THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 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 ~~also~~ 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: Rebecca Jane Rylett
SIGNATURE: Rebecca Jane Rylett
DEPARTMENT: Molecular Brain Research Group
ADDRESS: Robarts Research Institute
PHONE NUMBER: 519-931-5777 ext 24078
EMERGENCY PHONE NUMBER(S): 519-931-5777
EMAIL: jane.rylett@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Robarts Institute Room(s) 3rd floor

*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): Regulation of choline acetyltransferase at the cholinergic neuron

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.

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

Ewa Jaworski
Daisy Wong
Kathy James
Stefanie Black
Fatima Abji
Kirk Young
Ventzi Hristova
Alexis Gordon
Elizabeth Banasikowska

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

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 O NO
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? ⊗ 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 in the table below

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	O Yes ⊗ No		
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⊗ Yes O No	HEK 293, SH-SY5Y	ATCC
Rodent	⊗ Yes O No	PC12	ATCC
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 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 Known to Be 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		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 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 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
DH5alpha	pcDNA3.1	Clontech	choline acetyltransferase	. no apparent change except expression of protein

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV 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 using the viral vector in 4.0? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O 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 O YES, specify: _____ O NO

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

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*? _____

*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 ⊗ 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? O Initiate and maintain colony, give location: _____
O "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:
_____
_____
_____

*** 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 Ø 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

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 10.0 Ø 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

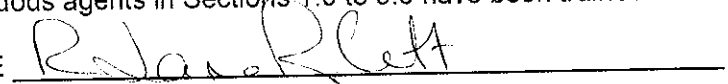
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

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 

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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 ⊗ 2 O 3

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

⊗ YES, permit # if on-campus_____
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 R Jane R Scott Date: 6 May 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:

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

Hello Jennifer

Here is a statement about our work:

Studies are focussed on changes in brain chemistry associated with normal aging and degenerative neurological and psychiatric diseases. This provides an assessment of how nerve cells communicate and conditions that promote healthy brain aging, and therapeutic interventions that may be beneficial for treatment of dusfunction. Experimental models involve cellular and molecular approaches, protein chemistry and function, trafficking of proteins in cells and interactions of cellular constituents and their role in regulation of cell function.

I hope that this helps
Jane Rylett

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

Subject: Re: Biohazardous Agents Registry Form: Rylett
Date: Tue, 14 Jul 2009 09:40:13 -0400
From: Jane Rylett <jane.rylett@schulich.uwo.ca>
To: jstanle2@uwo.ca
References: <4A5C527D020000C800018EB0@draco.med.uwo.ca>
<4A5C527D020000C800018EB3@draco.med.uwo.ca>

Yes that is correct. We normally do 100 ml or 250 ml cultures

Jane Rylett

-----Original Message-----
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Rylett, Jane <Jane.Rylett@schulich.uwo.ca>

Sent: 7/14/2009 9:38:35 AM
Subject: Biohazardous Agents Registry Form: Rylett

Thanks Dr. Rylett:
I noticed you said "yes" to question 1.1 (the use of micoorganisms or biological agents). However, Table 1.2 was not completed. I suspect that the only micoorganism that you use is E.coli dh5 alpha (less than 1 litre of it cultured at one time)...can you confirm this?
- Jennifer

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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Cell Biology

ATCC® Number: CRL-1573™ Order this Item

Designations: 293 [HEK-293]

Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $256.00

Depositors: FL Graham

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

Source: **Organ:** embryonic kidney
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.

Related Cell Culture Products

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]
transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
viruscide testing [92579]

Receptors: vitronectin, expressed

Tumorigenic: Yes

DNA Profile (STR): Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments: Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]

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Product Description

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Cell Biology

ATCC® Number:	**CRL-2266™** Order this Item	**Price:**	**$264.00**
Designations:	SH-SY5Y	**Depositors:**	JL Biedler
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	mixed, adherent and suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
			PHOTO

Source: **Organ:** brain
Disease: neuroblastoma
Derived from metastatic site: bone marrow

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

Restrictions: NOTE: SH-SY5Y was deposited at the ATCC by June L. Biedler, Memorial Sloan-Kettering Cancer Center. SH-SY5Y is distributed for academic research purposes only. Memorial Sloan-Kettering releases the line subject to the following: 1.) SH-SY5Y or its products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of SH-SY5Y including any use by a for-profit entity must first be negotiated with Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1970

Applications: transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)

Antigen Expression: Blood Type A; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 11
D16S539: 8,13
D5S818: 12
D7S820: 7,10
THO1: 7,10
TPOX: 8,11
vWA: 14,18

Cytogenetic Analysis: modal number = 47; the cells possess a unique marker comprised of a chromosome 1 with a complex insertion of an additional copy of a 1q segment into the long arm, resulting in trisomy of 1q [22554]

Age: 4 years

Gender: female

Comments: SH-SY5Y cells have a reported saturation density greater than 1 X 10(6) cells/sq cm. They are reported to exhibit moderate levels of dopamine beta hydroxylase activity [PubMed ID: 29704].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is a 1:1 mixture of ATCC-formulated

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Product Description

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

Additional information about this cell line

Designations: PC-12 **Depositors:** B Patterson

Biosafety Level: 1 **Shipped:** frozen

Medium & Serum: See Propagation **Growth Properties:** loosely adherent, multicell aggregates

Organism: Rattus norvegicus (rat) **Morphology:** polygonal

PHOTO

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.

Related Cell Culture Products

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. [1163]
The cells do not synthesize epinephrine. [1163]

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 2.5%; horse serum to a final concentration of 15%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.1. Remove and discard old culture medium.2. Pipet 10 ml fresh medium over the cell sheet and scrape.3. Aspirate cells with a small bore pipette to break up clusters.4. Add appropriate aliquots of the cell suspension to new 75 cm2 flask with 15 ml fresh growth medium. Seed flask at 1.0 x 10(4) to 3.0x 10(4) viable cells / cm2.Or use subcultivation ratio of 1:3 twice weekly Subculture when cell density reaches between 1.0x 1 0(5) to 2.0x 10(5) viable cells / cm2.5. Place culture vessels in incubator at 37ºC.PC-12 cells adhere poorly to plastic and tend to grow in small patches of loosely attached cells.Attachment can be enhanced by coating the flasks with Bovine Collagen I or using Corning® CellBIND® Surface Flasks (Free Samples)
Subcultivation Ratio: 1:3 twice weekly