**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOHAZARDOUS AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: September 25, 2009**
**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 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 biohazards 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 Biohazard 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: Timothy Regnault
SIGNATURE:
DEPARTMENT: Obs/Gyn
ADDRESS: ~~DSB Int~~ / DSB 2021
PHONE NUMBER: 83528
EMERGENCY PHONE NUMBER(S):
EMAIL: tim.regnault@uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 2019

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Ref In utero origins of Adult insulin resistance

**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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.**

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

Lin Zhao
Jeni Thompson
David Kao

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

***Introduction:*** Insulin resistance is the primary determinant of obesity, diabetes and cardiovascular disease, components of the metabolic syndrome, and is evident before overt disease develops. Insulin resistance develops in key insulin target tissues, such as muscle, and is a result of reduced muscle oxygen utilization, fat accumulation and mitochondrial dysfunction. Up to one half of metabolic syndrome diseases can be attributed to an adverse *in utero* environment, such as placental insufficiency-induced Intrauterine Growth Restriction (IUGR), representing 8% of newborns. Studies of adults, born IUGR, highlight that they are seven-fold more likely to develop insulin resistance, suggesting in utero programming of muscle insulin sensitivity.

Mammalian target of rapamycin (mTOR), the peroxisome proliferator-activated receptor (PPAR) nuclear receptor family; and the PPARγ transcriptional co-activator PGC-1α, are essential for regulating and maintaining insulin sensitivity. We recently demonstrated that reduced oxygen tension (hypoxia), a component of placental insufficiency-induced IUGR, suppresses *in vitro* muscle cell mitochondrial oxygen uptake and PGC-1α/PPAR expression and have confirmed aspects of these important changes in a guinea pig model of induced IUGR. The recognition of this critical link between hypoxia and mitochondrial function and PGC-1α/PPAR pathways suggests a radical new approach to the understanding of *in utero* induction of insulin resistance. However, the molecular mechanisms that underpin fetal programming of insulin resistance in skeletal muscle are poorly understood. Furthermore, Western style high-fat diets contribute significantly to the severity of insulin resistance in postnatal life, but the interaction between high-fat diets and IUGR-induced insulin resistance is largely unknown. ***Our overall hypothesis is that placental insufficiency and a high-fat postnatal diet result in an increased risk of insulin resistance in later life.***

**Hypothesis # 1:** Placental insufficiency impairs fetal muscle mTOR/PGC-1α/PPAR signaling.
**Hypothesis # 2:** A high-fat diet in postnatal life, in combination with impaired mTOR/PGC-1α/PPAR signaling leads to muscle mitochondrial dysfunction and insulin resistance in IUGR offspring.
**Hypothesis # 3:** Pharmacologic intervention can restore muscle insulin sensitivity under IUGR-associated conditions of hypoxia and oxidative stress.

***Research summary:*** We will use the uterine vessel ligation induced-IUGR guinea pig as our model system. To address hypotheses #1 and #2, we will further characterize the *in vivo* effects of placental insufficiency on the regulators of fetal muscle insulin sensitivity and the effects these changes have on postnatal insulin sensitivity after exposure to a high-fat diet. Utilizing positron emission tomography (PET) and computer assisted tomography (CAT) we will study, non-invasively, whole body changes in body composition and muscle insulin sensitivity in IUGR pups exposed to control and high fat diets at critical time points during postnatal development. To determine the effects of placental insufficiency on the key regulators of muscle insulin sensitivity, mitochondrial functional studies, Mass Spectrometry, PCR, immunoblotting and ChIP will be undertaken in fetal and adult tissues to complement the PET and CAT studies. We expect that alterations in regulators of insulin sensitivity in IUGR offspring will precede the development of the postnatal phenotype characterized by altered muscle mitochondrial dysfunction and early onset insulin resistance, and will be exacerbated with a high-fat diet in IUGR adults. To test hypothesis # 3, tissues from hypothesis # 1 and 2 animals will be used in *in vitro* muscle culture systems. Firstly we will explore the cellular mechanisms linking components of placental insufficiency, hypoxia and oxidative stress, to impaired fetal muscle insulin sensitivity signaling and secondly we will examine a number of key positive modulators of the insulin signaling pathway, in fetal and postnatal cells to determine their effects in this system. *In vitro* modulation of impacted regulators, with therapeutic agents, will potentially improve insulin sensitivity laying the foundation for future preclinical animal intervention studies.

***Significance:*** The findings from this proposal will advance our understanding of fetal programming of insulin resistance and interactions with postnatal diet. Such knowledge will be invaluable in formulating novel therapeutic interventions aimed at preventing and, or reducing insulin resistance resulting from an adverse *in utero* environment in the increasing group of IUGR babies.

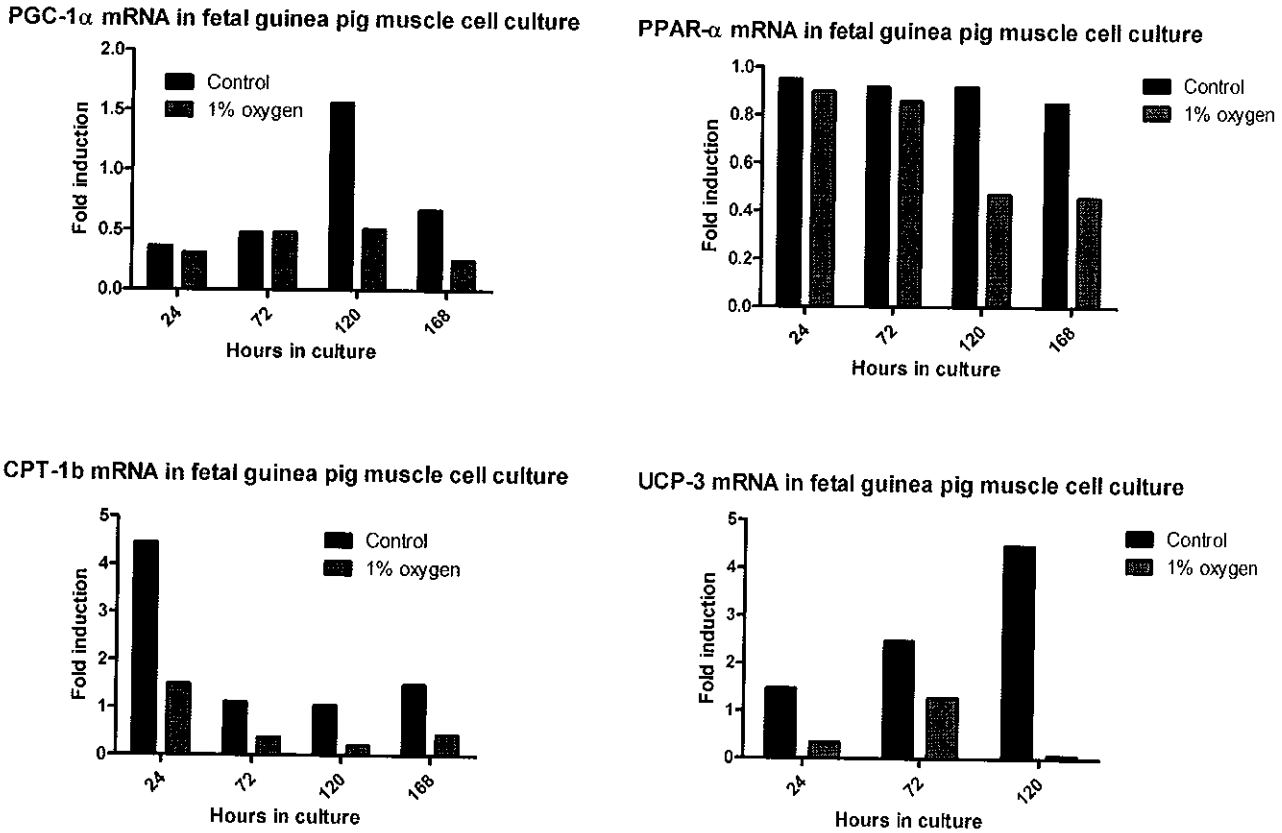
Individual muscle-myoblast cultures will be generated using established techniques (***Fig. 10***) [1;2]. From the fetal studies (Section 3.1.2), separate cultures will be generated from the IUGR pups and matched control pups. Additionally, cultures will be generated from postnatal animals (Section 3.2.2). Our fetal guinea pig muscle culture (***Fig. 10***), following experimental hypoxia and oxidative stress, will allow for confirmation of their influence upon the development of insulin sensitivity. The purpose of the postnatal cultures will be to compare fetal vs. postnatal cellular oxidative function and to look for evidence of programmed changes in oxidative metabolism and examine the possible manipulation of these changes in adult myoblast culture. From our preliminary data (***Fig. 10 and Appendix # 1***), we have calculated that it should be possible to detect significant differences from the proposed numbers of individual animal cultures (10 fetal control and 10 IUGR in specific aim # 1 and four groups of 10 cultures split between postnatal control/IUGR/control diet/high-fat diet treatments in specific aim # 2) and this is supported by other related ex *in vivo* muscle cell culture studies [3;4].

Muscle myoblast cultures will be established from each selected fetus/animal and within 3-6 passages grown to about 70% confluence in growth medium. Medium will then be replaced with differentiation medium (DM) at T0. Individual cultures, in triplicate, will be then be placed under recorded systematic oxygenation levels at muscle collection. That is, for the fetal cultures, myoblasts will be cultured under physiological hypoxia. Hypoxia will be introduced by placing appropriate cultures in an anaerobic incubator (Invivo 400 hypoxic work station, Rusken UK) set to provide hypoxic atmospheres of 1% and 5% $O_2$ (5% $CO_2$, balance $N_2$). The 5% and 1% treatments are selected as these are representative of fetal oxygenation in the normal situation (5% ~ 38 Torr) and in the hypoxic fetal growth-restricted situation (1% ~ 8 Torr) from our previous data [5] and as recently described by others for in utero culture [6]. While in vivo fetal muscle cell tension will be at lower concentrations, the use of relative concentrations is deemed to be suitable to highlight differential effects of altered oxygen tension [7]. Postnatal cultures will be conducted at standard conditions defined as 20% $O_2$ (80% $N_2$). To examine the direct effects of oxidative stress for comparison with hypoxia-induced oxidative stress, additional fetal cultures will be incubated with 25 μM $H_2O_2$ as described [8;9]. Cell samples will collected at T0, five (T5) and eight (T8) days in DM. These are based on literature [4;8;10;11], and responses in our downstream genes of interest at T5 (e.g. PGC-1α, CPT-1b and the UCPs) following other studies (***Appendix # 1***), along with maximal triglyceride accumulation at T8 (***Fig. 13***). For fetal and postnatal cultures at each collection point the experiments below will be undertaken.

Additional culture information

**Figure 10: Hypoxia-induced changes in PGC-1α, PPARα and downstream targets in fetal guinea pig muscle cell culture.**

This preliminary work (n=2), utilizing in-house designed and confirmed RT-PCR primer sets shows reductions in PGC-1α and PPARα mRNA in the upper two panels. In the lower two panels, mRNA levels from downstream fatty acid oxidation target genes, CPT-1b and UCP-2, are reduced in 1% oxygen.




**In fetal guinea pig muscle cultures we can detect guinea pig-specific PGC-1α, PPARα, CPT-1b and UCP-3 mRNA and under 1% oxygen, observe depressions similar to those observed for $C_2C_{12}$ cell cultures (Appendix # 1).**

Reference List

1. Arthur,P.G., J.J.Giles, and C.M.Wakeford. 2000. Protein synthesis during oxygen conformance and severe hypoxia in the mouse muscle cell line C2C12. *Biochim. Biophys. Acta* 1475:83-89.
2. Braems,G. and A.Jensen. 1991. Hypoxia reduces oxygen consumption of fetal skeletal muscle cells in monolayer culture. *J. Dev. Physiol.* 16:209-215.
3. Sun,C., F.Zhang, X.Ge, T.Yan, X.Chen, X.Shi, and Q.Zhai. 2007. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metab.* 6:307-319.
4. Gerhart-Hines,Z., J.T.Rodgers, O.Bare, C.Lerin, S.H.Kim, R.Mostoslavsky, F.W.Alt, Z.Wu, and P.Puigserver. 2007. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO Journal* 26:1913-1923.
5. Regnault,T.R., V.B.de, H.L.Galan, R.B.Wilkening, F.C.Battaglia, and G.Meschia. 2006. Development and mechanisms of fetal hypoxia in severe fetal growth restriction. *Placenta.* 28:714-723.
6. Casanello,P., B.Krause, E.Torres, V.Gallardo, M.Gonzalez, C.Prieto, C.Escudero, M.Farias, and L.Sobrevia. 2009. Reduced l-Arginine Transport and Nitric Oxide Synthesis in Human Umbilical Vein Endothelial Cells from Intrauterine Growth Restriction Pregnancies is Not Further Altered by Hypoxia. *Placenta.*
7. Yun,Z., Q.Lin, and A.J.Giaccia. 2005. Adaptive myogenesis under hypoxia. *Mol Cell Biol.* 25:3040-3055.
8. Ardite,E., J.A.Barbera, J.Roca, and J.C.Fernandez-Checa. 2004. Glutathione depletion impairs myogenic differentiation of murine skeletal muscle C2C12 cells through sustained NF-kappaB activation. *Am. J. Pathol.* 165:719-728.
9. Hansen,J.M., M.Klass, C.Harris, and M.Csete. 2007. A reducing redox environment promotes C2C12 myogenesis: implications for regeneration in aged muscle. *Cell Biol. Int.* 31:546-553.
10. Chevillotte,E., J.Rieusset, M.Roques, M.Desage, and H.Vidal. 2001. The regulation of uncoupling protein-2 gene expression by omega-6 polyunsaturated fatty acids in human skeletal muscle cells involves multiple pathways, including the nuclear receptor peroxisome proliferator-activated receptor beta. *J. Biol. Chem.* 276:10853-10860.
11. Dressel,U., T.L.Allen, J.B.Pippal, P.R.Rohde, P.Lau, and G.E.Muscat. 2003. The peroxisome proliferator-activated receptor beta/delta agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. *Mol. Endocrinol.* 17:2477-2493.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES ~~O~~ NO
(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? 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? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|---|---|---|---|---|---|---|
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 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? ~~O~~ 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:

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|---|---|---|---|
| Human | ~~O~~ Yes O No | Amnion | Not applicable |
| Rodent | ~~O~~ Yes O No | Fetal sheep + Guinea Pig muscle | N/A |
| Non-human primate | O Yes ~~O~~ No | | |
| Other (specify) | ~~O~~ Yes ~~O~~ No | | |

see emailed attached May 28/10

*** 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:

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Supplier / Source |
|---|---|---|---|
| Human | X Yes O No | Fetal Liver CRL-11609 | ATCC |
| Rodent | X Yes O No | $C_2C_{12}$ / HEK | ATCC |
| Non-human primate | O Yes X No | | |
| Other (specify) | O Yes X No | | |

May 28/10 See attached e-mail JL

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

Adult Kidney ~~CRL~~ PCS-400-012 ATCC

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

**3.0 Use of Human Source Materials**

3.1 Does your work involve the use of human source materials? X YES O 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 | Delivery Collection | O Yes X No O Unknown | N/A | O 1 X 2 O 3 |
| Human Blood (fraction) or other Body Fluid | Delivery Collection | O Yes X No O Unknown | N/A | O 1 X 2 O 3 |
| Human Organs or Tissues (unpreserved) | Delivery Collection | O Yes X No O Unknown | N/A | O 1 X 2 O 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? 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.

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

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | 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 NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

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

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

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted involving a biological agent? O YES ~~O~~ 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? O YES O 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? O YES, number: _____ O NO O PENDING

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used Guinea Pigs

6.3 AUS protocol # pending

May 28/10 see email attached

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

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

_____

_____

*** 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 O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

## 8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES O 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_____

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? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

## 9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES O 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? O Initiate and maintain colony, give location: _____
O "One-time" 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 O 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
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

**11.0 Import Requirements**

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

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 or plant 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 _____ 21st May 2020

*** 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. O 1 ~~O 2~~ O 3

13.2 Has the facility been certified by OHS for this level of containment?
- ~~O~~ YES, permit # if on-campus BIO UWO-0157
- O NO, please certify
- O 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 _____ Date: 21st May 2010.

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.
Sheep Containment Unit SOP where applicable (see attached)
_____
_____

M. May 28/10 see email

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:
OH wash of injury and contact PI then OHS
_____
_____

**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:

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

**Subject:** RE: Biohazardous Agents Registry Form - Regnault
**From:** Timothy Regnault <tim.regnault@uwo.ca>
**Date:** Fri, 28 May 2010 12:18:42 -0400
**To:** 'Jennifer Stanley' <jstanle2@uwo.ca>

Hi Jennifer, please see below.

Thanks

Tim

-----Original Message-----
From: Jennifer Stanley [mailto:jstanle2@uwo.ca]
Sent: Friday, May 28, 2010 10:30 AM
To: Timothy Regnault
Subject: Biohazardous Agents Registry Form - Regnault

Tim -
Thanks for your form. I have a couple of questions:
1. Section 2.2/2.3 - confirm that no NHP cells or cell lines are used.
confirm
2. Section 6.0 - You do not have an AUP number listed for your guinea
pig study - is it "pending"? It is pending

Also, do you (or will you in the near
future) have a sheep protocol? If so, please give me the AUS protocol
numbers(s). not at this stage.

3. Section 14.2 - I will add that you will use the established sheep
SOP's where applicable., many thanks

Tim

Regards
Jennifer

Western

**SAFETY PROCEDURE/GUIDELINES**
**Sheep Containment Unit**

**NUMBER: Procedure Guideline SF-06**
**SUBJECT: For Laboratories Outside of the SCU working with organs, tissues and/or blood from sheep used in the Health Sciences Sheep Containment Unit (SCU)**
**Occupational Health and Safety**

**PAGES: 2**
**EFFECTIVE DATE: January, 2007**
**SUPERSEDES: September, 2006**
**APPLIES TO: All Personnel**
**APPROVED: January, 2007, Biosafety Committee Chair**

## 1.0 Introduction

1.1 Sheep are a common reservoir of the zoonotic agent *Coxiella burnetti*, a rickettsial micro organism which, if transmitted can infect humans an may cause an illness called Q fever. About one half of people infected with *C. burnetti* show signs of clinical illness with a small percentage of clinical case developing pneumonia and some cases developing liver disease or rarely, endocarditis.

Human infection usually occurs through inhalation of contaminated dusts and aerosols generated by infected animals, their waste products, placental tissues and fluids and contaminated straw or bedding.

The risk of exposure for personnel is significantly higher when work with pregnant sheep where large numbers of organisms may be present in placenta, birth tissues and amniotic fluids of infected animals. Persons at risk (i.e. those with valvular heart disease, persons who are immune suppressed or pregnant women) should be advised of the risk of serious illness that may result from Q fever and should seek medical advice before working with pregnant sheep.

For more information on Q-Fever, please see:

(Health Canada) http://www.phac-aspc.gc.ca/msds-ftss/
(CDC) http://www.cdc.gov/ncidod/dvrd/qfever/

## 2.0 Measures to Reduce the Risk of Exposure to Q Fever

2.1 Sheep will only be admitted to the facility where there is no record of Q fever infection in the originating flock as determined by the best available testing methodology (See SOP 169).

2.2 The sheep housing facilities and operating rooms at UWO will be designed and operated at the standard required by Health Canada as stated in the "*Guidelines for Biomedical Facilities using Sheep as Research Animals" December 2000". This standard if available at: www.phac-aspc.gc.ca/ols-bsl/aminres_e.html*

2.3 Entrance to the sheep facilities will be limited to approved persons only.

2.4 ***These procedures and SOPs are intended to reduce the risk of exposure to Q Fever for all personnel who work in the Sheep Containment Facility at the University of Western Ontario. It is essential for the protection of all, that all procedures are followed at all times by all personnel.***

**3.0** Laboratories employing UWO staff who will handle organs, blood and/or tissues from sheep housed in the UWO Health Sciences Sheep Containment Unit must be listed with Occupational Health and Safety Services at UWO. The laboratory supervisor must complete a University Biohazardous Agents Registry Form and submit it to Occupational Health and Safety (OHS) at UWO for review. This will require the laboratory to be inspected by the safety officer for the facility.

Tissues or blood which have been decontaminated by a procedure approved by Occupational Health and Safety at UWO do not require Level 2 precautions.

Tissues or blood from animals verified by PCR analysis to be C. burnetti free do not require Level 2 precautions.

Tissues or blood from animals not verified by PCR analysis to C. burnetti free must follow Level 2 precautions, as stated in the UWO Biosafety Manual:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/lab_safety_idx.htm

All laboratory and transportation requirements must be met according to the Laboratory Health and Safety Manual for General Laboratory Practices:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/lab_safety/lab_safety_idx.htm

# MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

**ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.**

**ATCC Emergency Telephone:** (703) 365-2710 (24 hours)
**Chemtrec:** (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

**Description**

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

**SECTION I**
**Hazardous Ingredients**

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

**SECTION II**
**Physical data**

Pink or red aqueous liquid

**SECTION III**
**Health hazards**

**For Biosafety Level 1 Cell Lines**

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

**For Biosafety Level 2 Cell Lines**

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

**SECTION IV**
**Fire and explosion**

Not applicable

## SECTION V
**Reactivity data**

Stable. Hazardous polymerization will not occur.

## SECTION VI
**Method of disposal**

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

## SECTION VII
**Special protection information**

**For Biosafety Level 1 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

**For Biosafety Level 2 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

## SECTION VIII
**Special precautions or comments**

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

**THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.**


February 2002

ATCC® Primary Cell Solutions™

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|---|---|---|---|---|
| ATCC® Number: | **PCS-400-012** | Order this Item | Price: | **$579.00** |

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|---|---|
| Name: | Primary Renal Mixed Epithelial Cells; Normal, Human |
| Biosafety Level: | 1 |
| Shipped: | frozen |
| Organism: | *Homo sapiens* (human) |
| Source: | Kidney |
| Growth Properties: | Adherent |
| Age: | Batch-specific |
| Morphology: | Cuboidal with a characteristic pattern of swirled cells; refractile |
| Number of Viable Cells per Vial: | ≥ 500,000 in cryopreservation medium |
| Volume per Vial: | 1 ml |
| Gender: | Batch-specific |
| 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 Products: | Primary Renal Proximal Tubule Epithelial Cells; Normal, Human (ATCC PCS-400-010)<br>Primary Renal Cortical Epithelial Cells; Normal, Human (ATCC PCS-400-011)<br>Renal Epithelial Cell Basal Medium (ATCC PCS-400-030)<br>Renal Epithelial Cell Growth Kit-BBE (ATCC PCS-400-040)<br>Dulbecco's Phosphate Buffered Saline (D-PBS) (ATCC 30-2200)<br>Trypsin-EDTA for Primary Cells (ATCC PCS-999-003)<br>Trypsin Neutralizing Solution (ATCC PCS-999-004)<br>Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)<br>Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)<br>Phenol Red (ATCC PCS-999-001) |
| Comments: | A complete solution to propagate renal mixed epithelial cells with excellent morphology in low serum (0.5% FBS) conditions. |
| Applications: | Applications for use might include research related too hypertension; diabetes; oncology; renal fibrosis; inflammation; autoimmune disease; drug screening/development; and toxicology. |
| Viability: | ≥ 50% |

**Related Links ▸**

NCBI Entrez Search

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Product Information Sheet

Cell Biology

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|---|---|---|---|---|
| ATCC® Number: | **CRL-1772™** | Order this Item | Price: | **$256.00** |

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|---|---|
| Designations: | C2C12 |
| Biosafety Level: | 1 |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | *Mus musculus* (mouse) |
| Morphology: | myoblast   |
| Source: | **Strain:** C3H<br>**Tissue:** muscle<br>**Cell Type:** myoblast; |
| 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. |
| Applications: | transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) |
| Comments: | This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903]<br>The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953]<br>Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427]<br>Tested and found negative for ectromelia virus (mousepox). |
| 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%.<br>**Temperature:** 37.0°C |

**Related Links ▸**

- NCBI Entrez Search
- Cell Micrograph
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- Technical Support
- Related Cell Culture Products

Cell Biology

| | | | | |
|---|---|---|---|---|
| ATCC® Number: | **CRL-1573™** | Order this Item | Price: | **$256.00** |

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|---|---|
| Designations: | 293 [HEK-293] |
| Depositors: | FL Graham |
| Biosafety Level: | 2 [CELLS CONTAIN ADENOVIRUS ] |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | *Homo sapiens* (human) |
| Morphology: | epithelial   |
| Source: | **Organ:** embryonic kidney<br>**Cell Type:** transformed with adenovirus 5 DNA |
| 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. |
| Restrictions: | These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties. |
| Applications: | efficacy testing [92587]<br>transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)<br>viruscide testing [92579] |
| Receptors: | vitronectin, expressed |
| Tumorigenic: | Yes |
| DNA Profile (STR): | Amelogenin: X<br>CSF1PO: 11,12<br>D13S317: 12,14<br>D16S539: 9,13<br>D5S818: 8,9<br>D7S820: 11,12<br>THO1: 7,9.3<br>TPOX: 11<br>vWA: 16,19 |
| Cytogenetic Analysis: | |

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