety as Level 1.

2.1.1 Safety Precautions

- Follow the Standard Operating Procedure (SOP's) for the decontamination of samples entering the facility and the clean-up of animal excrement and surface disinfection. Disinfectants must be effective and safe to use on the equipment. The SOP's 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

- Gloves and other personal protective equipment must be changed if they have been in contact with animal wastes.
- Procedures such as injections, **surgery**, anesthetizing and euthanization can be done on the open bench. Scavenging devices must be used on equipment used for anesthetizing or euthanization 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, procedures such as life-saving measures 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 2 agent via ingestion, inhalation, injection or absorption or carry a Level 2 zoonotic agent. Examples of Level 2 pathogens include:
 - ◆ Viral vectors such as adenoviral vectors
 - ◆ Human cell lines such as HEK 293 or non-human primate cell lines such as cos-7 because they carry viral genes capable of cell transformation
 - ◆ Microorganisms such as Salmonella sp. or Pseudomonas sp.
 - ◆ Biological toxins such as pertussis and cholera toxin.
 - ◆ Contact the biosafety officer at biosafety@uwo.ca for the containment level of the project. For more information, please see www.uwo.ca/humanresources/biosafety

2.2.1 Safety Precautions

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

- Level 2 agents must be handled in a 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 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 Level 2 housing facility.

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

Waste is collected in the 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.

2.2.1.2 9.4T MRI Facility

2.2.1.2.1 Approach #1

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

Animals must be placed in an approved HEPA filtered imaging apparatus in a 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. ~~The bag is closed in the biological safety cabinet~~ and disposed of by the research personnel. Carcasses are disposed of by research personnel.

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.

2.2.1.2.1 Approach #2

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

Animals are brought to the 9.4T MRI facility for imaging where the rodents have been previously exposed to a Level 2 agent in an approved HEPA filtered transport cage on a cart and taken to the 9.4T suite where the animal can be placed in the appropriate imaging insert coils.

Approach #2 for MR and fiber optic imaging of Level 2 animals in the 9.4T MRI Suite and 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 9.4T MRI suite is HEPA filtered. Entrance is through a controlled air lock and 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 biological safety cabinet is not available. Protective clothing must be removed before leaving the 9.4T facility as stated in SOP 210. Decontamination procedures for the 9.4T suite are outlined in the Facility's SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for the Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 for the Center for the Brain and Mind. Personnel must be specially trained to work in the 9.4T MRI Level 2 containment suite.

Waste is collected in autoclaveable bags. ~~The bag is closed in the biological safety cabinet~~ and disposed of by the research personnel. Carcasses are disposed of by research personnel.

2.3 Imaging Involving Non-Human Primates

Approach #2 for MR and fiber optic imaging of Level 2 animals in the 9.4T MRI Suite and 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 9.4T MRI suite is HEPA filtered. Entrance is through a controlled air lock and 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 biological safety cabinet is not available. Protective clothing must be removed before leaving the 9.4T facility as stated in SOP 210. Decontamination procedures for the 9.4T suite are outlined in the Facility SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for the Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 and other Center for the Brain and Mind Rhesus Facility Standard Operating Procedures. Personnel must be specially trained to work in the 9.4T MRI Level 2 containment suite.

Once the space under renovation for the new Brain and Mind 3T and 7T magnets in the Cuddy wing is completed (as Level 2 containment laboratories using the same design features as the 9.4T suite) and certified by the Biosafety Officers for Robarts, the same SOP's will apply.

3.0 Introduction to *In vitro* Research Involving Imaging

Samples are prepared for imaging in a biosafety laboratory. Samples are brought in sealed, leak and shatter proof tubes or other containers to the imaging facility. 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 2+3 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 SOP's 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 biological safety cabinet.

3.3.1 Preclinical Imaging Suite and 3T MRI Facilities

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

3.3.2 9.4T MRI Facility

There is no biological safety cabinet in this facility. 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 facility. The sample is kept closed during transportation and imaging of the samples.

4.0 Imaging Involving Level 2 plus Level 3 Work

The researcher must have an approved, current Biohazardous Agents Registry Form on file with the biosafety office which reflects the research being done. For more information, see: www.uwo.ca/humanresources/biosafety

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 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 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), they must be decontaminated in a Wescodyne solution in a biological safety cabinet. The Wescodyne working solution has: 40% H₂O, 40% ethanol and 20% Wescodyne. (It can be prepared in advance)
- Submerge all the reusable instruments (surgical) in the labelled Wescodyne solution for 2 hours.
- Rinse the instruments after 2 hours with H₂O and let dry.
- After drying, pack in autoclave bags and autoclave 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.
- 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 Waste Management Handbook:
http://www.uwo.ca/humanresources/docandform/docs/ohs1/manuals/hazardous_handbook.pdf.