THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

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, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Edmund MK Lui
SIGNATURE
DEPARTMENT Phy & Pharm
ADDRESS Depart Physiology and Pharmacology, UWO
PHONE NUMBER 83320 (office) 86842 (Biotron lab)
EMAIL ed.lui@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Biotron Room(s) 5 and 20

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES:Ontario Rsearch Fund, Ministry of Research and Innovation

GRANT TITLE(S): New technologies for ginseng agriculture and product development

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

Medicinal herbal products generally contain multiple components and a spectrum of pharmacological activities. Generally these products have long history of cultural use and are considered as safe; although occasionally toxicity has been reported due to contamination or adulterated products. We focus on the immunomodulatory and pro-and anti-inflammatory effects as well as the vascular protective action of ginsengs and other Chinese medicinal herbs for the treatment of cancer, inflammation as well as metabolic and vascular diseases. We are use state-of- the-art methodology to We will use state-of- the- art methodology to i) prepare herbal extracts ; ii)

chemically characterize the extracts, iii) elucidate their pharmacological mechansim of action; iv) correlate activity with chemical profile; and v) isolate and identify pharmacologically active components.
All of the herbal medicines tested have a long history of cultural use and have been subjected to scientific investigations.
Extracts will be prepared with either water or ethanol. They are fractionated and analysed by gel filtration and HPLC methodology. We use different cell line to test the pharmacological activities with in vitro experiments with several cell lines (RAW 264.7, EA hy 926,B16, B16F10 melanoma cells) in mono-layers to test the anti-oxidative, immuno-modulatory, anti-angiognesis and apoptosis effects of herbal medicines. For immuno-modulation study, LPS is used as a positive control and is also used to induce inflammation and screen for anti-inflammatory activity.

Names of all personnel working under Principal Investigators supervision in this location:
1) Jirui Hou (PDF)
2) Chike Azike (GS)
3) Hua Pei (staff)
4) Yuan Liu (visiting scholar)

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)? O YES X NO

If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O 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:

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?
	Yes .No .	Yes .No .	.Yes.No	
	Yes .No .	Yes .No .	.Yes.No	
	Yes .No .	Yes .No .	.Yes.No	
	Yes .No .	Yes .No .	.Yes.No	

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

*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? **X 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 (Nil)

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
Vascular endothelial cell	Yes	EA.hy 926	Dr. Edgell from the Univeristy of North Carolina
Mouse melanoma	Yes	B16 F10 melanoma cells	Dr. Anne Chambers (London Regional Cancer Centre). It was tested negative for murine pathogens on Oct 25, 2005 by MU Research Animal Diagnostic Lab. Columbia MO. See attached report.
RAW264.7	Yes	mouse	Dr. Jeff Dixon(physiology & pharmacology university of western Ontario)
Other (specify)	No .		

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

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES XO 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		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (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 **X NO** If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

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

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below XO NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ♦ HIV — O YES, please specify _____ O NXO
- ♦ HTLV — 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NOX
- ♦ SV 40 Large T antigen — O YES O NOX
- ♦ E1A oncogene — O YES O NOX
- ♦ Known oncogenes — O YES, please specify _____ O NOX
- ♦ Other human or animal pathogen and or their toxins — O YES, please specify _____ O NOX

4.5 Will virus be replication defective? O YES O NOX

4.6 Will virus be infectious to humans or animals? O YES O NOX

4.7 Will this be expected to increase the containment level required? O YES O NOX

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES **X 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, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? OX NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_ ___

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals **YES**, specify: XO NO

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

7.0 Use of Animal species with Zoonotic Hazards

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

- ♦ Pound source dogs — O YES — **X NO**
- ♦ Pound source cats — O YES — **X NO**
- ♦ Cattle, sheep or goats — O YES — **X NO**
- ♦ Non- Human Primates — O YES, please specify species _____ — **X NO**
- ♦ Wild caught animals — O YES, please specify species & colony # _____ — **X NO**
- ♦ Birds — O YES **X NO**
- ♦ Others (wild or domestic) — O YES, please specify _____ — **X NO**

8.0 Biological Toxins

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

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES **X NO**
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES XO 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 plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____

_____

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

_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:

_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ XO NO
If no, please proceed to Section 10.0

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

11.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O _____NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 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. XO 1 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
O XNO
O NOT REQUIRED

14.0 Procedures to be Followed

14.1 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 have an up-to-date Position Hazard Communication Form, found at http://www.wpn.uwo.ca/

SIGNATURE _____ Date:_April 25, 2009___

15.0 Approvals

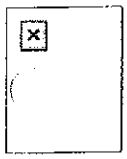
UWO Biohazard Subcommittee: SIGNATURE: _____
Date:_____

Safety Officer for Institution where experiments will take place: SIGNATURE:_____
Date:_____

Safety Officer for University of Western Ontario (if different from above):SIGNATURE:_____
Date:_____

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

Special Conditions of Approval:



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 8053-2005

RECEIVED ON: 10/19/2005
COMPLETED ON: 10/25/2005

SUBMITTED BY:

Dave Dales
London Regional Cancer-Programme
790 Commissioners Rd. E.
London, Ontario
N6A 4L6
Canada
(519) 685-8600 x53271
[519] 685=8646 (fax)

SPECIMEN DESCRIPTION:

SPECIES: mouse
DESCRIPTION: cells
NUMBER OF SPECIMENS: 11

PURCHASE ORDER #: CCE-117285

ID

1 -1 B16F1
B16F10
D2A1
D2OR
PAP2
4T1
66cl4
168 FARN
67 NR
MDA MB 468 LN
MDA MB 435 HAL

PROFILE/EXAM REQUESTED: IMPACT III PCR Profile

SUMMARY: No pathogens were detected by PCR assays.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

Subject: Re: Biohazardous Agents Registry From: Lui
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 05 May 2009 14:29:14 -0400
To: Ed Lui <Ed.Lui@schulich.uwo.ca>

Hi Dr. Lui
One more question - The B16F10 cell line was likely originally purchased from ATCC (www.atcc.org) - do you know if the cell line has been modified by the Chambers lab in anyway? If so, please send the details on any genetic modifications that have been done...
Thanks,
Jennifer

Jennifer Stanley wrote:

> Hello Dr. Lui
> I received your form -thanks! I have one question, is the "EA.hy 926" cell line a rodent cell line or a human cell line? Do you have a website or something on these cells?
> Thanks
> Jennifer

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item

Price: $264.00

Designations: B16-F10

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: melanocyte

PHOTO

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host (technology from amaxa)

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:
22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987