Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Personnel
(Please stroke out any personnel to be removed)

Boram Ham

Anthony Bruni

Sarah Spanton

Andrew Martins

Soon-Duck Ha

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	P. aeurogenosa, S. typhimurium	Lactobacillus rhamnosus, E.coli EC1000, E.coli BL21
Approved Cells	Human (primary), rodent (primary), human (established), THP-1, rodent (established), RAW 2649	
Approved Use of Human Source Material	Blood (whole), PARF-CFP, mRFP-Rab7, mRFP-Rab5	
Approved GMO		pTRK830, pOR128, pTRK669
Approved use of Animals	mice	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Apr 28, 2008

Signature of Permit Holder

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Toxin(s) cholera, diphtheria, CONT'D anthrax toxin

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Chair,Biohazards Subcommittee:

The MTA is to obtain vectors to transform Lactobacillus rhamnosus for probiotic study. We will transform L. rhamnosus using these vectors to identify genes involved in macrophage activation in vitro.

pTRK830 (EmR vector for Lactobacillus rhamnosus) -
pORI28 (integration targeting plasmid, EmR)
pTRK669 (helper plasmid, CmR)
E. coli EC1000
/Purpose is for cloning and integration experiments in Lactobacillus rhamnosus.

We prepare recombinant lethal toxin and protective antigen from E. coli. After purification, we use them cell lines or primary peritoneal or bone marrow-derived macrophages in vitro. All toxins are kept in -80 in our lab. Our lab is locked all the time, unless some one is in site.

We are using E. coli-BL21 to express PA and Bacillus megaterium for LF. I believe they are commercial strains.
Mostly we use both toxins to treat cells.

We use toxins to treat mouse primary and immortalized macrophages, but sometimes use human or mouse fibroblasts.

------- Original Message --------
Subject: Re: Containment Question, lethal toxin
Date: Fri, 23 Jan 2009 11:10:08 -0500
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Good morning Ms. Stanley,

The toxins you are describing me are 2 of the 3 components of anthrax toxin. There are too many variables for me to give you an answer. Although the toxins are produced separately in another host, the risk level is most probably the same, the toxins are probably as potent as if they were produced by B. anthracis. Once I receive the importation application, I will have to complete an in depth risk assessment, which will take some time.

However, I think this information will be useful to you.

Bacillus anthracis causes anthrax. B. anthracis requires 2 plasmids for its virulence. One plasmid contains the toxin genes (pX01) and the second plasmid contains the capsular genes (pX02). The exotoxins secreted by B. anthracis, encoded by pX01, are composed of three distinct components: protective antigen (PA), lethal factor (LF), and edema factor (EF). These proteins play a key role in the pathogenesis of anthrax. EF and LF have enzymatic functions but require PA, responsible for their transport into the host, to achieve their biological effects. These proteins individually cause no known physiological effects in animals but in pairs produce two toxic actions. Injection of PA with LF causes death of rats in 60 min, whereas PA with EF causes edema in the skin of rabbits and guinea pigs. S H Leppla, Anthrax toxin edema factor: a bacterial adenylate cyclase that increases cyclic AMP concentrations of eukaryotic cells. PNAS May 1, 1982 vol. 79 no. 10 3162-3166.

This is as much as I can do for now. I hope this information will help you.

Regards

Genevieve Lacroix
A/Head, Importation and Biosafety Program/
Chef Intérimaire, Importation et Services de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada/ Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 946-6982
Fax: (613)941-0596
genevieve_lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Personnel
(Please stroke out any personnel to be removed)

Sarah Spanton

Andrew Martins

Soon-Duck Ha

Additional Personnel
(Please list additional personnel here)

Anthony Bruni

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Approved GMO		
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** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Date of last Biohazardous Agents Registry Form Apr 28, 2008

Signature of Permit Holder:

BioSafety Officer(s): J Hartley Sept 30/08

Chair,Biohazards Subcommittee: S.M. Kilpatrick

Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Toxin(s)		

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Date of last Biohazardous Agents Registry Form Apr 28, 2008

Signature of Permit Holder:

BioSafety Officer(s): J Tanley Sept 30/08

Chair,Biohazards Subcommittee: G.M. Kidder

Bio - UWO - 0147

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: September, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR: Kim, Sung Ouk
SIGNATURE:
DEPARTMENT: Micro & Immuno
ADDRESS: SDRI Rm 119, 1400 Western Rd.
PHONE NUMBER: 82961
EMAIL: sung.kim@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) SDRI Room(s) 119

*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

GRANT TITLE(S):
~~- Dissecting signaling mechanisms of TNF-induced necrotic cell death~~
- Investigating the mechanisms of Nod-like receptor-induced cytokine release and cell death in macrophages

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES: CIHR

Names of all personnel working under Principal Investigators supervision in this location:

Soon-Duck Ha
Andrew Martins
Anthony Bauni
Sarah Spanton

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ✓YES O NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	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?	Source/ Supplier	Health Canada or CFIA Containment Level
P. aeruginosa	✓Yes O No	✓Yes O No	O Yes O No	2×10^9 cells		O 1 ✓2 O 3
S. typhimurium	✓Yes O No	✓Yes O No	O Yes O No	2×10^9 "		O 1 ✓2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ✓YES O 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	✓Yes O No	primary peripheral blood, ~~THP-1~~
Rodent	✓Yes O No	peritoneal, bone-marrow-derived macrophages
Non-human primate	O Yes O No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	✓Yes O No	THP-1	ATCC
Rodent	✓Yes O No	RAW 264.7	ATCC
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

2.4 For above named cell types(s) indicate HC or CFIA containment level required O 1 ✓2 O 3

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

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ✓ YES O 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 Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (if applicable)	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid	Volunteer	✓ Yes O No		O 1 ✓ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs (preserved)		O Yes O No		O 1 O 2 O 3

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? O YES ✓ NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

- HIV O YES O NO
  if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens O YES O NO
  if YES specify_____
- Other human or animal pathogen and or their toxins O YES O NO
  if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen O YES O NO If YES specify_____
- Known oncogenes O YES O NO If YES specify_____

4.4 Will a live viral vector(s) or bacterial plasmid be used for gene transduction O YES O NO
If YES name _____.
Please attach a Material Safety Data Sheet or equivalent.

4.5 List specific vector(s) to be used: _____

4.6 Will virus be replication defective O YES O NO

4.7 Will virus be infectious to humans or animals O YES O NO

4.8 Will this be expected to increase the Containment Level required O YES O NO

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? O YES O NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? O YES O NO O PENDING

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ✓ YES O NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used mouse, C57 BL/6

6.3 AUS protocol # pending

6.4 If using murine cell lines, have they been tested for murine pathogens? ✓ YES O NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:

- Pound source dogs O YES ✓ NO
- Pound source cats O YES ✓ NO
- Cattle, sheep or goats O YES ✓ NO
- Non- Human Primates O YES ✓ NO If YES specify species _____
- Wild caught animals O YES ✓ NO If YES specify species _____ colony # _____
- Birds O YES ✓ NO
- Others (wild or domestic) O YES ✓ NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ✓ NO
If no, please proceed to Section 9.0

8.2 If YES, please name the toxin_____

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.0 Import Requirements

9.1 Will the agent be imported? ✓ YES O NO
If no, please proceed to Section 10.0
If yes, country of origin USA

9.2 Has an Import Permit been obtained from HC for human pathogens? ✓ YES O NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ✓ YES O NO

9.4 Has the import permit been sent to OHS? O YES ✓ NO
If yes, Permit # _____

10.0 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
- Employee Health and Safety Orientatioin

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

SIGNATURE [signature]

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ✓ 2 O 3

11.2 Has the facility been certified by OHS for this level of containment? ✓ YES O NO

11.3 If yes, please give the date and permit number: Bio-UWO-0147

12.0 Approvals

UWO Biohazard Subcommittee.

Signature [signature] Date 28 April '08

Safety Officer for Institution where experiments will take place

Signature [signature] Date Apr. 28/08

Safety Officer for University of Western Ontario (if different from above)

Signature _____ Date _____

Expiry Date (3 years from Approval): April 28, 2011

DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED