THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 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	Daniel J. Belliveau
DEPARTMENT	Anatomy & Cell Biology
ADDRESS	Dental Sciences Building, room DSB 00060
PHONE NUMBER	Ext. 86830 OR 88235
EMERGENCY PHONE NUMBER(S)	519 473-6700 (cell: 519 852-3172)
EMAIL	dbellive@uwo.ca

Location of experimental work to be carried out: Building(s) Dental Sciences Building Room(s) 00060

*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:___Natural Sciences and Engineering Research Council
GRANT TITLE(S): ____Neurostorphic Control of Gap Junction Function in Neurons

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

Name	UWO E-mail Address	Date of Biosafety Training
Mandeep Sidhu	Msihdu6@uwo.ca	21-Jan-2009

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.

Retroviruses
Used in in vitro cell culture experiments in a level 2 certified biosafety cabinet and laminar flow hood. All agents are stored at -80 °C prior to use and all exposed materials and fluids decontaminated with 6% sodium hypoclorite solution.

Adenovirus
Used in in vitro cell culture experiments in a level 2 certified biosafety cabinet and laminar flow hood. All agents are stored at -80 °C prior to use and all exposed materials and fluids decontaminated with 6% sodium hypoclorite solution.

E. coii bacteria
Used to propagate plasmid vectors. Stocks grown in lauria Broth or LB-agar plates are stored at 4 °C for approximately 1 month after use and disposed of by decontamination with bleach or autoclaving (plates). Stock of competent bacteria are stored at -80 °C.

Please include a one page research summary or teaching protocol.

Neurons are specialized cells responsible for transmitting and receiving impulses that control our movements and sensations of the outside world. As neurons develop, they rely upon factors in the environment that support their survival. These factors, called neurotrophins have a number of family members, one of which is nerve growth factor or NGF. NGF regulates many neuronal functions including survival, growth of axons and cellular responses to the environment. Our laboratory is studying how NGF regulates the communication between neurons through specialized bridges called gap junctions. These bridges, or channels, allow small molecules to pass between cells, molecules important in many functions of the neuron.

Our research will examine how neurotrophins such as NGF open gap junction channels and what cellular mechanisms are specifically involved in stimulating the gap junctions to move to the surface of the neuron and contact its neighbor. It is very important to understand the methods used to move these molecules from the site of production (the control centre of the neuron, called the cell body) to the far-reaching processes that neurons extend to contact other neurons. This movement with be looked at by tagging the gap junction proteins with a glowing protein tag call green fluorescent protein. This will allow us to trace where the gap junctions are moving in the neuron and how this changes when the neurons are stimulated with NGF.

Gap junctions are important for proper development of the nervous system. In addition they help neurons coordinate information between one another. Knowing how gap junctions are used by neurons and how these channels open and close is essential in better understanding the importance of gap junctions during the development and normal functioning of neurons in the nervous system.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓ YES O 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? O 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)* (Be specific)	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
Mouse retrovirus (VSV-G)	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1 x 10^9 pfu per virus	Generated in house	O 1 ✓ 2 O 2+ O 3
Adenovirus (recombination difficient)	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1 x 10^{11} pfu per virus		O 1 ✓ 2 O 2+ O 3
E. coli bacteria	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			

*Please attach a Material Safety Data Sheet or equival

See Table 1.4.2 (E. coli)

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ✓ 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 ✓ No		Not applicable
Rodent	✓ Yes O No	Nervous tissue	2009-056
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	✓ Yes O No	SHSY5Y, IMR-32, SKNMC	Level 2	ATCC
Rodent	✓ Yes O No	PC12, N2A	Level 1	ATCC, collaborators
Non-human primate	O Yes ✓ No			
Other (specify)	O Yes ✓ 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 PHAC or CFIA containment level required O 1 ✓ 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ✓ 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 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? ✓ YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5-alpha JM109	*pCMV-6*	*Origine*	*Connexins*	*Cellular phenotype changes, growth rate changes*

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

See E-mail

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
✓ YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Retrovirus (VSV-G)		*Collaborator*	*Connexin genes*	*Cellular phenotype changes, growth rate changes*

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ✓ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ✓ NO
- SV 40 Large T antigen O YES ✓ NO
- E1A oncogene O YES ✓ NO
- Known oncogenes O YES, please specify _____ ✓ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ✓ NO

4.5 Will virus be replication defective? ✓ YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? 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? ✓ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used____rat_____

6.3 AUS protocol # ____2009-056_____

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

6.5 Will the agent(s) be shed by the animal: O 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)? O YES ✓ No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ 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, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O 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 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 details:_____ O 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? O 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? 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 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

10.0 Plants

10.1 Do you use plants? O 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? 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 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? O 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? 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 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. O 1 ✓ 2 O 2+ O 3

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

✓ YES, date of most recent biosafety inspection: _____

O NO, please certify

O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-UWO-0003

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures w[illegible] measures, that are unique to this agent.

_____

_____

_____

See E-mail

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

_____

_____

14.3 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:_____November 30, 2010 _____

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: Fwd: EXPIRED Biological Agents Registry Form: Belliveau - EXPIRED
Date: Thu, 09 Dec 2010 10:17:25 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Daniel J Belliveau <Daniel.Belliveau@schulich.uwo.ca>

Hi Dan

I found some information from Origene on the pCMV-6:
http://www.origene.com/cdna/trueclone/vectors.mspx

However, I still need some information on the vector(s) in Table 4.3.

Also, question 14.2 was not completed. If you could send an e-mail, that would be great:
Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash

Regards
Jennifer

-------- Original Message --------
Subject:EXPIRED Biological Agents Registry Form: Belliveau - EXPIRED
Date: Wed, 01 Dec 2010 16:38:07 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Daniel J Belliveau <Daniel.Belliveau@schulich.uwo.ca>

Hi Dan -

This pdf version is fine. I just have a couple of questions.

1. Do you have any information on the vectors (Table 4.3).
2. Question 14.2 was not completed. If you could send an e-mail, that would be great:
Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash

Regards,
Jennifer

E-mail

Cell Biology

ATCC® Number:	**CCL-127™**	Order this Item	Price:	**$264.00**

Designations:	IMR-32
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast; neuroblast 
Source:	**Organ:** brain **Disease:** neuroblastoma **derived from metastatic site:** abdominal mass
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.
Isolation:	**Isolation date:** April, 1967
Applications:	transfection host (technology from amaxa)
Virus Resistance:	echovirus 11
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9 D16S539: 8 D5S818: 11,12 D7S820: 9,10 THO1: 7,9.3 TPOX: 11 vWA: 15
Cytogenetic Analysis:	Stable male karyotype with stemline number of 49. Two large marker chromosomes with submedian centromeres. A deletion in one number 1 chromosome: One number 16 chromosome missing; two extra chromosomes in C group. Sublines with 50 and 48 chromosomes differ from those with 49 chromosomes by having an extra or missing C group chromosome respectively.
Isoenzymes:	G6PD, B
Age:	13 months
Gender:	male
Ethnicity:	Caucasian

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

Cell Biology

ATCC® Number:	**CRL-1721™**	Order this Item	Price:	**$256.00**

Designations:	PC-12
Depositors:	B Patterson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	floating clusters; few scattered lightly attached cells.
Organism:	Rattus norvegicus (rat)
Morphology:	small irregularly shaped cells 
Source:	**Organ:** adrenal gland **Disease:** pheochromocytoma
Cellular Products:	catecholamines; dopamine; norepinephrine [1163]
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 (Roche FuGENE® Transfection Reagents technology from amaxa)
Receptors:	nerve growth factor (NGF), expressed
Tumorigenic:	Yes
Cytogenetic Analysis:	40 chromosomes; 38 autosomes plus XY [1163]
Gender:	male
Comments:	The PC-12 cell line was derived from a transplantable rat pheochromocytoma. [1163] The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. [1163] The cells do not synthesize epinephrine. [1163]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: • heat-inactivated horse serum to a final concentration of 10% • fetal bovine serum to a final concentration of 5% **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Product Information Sheet

BioProducts
- Cell, microbial and molecular genomics products for the life sciences

BioServices
- Bio-materials management; basic repository to complex partnership-level services

BioStandards
- Biological Reference Material and Consensus Standards for the life science community

OriGene has two types available to our customers. All OriGene TrueClones are provided in a pCMV6 Cloning Vector and all are available for purchase as negative controls.

pCMV6-XL4
PCMV6-XL5
PCMV6-XL6