

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: October 14, 2010  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://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, 1<sup>st</sup> 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](mailto: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/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR	<u>Jin Zhang</u>
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Location of experimental work to be carried out: Building(s) Spencer Engineering building Room(s) 2021

\*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: NSERC DG  
GRANT TITLE(S): "Development of Biocompatible Nanocomposites"

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Longyan Chen	<u>lchen266@uwo.ca</u>	<u>17-Sept-2010.</u>
(Robert) Bi	<u>Ybi24@uwo.ca</u>	<u>23-Jun-2011</u>
Pei Yin	<u>Pyin7@uwo.ca</u>	<u>11-May-2011</u>
Hong Hai	<u>hhai2@uwo.ca</u>	<u>11-May-2011</u>
Yi Chen	<u>yChen845@uwo.ca</u>	<u>19-Dec-2011</u>

Change(s) to workers

**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.

**Sub-project 1 supported by NSERC DG: “development of magnetic nanocomposite-based device for the detection and capture of microorganisms”**

**Description:** The goal of this project is to develop a nanocomposite-based device to capture and detect the microorganism in short period. Meanwhile, the device will act as antibiotics to kill bacteria. Our target is to detect low concentration of *E. coli*,  $10^3$  cell/L.

The bacterial culture received from ATCC is freeze-dried and stored in a vial at 4 °C, or lower.

The freeze-dried bacterial culture is revived using the standard procedure for further application or storage. We revive the freeze-dried bacterial culture using standard procedure. Briefly, we rehydrate the entire pellet by 1 mL LB broth, which is further transferred into 5 mL LB broth in a sterile test tube using a sterile pipette, following that, incubate the tube at 37 °C for 7 hr. The culture can be used for further applications and storage described below.

**Use:** The *E. coli* will be grown for 24 hours in broth media at room temperature to obtain an approximately  $10^7$  cfu/mL. The cells are harvested by centrifugation (8000 rpm, 5 min) and further re-suspended in Phosphate Buffered Saline (PBS, 0.01 M, pH 7.4) buffer. To study the response of *E. coli* to the biomodified nanomaterials, diluted suspension of cell ( $<10^4$  cfu/mL) will mix with our developed bio-modified magnetic nanomaterials. Then, we use magnets to separate the solid nanomaterials, which recognize and capture *E.coli*, from the liquid medium. Both supernatants and re-dispersed nano-colloids are spread onto agar plates (petri dishes). All the agar plates are incubated overnight at 37°C to calculate the number of colony-forming unit (CFU) of *E. coli*.

**Storage of *E. coli*:**

For short-term storage of *E. coli culture*: the procedure is as follows: prepare a nutrient agar medium and boil it to completely melt; cool the agar to below 50 °C. Following that, 1 ml agar is introduced into a 2 ml screw-cap vial with good sterile conditions until the agar is solidified. A sterile straight wire is used to pick a single colony from a freshly grown plate and quickly move it from bottom to the surface of the soft agar several times. Then, the vial is incubated at 37 °C for 8-12 h without tightening the cap. Finally, seal the vial tightly and store in the dark at 4 °C.

For long-term storage: bacterial culture is mixed with sterile glycerol (sterilized by autoclave, final glycerol concentration) in a sterile test tube. Then, store the tube in -20 °C for medium-term storage in SEB 2012. A shared -80 °C freezer is available in my Department for long-term storage.

**Disposal:**

All containers used for storing and growing cultures should be autoclaved after use to ensure all microorganisms are killed. All contaminated items should be left fully immersed in the disinfectant for 24 hrs. After use, disinfectant in the waste jars should be poured away. Solid wastes should be disposed into orange bags, which are autoclaved and placed in a red biohazard bag for final disposal.

The bio-hazardous materials must be transported in sealed primary container inside a sealed durable and leak proof secondary containment labeled with a biohazard sticker.

*E. coli* W3110 is purchased from ATCC through the sale representative in Canada, Cedarlanelabs ([www.cedarlanelabs.com](http://www.cedarlanelabs.com)). The information of the product can be found at the end of the Form.

Changes to summary

## Sub-Project 2. “ Development of luminescent and biocompatible nanocomposites”

**Description:** An optical nanocomposite-based transducer incorporated with biopolymer materials for non-invasive diagnostic tools.

**Use:** (1) HUVEC cell is going to be used to study the lens sensor’s biocompatibility, HUVEC are human umbilical vein endothelial cells. Each vial of this product contains  $\sim 5 \times 10^5$  cells that have been cryopreserved at the end of the primary culture stage in a medium containing 10% DMSO. During the culture period, no contamination by bacteria, yeast, or fungi was detected. Upon thawing, the cells are guaranteed to be >70% viable (trypan blue), and to have a potential of >16 population doublings when handled according to the directions provided in this document.

**Storage:** “Cryopreserved HUVEC should arrive frozen on dry ice. If the cells are not to be used immediately, the user should prepare a space for storage of the vial in the vapor phase of a liquid nitrogen freezer. While wearing protective eyewear, gloves, and a laboratory coat, remove the vial from its shipping container and place immediately in the liquid nitrogen freezer. Although the viability of cryopreserved cells decreases with time in storage, useful cultures can usually be established even after 2 years of storage at liquid nitrogen temperatures” –based on the information provided by the supplier.

### Procedure for Cell culturing and maintenance:

- The cell line samples can be purchased from ATCC, through Cederlane Labs.

### Starting Cell culturing:

- T-75cm flask are coated with 0.1% gelatine and left to coat for more than 1hr at 37°C .

- The gelatine is removed and 12mL of M-131 or similar endothelial media containing adequate Growth factors is added to the flasks.

- The frozen cell sample is thawed slightly in water bath and as quickly transferred into the T-75 flask containing the media and kept at 37°C incubator.

- The cells are observed for growth, and media is changed every two days. Old media is discarded and the cells are ideally washed with 10mL of Dulbecco’s PBS solution and new media added to replace the removed old media.

- The procedure of changing media is continued till the cells have reached 80-85% confluency (where the cells cover almost the entire surface of the flask’s inner surface ).

- Once confluent, the cells have 3 options:

a) Use the cells for experiment.

b) Split the cells and maintain the cell culture.

c) Freeze the cells (especially earlier passages) for future use.

### Splitting cells:

- T- 75cm flasks that are confluent can be split to two or more T 75 flasks depending upon the speed of growth in cells required(faster growth requires more cells /flask), whereas T150cm flasks of confluent cells can be split to three T-75cm flasks.

- The required T flask are coated with gelatin (0.1%) and kept for incubation at 37°C for at least 1 hour.

- Add media to the flasks after incubation and removal of gelatine.

- The 80%conflunet plates are washed with PBS, and 3ml of Trypsin added to the flasks for detaching the cells. (T-150cm requires 4mL).Leave the plates in hood for 2-5 mints.

- Add 4mL of Trypsin Neutralizing solution and 7mL of Media.

- Scrap the cells from the flask using a cell scraper and as the cells+media volume is about 14ml, Divide the volume into the the flasks of the required number of coated flasks.

- The flasks are then observed under microscope and left to grown in the 37°C incubator.

**Disposal:** According to standard biohazard waste disposal procedures; autoclaving (steam sterilization) is generally the surest method of inactivating biological agents and should be used whenever possible. Liquid waste containers designed to withstand autoclaving temperatures must be used. Containers of liquid waste must be placed into a tray or pan of sufficient capacity to contain all liquid in the event of vessel failure or breakage inside the autoclave chamber.

The information about the cells can be found at the end of the Form

#### **Other information**

All students and researchers in Dr. Zhang's lab are demanded to obtain the Biosafety Certificate. The personal protective equipment (PPE), and the guidance of operating the lab of SEB 2021 are available to all members. Also, there is a biosafety cabinet II in SEB2021.

#### **Please include a one page research summary or teaching protocol.**

Biocompatible nanocomposites composed of inorganic nanoparticles and biopolymer matrixes enable to perform advanced optical, magnetic, mechanical, and biocompatible properties as one entity. They have diverse applications in lightweight devices, particularly in the field of point-of-care (POC) biomedical devices. Since 2008, the principle investigator (PI), has been leading a research team at Western to develop biocompatible multifunctional nanocomposites. That is, nanoparticles (NPs), e.g. mesoporous silica (SiO<sub>2</sub>) NPs and iron oxide (Fe<sub>3</sub>O<sub>4</sub>) NPs, loaded with multi-functional agents, e.g. growth factor, fluorophore, etc., have been incorporated into biocompatible hydrogel films by using the chemical and photo-polymerization. The PI's group has gained experience in applying the multifunctional nanocomposites for the controlled releasing of protein drug, and for the non-invasive biomolecular detection. To date, "bottom-up" process, including chemical solution coating, and self-assembly techniques, are mainly used to generate nanocomposites films. It is difficult, however, to consistently obtain uniform films on a large scale, partially because of the uncontrollable evaporation dynamics in these solution-based techniques. Another hurdle in promoting the use of the new nanocomposites in the market is the difficulty of the large-scale production in a desired period. There is a critical need to establish new processing techniques that effectively manipulate inorganic and organic molecules, yet are applicable to macroscopic processing.

Compared to conventional chemical and physical processes, *in situ* techniques enable to produce uniform dispersion of nanoparticles in biopolymers more efficiently. Thus, our long-term goal is to develop novel techniques for producing hydrogel nanocomposite-based devices for the biochemical and biomedical applications, including bio-chips, bio-imaging, and the biosensors. In the next 5 years, the PI is going to combine the physical and chemical techniques to produce the advanced hydrogel nanocomposites. Using near infrared (NIR) laser-assisted deposition system to produce hydrogel nanocomposites is a new, but an invaluable tool. The NIR laser system has the wavelength in the range of 800-2400 nm. Biomolecules and biopolymers can keep their fully functional structures and properties under the radiation of NIR laser. Furthermore, the NIR laser beam has the strong capability for tailoring the surface and interface at micro-, even nano-scale. The planned sub-projects include: (1) Study on interaction between NIR laser and biomaterials/biopolymers. (2) Development of non-invasive biosensors based on multifunctional nanocomposite produced by the NIR-laser assistant technique. (3) Development of powerful nanocomposite-based device for rapid microbial capture and detection. The efforts of the PI's research group will be focused

on investigating the *in situ* deposition with a fundamental understanding of the interaction between the NIR laser and nanocomposites composed of nanostructures, e.g. silica, iron oxide, etc., and biopolymers, e.g. gelatin, and collagen, and then will exploit the unique properties of NIR lasers and hybrid biomaterials to produce and characterize new nanocomposites with multifunctional properties, including optical, magneto-conductive, and/or thermal-stable properties. All trainees in Dr. Zhang's laboratory will get the benefit by working on the exciting research supported by NSERC discovery grant for their future career in academia, nanotechnology, and biomaterials industry.

### 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: \_\_\_\_\_

Change(s) to 1.2

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
E. Coli strain W3110  It is not considered pathogenic to animal.	○ Yes ● No	○ Yes ● No	○ Yes ● No	~10 <sup>10</sup> cells in 100 mL (0.1L) with 10 <sup>8</sup> cells/mL	ATCC/ Cedarlane Laboratories	● 1 ○ 2 ○ 2+ ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 2+ ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 2+ ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 2+ ○ 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 checked="" type="radio"/> Yes <input type="radio"/> No	Human umbilical vein endothelial cells (no rodent/NHP cells)	Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input 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 checked="" type="radio"/> Yes <input type="radio"/> No	Human umbilical vein endothelial cells	Cedarlane Laboratories
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 type="radio"/> No		

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

2.4 For above named cell types(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/UNKNOWN	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)		<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 (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) of bacteria and/or cells 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?

4.7 Will this be expected to increase the containment level?

5.1 is 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 \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_

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 Will live animals be used?  YES  No

7.3 If yes, 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

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?  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<sub>50</sub> (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](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:

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### 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:

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10.8 Is the CFIA permit attached?  YES  NO  
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 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 \_\_\_\_\_ *Jim Zhang* \_\_\_\_\_

**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.  1  2  2+  3

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

- YES, \_\_\_\_\_
- NO, please certify
- NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): \_\_\_\_\_

**14.0 Procedures to be Followed**

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

\_\_\_\_\_ 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/>

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:

\_\_\_\_\_ No specific measures is required \_\_\_\_\_

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: \_\_\_\_\_ Feb 15, 2012 \_\_\_\_\_

**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:



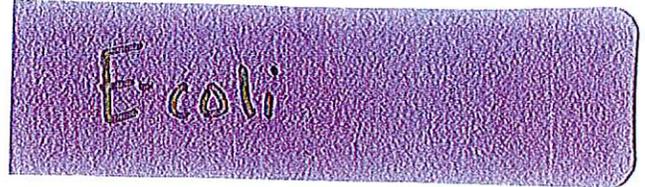
Office of Biohazard Containment and Safety  
Science Branch, CFIA  
53 Somerset Drive, Ottawa, Ontario K1A 0Y9  
Tel: (613) 221-7053 Fax: (613) 228-6129  
Email: InocorZoocont@inspection.gc.ca

Bureau du confinement des biohazards et sécurité  
Direction générale des sciences, ADA  
53 promenade Carleton, Ottawa, Ontario K1A 0Y9  
Tel: (613) 221-7053 Téléc: (613) 228-6129  
Courriel: InocorZoocont@inspection.gc.ca

October 20<sup>th</sup>, 2009

Ms. Shamila Survery / Mr. Michael Decosimo  
Cedariane Laboratories Ltd  
4410 Paletta Court  
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020



SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are considered to be level 1 animal pathogens:

- |               |                    |           |                   |                |
|---------------|--------------------|-----------|-------------------|----------------|
| • 5K          | • CIE85            | • J52     | • MC4100 (MuLac)  | • U5/41        |
| • 58          | • DH1              | • J53     | • MG1655          | • W208         |
| • 58-161      | • DH10 GOLD        | • JC3272  | • MM294           | • W945         |
| • 679         | • DH10B            | • JC7661  | • MS101           | • W1485        |
| • 1532        | • DH5              | • JC9387  | • NC-7            | • W3104        |
| • AB284       | • DH5-alpha        | • JF1504  | • Nissle 1917     | • W3110        |
| • AB311       | • DP50             | • JF1508  | • One Shot STBL3  | • WA704        |
| • AB1157      | • DY145            | • JF1509  | • OP50            | • WP2          |
| • AB1206      | • DY380            | • JJ055   | • P678            | • X1854        |
| • AG1         | • E11              | • JM83    | • PA3C9           | • X2160T       |
| • B           | • EJ183            | • JM101   | • PK-5            | • X2541        |
| • BB4         | • EL250            | • JM109   | • PMC103          | • X2547T       |
| • BD792       | • EMG2             | • K12     | • PR13            | • XL1-BLUE     |
| • BL21        | • EPI 300          | • KC8     | • Rri             | • XL1-BLUE-MRF |
| • BL21 (DE3)  | • EZ10             | • KA902   | • RV308           | • XL0LR        |
| • BM25.8      | • FDA Seattle 1946 | • KAM32   | • S17-1λ -PIR     | • Y10          |
| • C           | • Fusion-Blue      | • KAM33   | • SCS1            | • Y1090 (1090) |
| • C-1a        | • H1443            | • KAM43   | • SMR10           | • YN2980       |
| • C-3000      | • HF4714           | • LE450   | • SOLR            | • W3110        |
| • C25         | • HB101            | • LE451   | • SuperchargeEZ10 | • WG1          |
| • C41 (DE3)   | • HS(PFAMP)R       | • LE452   | • SURE            | • WG439        |
| • C43 (DE3)   | • Hfr3000          | • MB408   | • TOP10           | • WG443        |
| • C600        | • Hfr3000 X74      | • MBX1928 | • TG1             | • WG445        |
| • Cavalli Hfr | • HMS174           | • MC1061  |                   |                |

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cynthia Labrie  
Head, Animal Pathogen Importation Program  
Office of Biohazard Containment & Safety

Canada

# Info on Cell (s)

## Cell Biology

ATCC® Number: **CRL-1730™** | Order this Item | Price: **\$279.00**

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

Cellular Products: factor VIII [23284]

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 ([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  
 TH01: 6,9.3  
 TPOX: 8,11  
 vWA: 16

Cytogenetic Analysis:

## Related Links ▶

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Dear Biohazards Subcommittee,

Many thanks for your time and constructive comments on my Biological Agents Registry Form. Responses to the review comments are listed below. I have revised the Form according to all the comments. Detailed response can be found in the following point-to-point responses.

*Comments:* Please identify the person who helped you fill in the form.

*Response:* I asked my colleagues, Dr. Amarjeet Bassi, to read the Form.

*Comments:* "microbial" is an adjective, not a noun. Therefore correct the text to say that you are trying to detect and kill microorganisms.

*Response:* I apologize for this mistake. The microorganism is used in the Form as per the suggestion.

*Comments:* Antibiotics do not 'de-contaminate' bacteria. Objects are decontaminated.

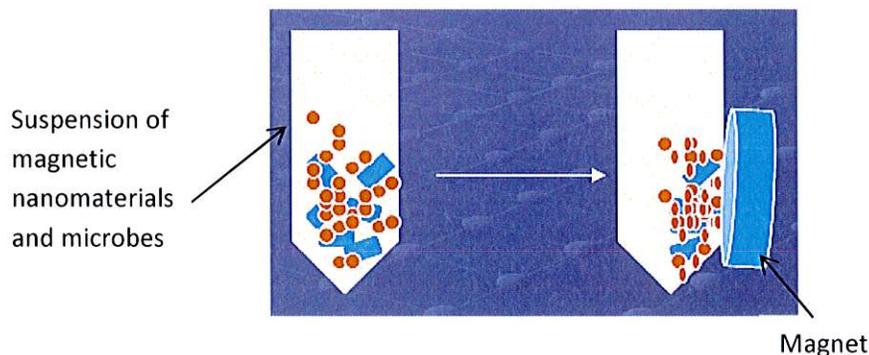
*Response:* "de-contaminate" is removed.

*Comments:* Most importantly, bacteria are not in solution. If they were, you would not have cells. You have a 'suspension' of cells.

*Response:* The "solution" is changed to "suspension" in this Form.

*Comments:* 'Samples' are not separated from the 'solution'. What is separated?

*Response:* The research aims at developing nanomaterials for microbial capture, detection and decontamination. Our developed sample, *i.e.* biomodified magnetic nanomaterials, mix with cultured *E. coli*. We then use magnets to separate the solid nanomaterials which recognize and capture microbes from the liquid medium as shown in the illustration below.



*Comments:* What is a magnetic 'confinement'?

*Response:* It refers to capture microorganisms from liquid medium by using magnets as shown in above illustration.

*Comments:* For 'Storage': what are you storing in the 'original container'? What has an expiration date?

*Response:* Typically, the bacterial culture received from ATCC is freeze-dried and stored in a vial at 4 °C, or lower. In addition, we may use protein to modify the surface of magnetic nanomaterials. Keep those protein containers with expiration date in refrigerator as per the recommendations. I apologize that my previous form includes non-relevant information. For the Biological Agents Registry Form, it should focus on *E.coli* for the project one. The revision is able to be found in the Form.

*Comments:* Do not include the Product Description for the *E. coli* strain here. It is to be added at the end.

*Response:* Thanks. It is now moved to the end.

*Comments:* Delete the copy and paste 'standard process', Handling and Disposal Precautions, and Safety Equipment text from Quiagen and write in your own words how and where you will store and use the *E. coli* in your lab.

*Response:* It is revised.

Again, thank you for your time and consideration.

Yours sincerely,



Jin Zhang, Ph.D.  
Assistant Professor  
Dept. of Chemical & Biochemical Engineering  
University of Western Ontario  
London ON, Canada N6A 5B9  
Tel: (519) 661 2111 ext. 88322

## Information of *E. coli* W3110 used in the project

Link- <http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=35339&Template=bacteria>)



[ATCC Advanced Catalog Search](#) » [Product Details](#)

### 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.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macao, 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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### Bacteria

ATCC<sup>®</sup> Number: 35339™ [Order this Item](#) Price: \$255.00

Organism: *Escherichia coli* (Migula) Castellani and Chalmers

Designations: ECOR 20

Isolation: Steer, Bali

Depositor: H Ochman

History: ATCC<<-H Ochman<<-R. Milkman RM2131(e)

Biosafety Level: 1

Shipped: freeze-dried

Growth Conditions: [ATCC medium3](#); Nutrient agar or nutrient broth  
Temperature: 37.0°C

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.

Comments: reference strain [0410](#)

References: 0410: Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. *J. Bacteriol.* 157: 690-693, 1984. PubMed: [0303394](#)

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## Information of HUVEC cell used in the project

<http://www.cedarlanelabs.com/canada/products.asp?view=viewitem&id=CRL-1730>

<http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CRL-1730&Template=cellBiology>