

**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 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 mechanism 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-angiogenesis 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)? 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?
	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***
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*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?
If no, please proceed to Section 3.0

YES

NO

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 University 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		

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

*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? YES, complete table below 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.

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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? YES, please specify _____ NXO
- ◆ HIV YES, specify _____ NOX
 - ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NOX
 - ◆ SV 40 Large T antigen YES NOX
 - ◆ E1A oncogene YES NOX
 - ◆ Known oncogenes YES, please specify _____ NOX
 - ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NOX
- 4.5 Will virus be replication defective? YES NOX
- 4.6 Will virus be infectious to humans or animals? YES NOX
- 4.7 Will this be expected to increase the containment level required? YES NOX

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 _____
- 6.3 AUS protocol # _____
- 6.4 Will any of the agents listed be used in live animals YES, specify: NO

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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 YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES NO
- ◆ Non- Human Primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES NO
- ◆ Others (wild or domestic) YES, please specify _____ 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(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? YES 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? Initiate and maintain colony, give location: _____
 "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:

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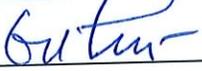
10.0 Plants Requiring CFIA Permits

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. _____

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:

Hi Jennifer,
The B16F10 cell line has not been modified.
EA.hy926 cells are a hybrid of HUVEC and a human lung carcinoma cell
line A549 (Edgell et al., 1983. Proc Natl Acad Sci 80: 3734-3737). This
cell line has been used by several groups
to carry out Matrigel-based tubulization of endothelial cells.
I hope this is what you need for documentation.
Thanks
Ed

>>> Jennifer Stanley <jstanle2@uwo.ca> 05/05/2009 2:29 pm >>>

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
>

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

Subject:Re: Cell line: EA.hy926

Date:Tue, 14 Jul 2009 14:46:52 -0400

From:Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer,

Again the same process applies, if this cell line has not been transformed with a human pathogen or if it does not contain human pathogen, than we can consider it a risk group 1. As far as I can judge with the provided information, I would classify it as a RG1 but again, you should perform a risk assessment to determine the risk group.

Regards

Genevieve Lacroix, M.Sc.

A/Head, Importation and Biosafety Programs

Chef intérimaire/Importation et service 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>



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Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a [local distributor](#) for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number:	CCL-185™	<input type="button" value="Order this Item"/>	Price:	\$256.00
Designations:	A549		Depositors:	M Lieber
<u>Biosafety Level:</u>	1		Shipped:	frozen
Medium & Serum:	See Propagation		Growth Properties:	adherent
Organism:	<i>Homo sapiens</i> (human)		Morphology:	epithelial
				
Source:	Organ: lung Disease: carcinoma			
Cellular Products:	keratin			
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.			
			<u>Related Cell Culture Products</u>	
Isolation:	Isolation date: 1972			
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)			
Reverse Transcript:	negative			
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9.3 TPOX: 8,11 vWA: 14			
Cytogenetic Analysis:	This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6)(q11;q27); ?del(6)(p23); del(11)(q21), del(2)(q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.			
Isoenzymes:	G6PD, B			
Age:	58 years			
Gender:	male			
Ethnicity:	Caucasian			
Comments:	This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a			



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Cell Biology

ATCC® Number: CRL-6475™

[Order this Item](#)

Price: \$264.00

Designations: B16-F10

Shipped: frozen

Biosafety Level: 1

Growth Properties: adherent

Medium & Serum: [See Propagation](#)

Morphology: melanocyte

Organism: *Mus musculus* (mouse)



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

Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

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



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Cell Biology

ATCC® Number: CRL-2873™

Designations: HUVEC-CS

Biosafety Level: 1

Medium & Serum: [See Propagation](#)

Organism: *Homo sapiens* (human)

Price: \$569.00

Depositors: IM Bird

Shipped: frozen

Growth Properties: adherent

Morphology: endothelial



Source: **Organ:** umbilical vein
Tissue: vascular endothelium
Disease: normal
Cell Type: endothelial

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](#)

Isolation: **Isolation date:** August, 1998

Age: newborn

Comments: HUVEC-CS was derived from HUV-EC-C (ATCC CRL-1730?) by initially passaging in tissue culture plastic flasks without additional growth factor like ECGS. Subsequently the HUVEC-CS cell line was plated on gelatin-coated flasks. It exhibits positive acetylated low-density lipoprotein (AcLDL) uptake and expresses eNOS, CD31 and v-cadherin (classical markers of endothelial cells).It spontaneously forms capillary-like structures when grown on Matrigel. Receptors were detected for angiotensin II (AII), bradykinin and ATP.[PubMed: 15350190]. Distribution freeze lots have a doubling potential of 5 to 8 PDLs.

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 20%.

Temperature: 37.0°C

Growth Conditions: Use gelatin-coated flasks.

Subculturing: **Protocol:** Volumes used in this protocol are for 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.1. Remove and discard culture medium.2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin- 0.53 mM EDTA solution (GIBCO Cat# 25300-054) to remove all traces of serum that contains trypsin inhibitor.3. Add 1.0 to 2.0 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.Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. To remove trypsin-EDTA solution, transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new porcine gelatin coated culture vessels. An inoculum of 1X 10(4) to 2X 10(4) viable cells/cm2 is recommended. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: 1: 3 to 1: 4

Preservation: **Freeze medium:** Culture medium,90%; DMSO, 10%
Storage temperature: liquid nitrogen vapor phase



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 F1 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.