

Modification Permit for Permit BICO (UAWCO) 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)

Approved Microorganisms

Please stroke out any approved Biohazards to be removed below

~~P. aurogenosa, S. typhimurium~~

Write additional Biohazards for approval below. *

*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

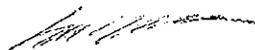
~~mice~~

* 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

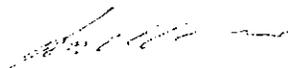
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BioSafety Officer(s):

Chair, Biohazards Subcommittee:

----- Original Message -----

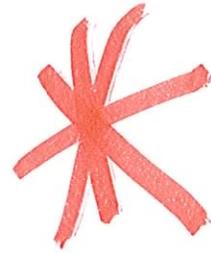
Subject:[Fwd: [Fwd: Re: Anthrax toxin project]]

Date:Thu, 28 May 2009 12:15:28 -0400

From:Sung Kim <Sung.Kim@schulich.uwo.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

References:<4A1EB7A8.60609@uwo.ca>



Well, we have to dig up data from Japanese human experiments done during the 2nd world war.

But it was banned from access and citation.

SK

>>> Jennifer Stanley <jstanle2@uwo.ca> 28/05/2009 12:11 pm >>>

Hi Dr. Kim

If at all possible, please look up the lethal dose. I have been looking on-line and I can not find any information for the lethal dose for the toxin itself.

Thanks

Jennifer

----- Original Message -----

Subject: Re: Anthrax toxin project

Date: Thu, 28 May 2009 11:25:23 -0400

From: Sung Kim <Sung.Kim@schulich.uwo.ca>

To: Jennifer Stanley <jstanle2@uwo.ca>

References: <4A1EAAFD.4040603@uwo.ca>

No. We bleach them.

SK

>>> Jennifer Stanley <jstanle2@uwo.ca> 28/05/2009 11:17 am >>>

Hi Dr. Kim

For the toxins that you use, do you know the human lethal dose?

Also - how do you dispose of the contaminated materials?

Thanks

Jennifer

Re: Use of lethal toxin

Subject: Re: Use of lethal toxin
From: Sung Kim <Sung.Kim@schulich.uwo.ca>
Date: Fri, 06 Feb 2009 11:22:01 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>



See below:

>>> Jennifer Stanley <jstanle2@uwo.ca> 2/6/2009 11:18 AM >>>
Thanks Dr. Kim

I understand from this that you store ~ 1 mg of each component. Total we have. We have in aliquots. Do you have any LD50 information? about 100 micg/20g wt. in mice. How do you dispose of the material when the experiments are complete? Toxin is inactivated in 3h at room temp. Bleach them, anyway.

Jennifer

Sung Kim wrote:

> Hi Jennifer:
>
> >>> Jennifer Stanley <jstanle2@uwo.ca> 2/5/2009 9:36 AM >>>
> Hi Dr. Kim
> Just a couple more questions:
> - how much of the toxins (PA and LF) do you keep on hand at once? ~1
> mg each
> - how much of each do you usually use at once? 5 micg What is the
> concentration of toxin? 50 micg/ml
> Thanks!
> Jennifer

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

101 St. Patrick St. AL: 611A, Ottawa, Ontario, Canada, K1A 0X9

Tel: 613 946-6901

Fax: 613 941-1196

genevieve.lacroix@phac-aspc.gc.ca

http://www.phac-aspc.gc.ca/lis-lsa/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

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	P. aeruginosa, S. typhimurium	
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		
Approved use of Animals	inico	

* 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.

Date of last Biohazardous Agents Registry Form Apr 28, 2008

Signature of Permit Holder:

Biosafety Officers: *[Signature]*
Chair, Biohazards Subcommittee

[Signature]
Sept 30/08
G.H. K. R. D. S.

Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Toxin(s)

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PLEASE ATTACH 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):

Q. Hanley Sept 30/08

Chair, Biohazards Subcommittee:

E.M. Kiddes

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 & Immunology
ADDRESS SPR1 Rm 119, 1400 Western Rd.
PHONE NUMBER 82961
EMAIL sung.kim@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) SPR1 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 TGF- β 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:
Sean-Duck Ha
Andrew MacKinnon
Anthony Blain
Sarah Spanton

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 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 <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Is it known to be an animal pathogen? YES/NO <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Is it known to be a zoonotic agent? YES/NO <input type="checkbox"/> Yes <input type="checkbox"/> No	Maximum quantity to be cultured at one time?	Source/Supplier	Health Canada or CFIA Containment Level 0 1 2 3 <input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 3
<i>P. aeruginosa</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	2 x 10 ⁹ cells		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 3
<i>S. typhimurium</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	2 x 10 ⁹ "		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 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 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? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Source of Primary Cell Culture Tissue
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	primary peripheral blood, marrow
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	peritoneal, bone-marrow-derived macrophages
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> 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? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Specific cell line(s)	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	TMP-1	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	RAW 264.7	ATCC
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

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

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www3022.org.)

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 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	<i>Volunteer</i>	<input checked="" type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs (preserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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? YES NO
 If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved: YES NO

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

4.3 Will intact genetic sequences be used from

- ♦ SV 40 Large T antigen YES NO If YES specify _____
- ♦ Known oncogenes YES NO If YES specify _____

4.4 Will a live viral vector(s) or bacterial plasmid be used for gene transduction YES 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 YES NO

4.7 Will virus be infectious to humans or animals YES NO

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES 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? YES 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? YES NO PENDING

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? YES 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 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 YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES NO
- ◆ Non-Human Primates YES NO If YES specify species _____
- ◆ Wild caught animals YES NO If YES specify species _____
colony # _____
- ◆ Birds YES NO
- ◆ Others (wild or domestic) YES NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin _____

8.3 What is the LD₅₀ (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 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 NO

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

9.4 Has the import permit been sent to OHS? 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
- † WHIMIS
- † 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 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 01 02 03

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

11.3 If yes, please give the date and permit number B/10 - 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 23/08

Safety Officer for University of Western Ontario (UWO) for above

Signature _____ Date April 28, 2011