The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: August 12, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**David Holdsworth**
DEPARTMENT:	**Imaging Research**
ADDRESS:	**Robarts Research Institute Rm. 1254C**
PHONE NUMBER:	**x24154**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**david.holdsworth@imaging.robarts.ca**

Location of experimental work to be carried out :

Building :	**Robarts Research**	Room(s):	**3232, 3232B, 3232C,**
Building :	**Robarts Research**	Room(s):	**2251**
Building :		Room(s):	

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR**

GRANT TITLE(S): **Advanced anatomical and dynamic micro-computed tomography for arthritis research**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Chris Norley	**cnorley@imaging.robarts.ca**	
Hristo Nikolov	**hnikolov@imaging.robarts.ca**	
Craig Tschirhart	**ctschirh@uoguelph.ca**	

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Samples (frozen human bone cleaned of all tissues by collaborators and instrumented with the required mechanical load testing apparatus) will be transported to Robarts in sealed containers that have been double bagged in sealed plastic bags or sealed in plastic wrap. The samples will be mounted on the required scanner and scanned while sealed.

For some experiments that will require the meniscus to be torn, the sealed specimens will be moved to an approved biohazard ~~fumehood~~ Safety Cabinet RD, the plastic wrap will be removed and a meniscal beak tear will be induced. The joint will be resealed in plastic wrap and returned to the scanner for additional testing.

Once all scans are complete, the sealed bone specimens will be returned to the collaborators. The scanners will be cleaned with quatricide, as is performed following all scans.

At all times, protocols will confirm to the standard operating procedures outlined in "Use of Robarts imaging suites: Biosafety requirements for in vivo and in vitro work," section 3.0, pages 7-8 (Approved February 2009).

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

Musculoskeletal disorders, such as arthritis, are the most common cause of severe long-term pain and physical disability affecting hundreds of millions of people around the world. As the world's population ages, the extent of the problem will increase, placing huge burdens on societies and health care systems. This proposal describes the development of new imaging techniques – using high-resolution computed tomography (CT) – to be used in research applications related to arthritis. Arthritis is a significant cause of pain and disability, and there are few effective treatments available. Basic research to understand arthritis and develop improved treatments is often carried out in small laboratory animals (such as the rat) and on human bone specimens (obtained from cadavers). Our group has experience with animal models of arthritis, in surgical treatment of arthritis, and in the development of CT imaging systems for clinical applications and basic research. Over the next five years, we intend to develop innovative CT-based tools that will address fundamental challenges associated with basic OA research using micro-CT. We have four major objectives: 1) to implement a novel "tilted-detector" approach, increasing the volumetric spatial resolution of benchtop micro-CT systems by up to an order of magnitude; 2) to develop and test a new intra-vascular contrast agent that is optimized for dual-energy imaging of blood vessels near bone; 3) to develop a new procedure for characterizing joint replacement components using micro-CT prior to implantation; and 4) to develop techniques to see inside human bones while they are being tested for mechanical strength.

Related to this research program, Objectives 1 and 3 do not involve biohazardous materials. Objective 2 involves micro-imaging of normal Spraque-Dawley rats with no known pathogens.

Objective 3 involves the use of cadaveric bone specimens, which have been obtained from a source (Life Legacy Foundation, Tuscon, AZ) that tests for major infectious diseases prior to shipment. The cadaveric bone specimens are imaged while undergoing mechanical testing in a sealed environment.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☐ YES ☒ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☐ NO
If YES, please give the name of the species ______
What is the origin of the microorganism(s)? ______

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ______

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐ YES ☒ NO
(If NO, please proceed to Section 3.0)

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☐ No		Not applicable
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

**Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)*

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒ YES ☐ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)	**Human Bone**	☐ Yes ☒ Unknown		☐1 ☒2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: **All specimens have been tested for infectious agents (HIV, Hepatitis) and specimens remain in double-sealed containers as per SOP described in**

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☐ YES ☒ NO If **NO**, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☐ YES, complete table below ☐ NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

Please attach a Material Safety Data Sheet or equivalent if available.
****Please attach a plasmid map.***
*****No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Rat**

7.3 AUS protocol # **2007-003-02 (experiments complete)**

7.4 Will any of the agents listed in section 4.0 be used in live animals
☒ NO ☐ YES, specify:

7.5 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

♦ Pound source dogs	☐ YES	☐ NO
♦ Pound source cats	☐ YES	☐ NO
♦ Cattle, sheep or goats	☐ YES, species	☐ NO
♦ Non-human primates	☐ YES, species	☐ NO
♦ Wild caught animals	☐ YES, species & colony #	☐ NO
♦ Birds	☐ YES, species	☐ NO
♦ Others (wild or domestic)	☐ YES, specify	☐ NO

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

10.2 If YES, please give the name of the species.

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name David Holdsworth ***Date:*** October 12, 2011

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☐ 1 ☒ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **January 13, 2011**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-RRI-0050**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
The wound will be expressed to bleed then washed with soap and water and then the individual will be sent to Staff Health.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/newposition.htm

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name David Holdworth ***Date:*** October 12, 2011

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Konrad Noseworthy
Date: Oct. 25, 2011

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

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

Approved: February, 2009
University of Western Ontario Biosafety Committee
(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 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.

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 SOPs take effect.

- This document applies only to containment level 1 (CL1), level 2 (CL2) or level 2 with level 3 operations. Research requiring level 3 containment must contact the Biosafety Officer at biosafety@uwo.ca. Level 2 research involving live non-human primates must follow the Standard Operating Procedures (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 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 Standard Operating Procedures (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 the Health and Safety Training found at: http://www.uwo.ca/humanresources/facultystaff/h_and_s/training/training_idx.htm

- Personnel using each Imaging 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, animal waste and carcasses, must follow the Hazardous Material Management Handbook: http://www.uwo.ca/humanresources/facultystaff/h_and_s/enviromental_prog/environmental_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 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 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 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 those not exposed to a 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.

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 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 (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 Standard Operating Procedure (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 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 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 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 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.

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

Waste is collected from 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. Waste is disposed of per the Hazardous Materials Management Handbook.

2.2.1.2 9.4T MRI Facility and Human High Field MRI Laboratory (3T & 7T)

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 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.

2.2.1.2.2 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 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.

2.2 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 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 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 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 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 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 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) 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 Waste Management Handbook: http://www.uwo.ca/humanresources/docandform/docs/ohs1/manuals/hazardous_handbook.pdf.