

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Blohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Qingping Feng
DEPARTMENT PHYSIOLOGY AND PHARMACOLOGY
ADDRESS MSB 254
PHONE NUMBER 82989
EMERGENCY PHONE NUMBER(S) 519-933-9289
EMAIL Qingping.feng@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 253

*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: CIHR, HSFO
GRANT TITLE(S): 1. Rac1 signaling in myocardial TNF-alpha expression in sepsis
2. Heart development in diabetes: Role of NO
3. Cardioprotection by erythropoietin: Role of NO

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> |
|--------------|---------------------------|-----------------------------------|
| Sharon Lu | Sharon.lu@schulich.uwo.ca | July 14, 2008 |
| Lily Xiang | Fxiang2@uwo.ca | May 26, 2008 |
| Yin Liu | Yliu258@uwo.ca | Registered for refresher training |
| Ting Zhang | Tzhang53@uwo.ca | May 26, 2008 |
| Carmen Leung | Cleung73@uwo.ca | Registered for refresher training |
| Hoda Moazzen | hmoazzen@uwo.ca | Registered for refresher training |
| Yan Wu | Ywu287@uwo.ca | Registered for refresher training |
| Murong Liu | Mliu223@uwo.ca | July 14, 2008 |
| | | |
| | | |
| | | |

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.

Adenovirus' are stored in DMEM media with 2.5% glycerol at -80°C. Amplification of adenovirus is performed using HEK 293 cells by infecting the cells with 10 µl of the adenovirus in a 60 mm dish. The collection of cells are frozen and thawed three times and the supernatant is separated from cell debris for infection of target cells, which are neonatal cardiomyocytes. Any solution or containers that handled adenovirus are washed with bleach and autoclaved before disposal.

Lentivirus' are stored in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 buffer at -80°C. Cocktails containing a combination of 6 lentivirus', packaged in VSVG envelope, (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4, Nanog) are infected into isolated fibroblasts from one of four cell sources: mouse neonatal skin, mouse embryo E14.5, mouse neonatal heart and the mouse adult heart. The isolated fibroblasts are grown until confluent and transfected at passage 2 or 3. Cells are incubated with the lentivirus for 24 hours at 37°C and 5% CO₂ and each virus is used at a range of multiplicity of infections (MOIs) or 5-20. After 24 hours the lentivirus is removed from the cells and inactivated with 10% bleach. Cells are then monitored for viability and characteristic changes of differentiation from fibroblasts into cardiomyocytes. Following our *in vitro* studies with the lentivirus, we will establish which combination of lentivirus (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4 and/or Nanog) will be most effective in reprogramming fibroblasts into cardiomyocytes. Upon approval of the protocol from the UWO animal subcommittee, we will inject infected cells that demonstrate characteristic changes into live animals for heart failure therapy. Specifically, the protocol will follow the details used in our *in vitro* work to develop viable cells with characteristic changes. The cells will then be injected into the pericardium cavity of mice and their ability to differentiate into cardiomyocytes will be evaluated using immunohistostaining. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. After the successful injection of infected cells into the heart of mice has been shown, the established combination of lentivirus from our *in vitro* studies will be directly injected into the pericardium cavity of mice to demonstrate reprogramming of cells into cardiomyocytes. Immunohistostaining will be used to evaluate the ability of cells to differentiate into cardiomyocytes. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. Injection of lentiviral infected cells or direct injection of lentivirus into the cardiac region of mice will demonstrate *in vivo* reprogramming of cells into cardiomyocytes, which will be beneficial for heart function following myocardial infarction. Any solution or containers that handled lentivirus are washed with bleach and autoclaved before disposal.

Directed cellular reprogramming of cardiac fibroblasts to cardiomyocytes

Qingping Feng

Department of Physiology and Pharmacology, University of Western Ontario
London, Ontario, Canada

Qingping.Feng@schulich.uwo.ca

Tel. 519-850-2989

With cardiovascular disease as the global leading cause of death, the development of cellular therapies to regenerate a damaged heart is imperative. Following myocardial infarction (MI), large numbers of cardiomyocytes are lost and fibroblasts proliferate rapidly, leading to impaired heart function. Many autologous cell types have been studied for their innate cardiogenic potential but none have been truly successful. It has been demonstrated that cardiomyocytes can be derived from human embryonic stem cells and induced pluripotent stem cells. However, these cell types can form teratomas when transplanted due to their original pluripotent nature. Studies on transdifferentiation or cellular reprogramming using defined factors have been promising because an abundant cell type can be directly reprogrammed into another important cell type without first becoming pluripotent. The present study proposes that cardiac fibroblasts, which proliferate extensively after MI, could be directed to transdifferentiate into cardiomyocytes with cardiac specific genes, therefore potentially improving cardiac function. The objectives of this study are to evaluate the ability of cardiac fibroblasts to reprogram into cardiomyocytes and to investigate if transplantation of these reprogrammed fibroblasts can improve heart function post-MI in a mouse model. Different combinations of lentiviral transductions with *Baf60c*, *Gata4*, *Tbx5*, *Nkx2.5*, *Oct3/4* and *Nanog* genes will be performed on cultured fibroblasts and monitored for spontaneous beating. Subsequently, immunofluorescence and western blot analysis will be carried out to analyze cardiomyocyte specific protein expression. The reprogrammed cells will be transplanted into the peri-infarct region of the heart after MI and heart function will be measured by Millar pressure-volume relationships. It is expected that transplantation of the lentiviral transduced fibroblasts will lead to cardiomyocyte regeneration, improve cardiac repair and cardiac function post-MI. This study may have a major impact on the regenerative medicine and treatment of MI.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

| Name of Biological agent(s)* | Is it known to be a human pathogen? YES/NO | Is it known to be an animal pathogen? YES/NO | Is it known to be a zoonotic agent? YES/NO | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|------------------------------|--|--|--|---|------------------------------|--|
| Adenovirus | <input checked="" type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | 0.1L | Applied Biomedical Materials | <input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| Lentivirus | <input checked="" type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | 0.1L | Applied Biomedical Materials | <input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| E. coli (055iB5) | <input checked="" type="radio"/> Yes <input type="radio"/> No | <input checked="" type="radio"/> Yes <input type="radio"/> No | <input checked="" type="radio"/> Yes <input type="radio"/> No | 0.2L | ATCC | <input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|-------------------|---|---------------------------------------|---------------------|
| Human | <input type="radio"/> Yes <input checked="" type="radio"/> No | | Not applicable |
| Rodent | <input checked="" type="radio"/> Yes <input type="radio"/> No | Mice heart, skin | 2007-011-03 |
| Non-human primate | <input type="radio"/> Yes <input checked="" type="radio"/> No | | |
| Other (specify) | <input type="radio"/> Yes <input checked="" type="radio"/> No | | |

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

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Supplier / Source |
|-------------------|--|------------------------|---------------------|
| Human | <input type="radio"/> Yes <input type="radio"/> No | HEK 293 | ATCC (already have) |
| 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 | | |

*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 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

| Human Source Material | Source/Supplier /Company Name | Is Human Source Material Infected With An Infectious Agent? YES/NO | Name of Infectious Agent (If applicable) | PHAC or CFIA Containment Level (Select one) |
|--|-------------------------------|--|--|---|
| Human Blood (whole) or other Body Fluid | | <input type="radio"/> Yes <input type="radio"/> Unknown | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| Human Blood (fraction) or other Body Fluid | | <input type="radio"/> Yes <input type="radio"/> Unknown | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| Human Organs or Tissues (unpreserved) | | <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 transfection |
|-----------------------------|---------------|-------------------|------------------|--|
| | | | | |

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

** Please attach a plasmid map.

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

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | Describe the change that results from transduction |
|------------------------------------|------------------------------|------------------|---|---|
| Adenovirus | Adenoviral expression system | ABM | Cre | Gene knockdown of specifically floxed genes; cells remain healthy and do not turn cancerous. |
| Lentivirus | Lentivirus expression system | ABM | Oct 3/4, Tbx5, Gata4, Baf6-c, Nkx2.5, Nanog | Gene upregulation; cells change phenotype to cardiomyocyte-like; cells remain health and do not turn cancerous. |

* 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? BOTH YES NO

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used C57BL/6 mice

6.3 AUS protocol # 2007-011-03

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

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO
If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

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

11.0 Import Requirements

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

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

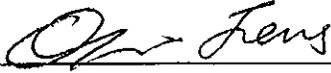
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____  _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus BIO-UWO-0089
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE *Q. J. Jany* Date: Sept. 3, 2010

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

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

Contact UWO Health Services for medical education

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:

Public Health
Agency of CanadaAgence de la santé
publique du Canada**Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3rd Ed., 2004***

This letter serves to confirm that the Public Health Agency of Canada, Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

HPTA Registration Number: R-06-000598

Facility: University of Western Ontario
Department of Physiology and Pharmacology

Attention: Dr. Qingping Feng

Address: 1151 Richmond Street
London, ON
N6A 3K7
Tel: 519-661-2111 ext 82989

Laboratory Room Number(s): DSB 6027

Type of work: *in vitro* only
 in vitro & *in vivo* (small lab animals only)*

Compliance Letter Number: C-00195

Compliance Letter Expiry Date: July 7, 2012.

To renew your compliance letter please complete a CL2 checklist and fax it to (613) 941-0596. The checklist can be obtained from the following website:
www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz
Chief, Importation and Regulatory Affairs

JULY 20, 2010

Date

**In vivo* work is only authorized for mice, rats, guinea pigs and insects. The Pathogen Regulation Directorate must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses. Please contact CFIA for all *in vivo* work.

Canada



Public Health
Agency of Canada

Agence de la santé
publique du Canada

Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3rd Ed., 2004*

This letter serves to confirm that the Public Health Agency of Canada, Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

HPTA Registration Number: R-06-000598

Facility: University of Western Ontario
Schulich School of Medicine & Dentistry
Department of Physiology and Pharmacology

Attention: Dr. Qingping Feng

Address: Medical Science Building
1151 Richmond Street
London, ON
N6A 5C1
Tel: 519-661-2111 ext 82989

Laboratory Room Number(s): 253A

Type of work: *in vitro* only
 in vitro & *in vivo* (small lab animals only)*

Compliance Letter Number: C-00047

Compliance Letter Expiry Date: April 27, 2012.

To renew your compliance letter please complete a CL2 checklist and fax it to (613) 941-0596. The checklist can be obtained from the following website:
www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz
Chief, Importation and Regulatory Affairs

MAY 25, 2010

Date

*In vivo work is only authorized for mice, rats, guinea pigs and insects. The Pathogen Regulation Directorate must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses.
Please contact CFIA for all in vivo work.

Canada

Bacteria

ATCC[®] Number: **12014™** [Order this Item](#) Price: **\$155.00**

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

Designations: CDC 5624-50 [NCTC 9701]

Depositor: CDC

Biosafety Level: 2

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.

Antigenic Properties: serotype O55:K59(B5):H-

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Workplace Health

Policy and Procedure for persons working with Lentivirus and Lentivirus-based vectors

The potential for human inoculation and uncertainties concerning the potential infectivity of agents transduced by the Lentivirus system dictate the need for a simple medical surveillance program.

All persons working with Lentivirus and Lentivirus-based vectors will be seen at Workplace Health prior to the commencement of work with the virus.

A reference serum specimen will be collected and banked.

No annual follow up will be required.

In the event of a needle stick injury in which self-inoculation with Lentivirus or Lentivirus-based vectors is a possibility, the individual will report to Workplace Health and a serum sample for HIV and / or other pathogens will be obtained and tested. All serum specimens will be coded and the test result will be known only to the Workplace Health physician and the donor individual.

Approved Biohazards Committee December 6, 2007

Applied Biological Materials Inc.
MATERIAL SAFETY DATA SHEET - BIOLOGICAL SUBSTANCES

Date Updated: 09/03/2008
Version 1.0

SECTION I - BIOLOGICAL AGENT

NAME: Lentivirus Expression System

SOURCE NAME: *Human Immunodeficiency Virus*

SYNONYM OR CROSS REFERENCE: HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV

GENERAL CHARACTERISTICS: Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase

RECOMBINANT CHARACTERISTICS: 3rd-generation of self-inactivating recombinant lentiviral vectors with enhanced biosafety and minimal relation to the wild-type, human HIV-1 virus. The lentiviral particles produced with this system are replication-incompetent and designed with a number of safety features to enhance its biosafety.

An enhancer deletion in the U3 region of 3' Δ LTR ensures self-inactivation of the lentiviral vector following transduction and integration into genomic DNA of the target cells. Utilization of an RSV promoter upstream of 5'LTR allows efficient Tat-independent production of viral RNA.

The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (*gag*, *pol*, *rev*), and their expression is derived from different plasmids, which all lack packaging signals. These plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus. None of the *gag*, *pol*, or *rev* genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency

EPIDEMIOLOGY: First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

INCUBATION PERIOD: Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years

COMMUNICABILITY: Period of communicability extends from asymptomatic period through appearance of opportunistic diseases

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Several reverse transcriptase and protease inhibitors now licensed

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PHYSICAL INACTIVATION: Effectiveness of 56°C - 60°C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56°C before serologic testing reduces residual infectivity to below detectable levels

SURVIVAL OUTSIDE HOST: Drying in environment causes rapid (within several hours) 90-99% reduction in HIV concentration

SECTION V - MEDICAL

SURVEILLANCE: Serological monitoring for evidence of HIV infection

FIRST AID/TREATMENT: Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV

IMMUNIZATION: None available

PROPHYLAXIS: Experimental prophylaxis with AZT/DDI or other appropriate drug

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 5 reported laboratory-acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwide

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products

PRIMARY HAZARDS: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and splashing infected materials - virus should be presumed in/on all equipment and devices coming in direct contact with infected materials

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and non-cultured procedures (primary containment devices may be indicated eg. biological safety cabinets) and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV; Biosafety level 3 practices, containment equipment and facilities for all work culturing HIV

PROTECTIVE CLOTHING: Gloves should be worn when handling potentially infectious specimens, cultures or tissues; laboratory coats, gowns or suitable protective clothing should be worn

OTHER PRECAUTIONS: Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal - steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labeled

SECTION IX - MISCELLANEOUS INFORMATION

The information contained in this Material Safety Datasheet is believed to be accurate but it is the responsibility of the user or supplier to determine the applicability of these data to the formulation of necessary safety precautions.

Applied Biological Materials Inc. shall not be held responsible for any damage resulting from the use of the above product or the information contained in this Material Safety Datasheet.

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

Subject:Re: Biological Agents Registry Form: Feng lab

Date:Mon, 30 Aug 2010 13:35:42 -0700

From:Carmen Leung <cleung73@uwo.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

CC:Qingping Feng <Qingping.Feng@schulich.uwo.ca>, Sharon Lu
<Sharon.Lu@schulich.uwo.ca>

Hi Jennifer,

The pCAG-ERT2CreERT2 is a plasmid that will be used to generate a transgenic mouse and will not be used to generate a virus. Therefore, I believe there should not be any biohazard concerns associated with it. If otherwise, please let me know what needs to be addressed regarding use of this material. Thanks.

Sincerely,

Carmen

Subject: Fwd: Re: Fwd: Re: Biological Agents Registry Form: Feng lab
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Fri, 08 Oct 2010 17:25:04 -0400
To: Qingping Feng <Qingping.Feng@schulich.uwo.ca>

Dr. Feng

Thank you for your submission

Do you do any genetic modifications using E. coli? If so, please complete this table:

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 |
|-----------------------------|---------------|-------------------|------------------|---|
| | | | | |

Thanks, and have a great Thanksgiving!
Jennifer