

**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
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Location of experimental work to be carried out: Building(s) MSB and DSB_ Room(s) MSB-521 & 496; DSB 2003

*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 will focus on the anti-oxidative, immunomodulation, vascular protective and anti-inflammatory effects of ginsengs and other Chinese medicinal herbs for the treatment of cancer, inflammation as well as metabolic and vascular diseases. We use state-of-the-art methodology to i) elucidate their pharmacological mechanism of action; ii) correlate biological activity

with chemical profile; and iii) isolate and identify pharmacologically active components. All of the herbal medicines to be tested have a long history of cultural use and have been subjected to scientific investigations. We use different cell lines (RAW 264.7, EA hy 926, B16, B16F10 melanoma cells) in mono-layers to test the pharmacological activities in vitro of herbal medicines: such as anti-oxidative, immuno-modulatory, anti-angiogenesis and apoptosis effects. For immuno-modulation study, LPS is used as a positive control and is also used to induce inflammation to screen for anti-inflammatory activity.

In addition to in vitro experiments, in vivo animal experiments are used to validate in vitro findings. The following is a brief protocol for the in vivo experiments.

Part I. To determine the protective effect against Melanoma cell-induced liver metastasis, B16F10 melanoma cells will be injected to adult C57bl mice (Day 0), which will be examined for tumor yield in the liver 21 day later. For each extract or fraction three experimental groups (Low dose, Medium dose, and High dose of the herbal medicines) and 1 control (saline) will be required. (3 dose levels are usually required to define a dose-response relationship). From our experience with variability in the induction of liver metastasis, a N value of 8-10 is required.

Herbal medicine treatment protocol:

1. Standard: One day after melanoma cell injection and continues daily (except week end) for 20 days.
2. Pretreatment. Same as in 1 except with a pretreatment for 3 days before melanoma cell injection. This pretreatment protocol is to determine whether activation of immune function (more specifically Kupffer's cells) would enhance the protective effect. This could be done at one dose level for comparison with the standard treatment protocol.

Part I a. Estimated animals required per test extract by the standard protocol at 3 dose levels: 45 animals.

Part I b. Estimated animals required per test extract by the pretreatment protocol at one dose level: 35 animals.

Part II. To evaluate treatment effect on microvascular function in liver tumor and non-tumor sites by Intravital video microscopy (IVVM), 3 animals per treatment groups will be required.

Part III. To evaluate treatment effect on Kupffer cell function, mice will be pretreated with herbal product fractions or saline for 3 days prior to melanoma cell injection and monitored for 5 hrs (migration rate and interaction with melanoma cells in liver) by IVVM. Two dose levels that have previously been established in the metastasis model will be used. N=5

Part 2. To determine the protective effect of ginseng against homocysteine-induced vascular injury, 3-week-old Sprague-Dawley rats (40–50 g) will be used. The normal control group (n=6) will have free access to standard tap water, while the treatment group (n=6) will receive homocysteine in tap water (50 mg/kg body weight per d) for 42 d to induce vascular injury. To evaluate the protective effect, two groups will be given 500mg/kg and 1000 mg/kg of different ginseng extracts (aqueous and organic ones) by gastric gavage daily. Body weight will be recorded daily. Rats are killed at the end of the experiment and blood taken to prepare serum for quantitation of cytokines and oxidant stress markers. Liver will be taken for the examination of tissue cytokines and oxidant stress. The aorta will be removed for histological and organ bath studies to assess contractile-relaxation response. (This protocol will be reinstated from a previous one (2003-101-11) by a modification of the current protocol: 2008-02-01) protocol.

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

- 1) Jirui Hou (PDF)
- 2) Chike Azike (GS)
- 3) Hau 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)? YES NO

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:

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?

*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?	Source of Primary Cell Culture Tissue
Macrophage	Yes	Mouse abdominal macrohgage
Lymphocyte	Yes	Spleen of mouse
Kupffer's cell	Yes	Liver of mouse

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

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 cells	Yes	EA.hy 926	Dr. Edgell from the Univeristy of North Carolina
Melanoma cells	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.
Macrophage	Yes	mouse RAW264.7	Dr. Jeff Dixon (Physiology & Pharmacology University of Western Ontario)
Other (specify)	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 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? 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		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input 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 or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (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 modification(s) involving plasmids be done? <input type="radio"/> YES, complete table below <input type="radio"/> 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? YES, complete table below 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 YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

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

5.0 Human Gene Therapy Trials

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used MOUSE & RAT

6.3 AUS protocol # 2008-018

6.4 Will any of the agents listed be used in live animals YES, specify: Tested negative for murine pathogens on Oct 25, 2005 by MU Research Animal Diagnostic Lab. Columbia MO. NO

* 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? 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 plant? _____

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

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

10.6 Do you do any modifications to the plant? YES 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? YES NO

10.9 Please describe any CFIA permit conditions:

11.0 Import Requirements

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

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

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

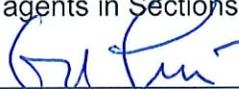
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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 _____


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

13.0 Containment Levels

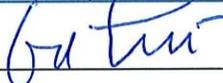
11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. XO 1 2 O 3

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

- YES, permit # if on-campus _____
- NO
- 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.wph.uwo.ca/>

SIGNATURE  Date: April 25, 2009 _____

15.0 Approvals

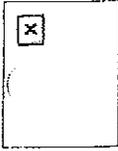
UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

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 B16F1
B16F10
D2A1
D2OR
PAP2
4T1
66c14
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.

Re: Biohazardous Agents Registry From: Lui

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™	<input type="button" value="Order this Item"/>	Price:	\$264.00
Designations:	B16-F10			
<u>Biosafety Level:</u>	1		Shipped:	frozen
Medium & Serum:	See Propagation			Growth Properties:
Organism:	<i>Mus musculus</i> (mouse)			Morphology:



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 30-2002

References: 22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1129790
 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: 953937