

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: July 9, 2010
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is 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 Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) 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), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Patricia Luke
 DEPARTMENT Smear
 ADDRESS 3391 Windermere Rd
 PHONE NUMBER 519-463-3180
 EMERGENCY PHONE NUMBER(S) 519-857-8222
 EMAIL patricia.luke@uwo.ca

Location of experimental work to be carried out: Building(s) RR7 Room(s) 2298

*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 the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: PSI KFOC
 GRANT TITLE(S): Dendritic Cell Therapy combined with SCD83
Enhancement of Allograft Function with
CD83

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
<u>Jordan L. D'Amico</u>	<u>j.l.damico@uwo.ca</u>	<u>July 25/2007</u>

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

No agents used

See E-mail

Please include a one page research summary or teaching protocol.

Despite improvements in immunosuppressive therapy, the long-term survival of kidney transplants has not increased over the past decade. With limited survival, the kidney's lifetime is dependent upon wear during age-related use, damage as a result of the transplant process and immune-related injury, as well as development of non-specific chronic kidney transplant injury.

In animal models, carbon monoxide (CO) inhalation has been shown to protect organs by decreasing inflammation and preventing cell death during the transplantation process. However, carbon monoxide inhalation is difficult to regulate and may lead to serious injury to patients by preventing oxygen delivery to vital organs. Carbon monoxide releasing molecules (CORM) are agents that safely deliver CO without risk of oxygen deprivation to the patient. We have shown that CORM, like CO, improves kidney transplant function and survival when given to the kidney donor or when added directly to the kidney during its removal and storage.

Our goal is to optimize kidney transplant protection after the transplant as well. We hypothesize that CORM can improve kidney transplant function and longevity through regulation of the immune system and reduction of inflammation and factors that create kidney scarring.

By investigating new compounds such as CORM, we hope to minimize kidney damage during the kidney transplant process as well as in the post-transplant period. We believe that this will lead to minimization of irreversible damage and improvement in the long-term survival of kidney transplants.

Murine TEC will be plated and treated with 10 ng/ml TGF- β 1, 10 ng/ml EGF and 36 μ g/ml hydrocortisone to induce EMT (63). During the treatment, cells will also be treated with 1) CORM-3, 2) iCORM-3, 3) no treatment, 4) CORM-3 + sGC inhibitor, ODQ and 5) p38 MAPK inhibitor, SB203480. After 6 days, cells will be fixed and stained for zona occludins 1 (ZO-1) to visualize cell junctions or vimentin to visualize the cytoskeleton. Western blots of lysates from cells will be performed, probing for E-cadherin, α -smooth muscle actin (α -SMA), S100A4 (fibroblast specific protein-1) or β -actin. This will assess the ability of CORM to prevent the development of EMT in vitro.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? 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? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
NA	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No	Human	Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	TEC	2006-04-111
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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 type(s) indicate PHAC or CFIA containment level required 1 2 2+ 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 Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)	ATCC HIV EC	<input type="radio"/> Yes <input checked="" type="radio"/> Unknown		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

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 from transformation or tranfection

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

** Please attach a plasmid map.

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* 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 involving a biological agent? YES NO
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
 Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent 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 C57BL/6 / BALB/c Lewis / B6N rat

6.3 AUS protocol # 2006-041-11

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ 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 _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? 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 life stage of the insect? _____

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

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

9.7 Do you use insects that require a permit from the CFIA permit? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? 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
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

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 or plant 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____


13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 01 02 02+ 03

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

- YES, permit # if on-campus RR1 0035
- NO, please certify
- NOT REQUIRED for Level 1 containment

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 working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE [Signature] Date: 13/10/10

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

N/A

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

N/A
_____ 

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____ Date: _____

2) Safety Officer for the University of Western Ontario SIGNATURE: _____ Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO): SIGNATURE: _____ Date: _____

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

Special Conditions of Approval:

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

Subject:Re: Biological Agents Registry Form: Luke Lab

Date:Fri, 19 Nov 2010 13:28:26 -0500

From:Patrick Luke <Patrick.Luke@LHSC.ON.CA>

To:Jennifer Stanley <jstanle2@uwo.ca>

CC:Amanda Travers <Amanda.Travers@LHSC.ON.CA>

Jennifer

Sorry for the delay in reply.

1)page2: Because the HUVEC cell line(CRL-1730 from ATCC) is a level 1 cell line,no special control measures are utilized. All the rodent tissues and HUVEC cells will be disposed into the biohazard garbage bag and autoclaved before being discarded.

2)HUVEC CRL-1730 (ATCC) is the one we are currently using.

3)Section14.3: The University of Western Ontario Workplace Health provides several health surveillance,testing and immunoprophylaxis program. We will contact with the Workplace Health immediately if an accidental exposure to a biological agents occurs.

Thanks

I hope this is adequate. Please let me know.

p



E-mail

Patrick Luke MD, FRCS(C)
Associate Professor of Surgery
Co-Director, Multiorgan Transplant Program
Schulich School of Medicine
University of Western Ontario
London Health Sciences Centre
phone 519.663.3180
fax 519.663.3858

Cell Line Info

Cell Biology

ATCC® Number: **CRL-1730™** Price: **\$280.00**

Designations: HUV-EC-C

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)
endothelial

Morphology:



Organ: umbilical vein

Tissue: vascular endothelium

Disease: normal

Cell Type: endothelial

Source:

Cellular Products: factor VIII [23284]

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.

Permits/Forms:

Applications: transfection host ([technology from amaxa](#))

Tumorigenic: No

Amelogenin: X

CSF1PO: 11,12

D13S317: 9,11

D16S539: 11,12

DNA Profile (STR): D5S818: 11,12

D7S820: 8,12

THO1: 6,9.3

TPOX: 8,11

vWA: 16

This is a hypodiploid human cell line. The modal chromosome number was 45 occurring in 72% of cells counted. The rate of polyploid cells was 15.8%. All cells had monosomic N13 and the subclone with additional monosomic N15 predominates. Other coexisting subclones include those with 46,XX,-11,-13,i(11p),i(11q) and 46,XX,+11,-13 karyotypes. Both X chromosomes appear normal.

Cytogenetic Analysis:

Endothelial Cell Growth Supplement (ECGS) and unidentified factors from bovine pituitary, hypothalamus or whole brain extracts are mitogenic for this line. [23284]

Comments:

The cells have a life expectancy of 50 to 60 population doublings.

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Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated of F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: 0.1 mg/ml heparin; 0.03-0.05 mg/ml endothelial cell growth supplement (ECGS); adjust to a final concentration of 10% fetal bovine serum.
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%
Temperature: 37.0°C

Protocol:

- Subculturing:**
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 which 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 1:2 to 1:3 is recommended

Medium Renewal: Two to three times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC [30-2004](#)
 recommended serum: ATCC [30-2020](#)

23284: Hoshi H, McKeehan WL. Brain- and liver cell-derived factors are required for growth of human endothelial cells in serum-free culture. Proc. Natl. Acad. Sci. USA 81: 6413-6417, 1984. PubMed: [6333682](#)

References: 29192: Zahedi K. Characterization of the binding of serum amyloid P to laminin. J. Biol. Chem. 272: 2143-2148, 1997. PubMed: [8999915](#)

33021: Soker S, et al. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-encoded domain. J. Biol. Chem. 271: 5761-5767, 1996. PubMed: [8621443](#)

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