Modification Form for Permit BIO-UWO-0224

Permit Holder: Stephen Barr

Approved Personnel
(Please stroke out any personnel to be removed)

Sherry Xu

Jenna Kelly

Additional Personnel
(Please list additional personnel here)

Matthew Woods

Clayton Hattlmann

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	HIV-1, E. Coli (HB101), SIV, Adeno-associated virus (replication incompetent), lentivirus (HIV based)	
Approved Cells	Human (primary): blood. Human (established): HOS, U2OS, 143B, HeLa, Jurkat, U937, 293T, SupT1. Non-human primate (established): cos-1, Vero, cos-7, Phoenix cells, (Lentiviral) HUT78 (T cells).	
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid: Healthy volunteers	
Approved GMO	Plasmids: pcDNA, pFLAG, pCS2, pLKO.1. Vectors: NEO-loxP-3xFlag, deltaR9, VSVg, ppt, LTR, Gag, Env, accessory genes, pTetOn, pTRE2hyg-HIV protease, (tet-responsive) pLPCX (Retroviral), pLRCX	pMT-2.HTLV1 pHTLV1-K30
Approved use of Animals		
Approved Toxin(s)		

We will transfect the plasmids (pMT-2.HTLV1 and pHTLV1-K30)encoding human T-lymphotropic virus with plasmids encoding TRIM22 or HERC5 (interferon-induced proteins that block HIV infection) to see if they can block the release of HTLV1 virus from human cells. All work will be carried out in the BSL3.

Source of plasmids: pMT-2.HTLV1= Dr Greg Dekaban
pHTLV1-K30= NIH AIDS Research and Reference Reagents Program

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Classification: 3

Date of Last Biohazardous Agents Registry Form: Nov 11, 2008

Date of Last Modification (if applicable): Jun 26, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-0224

Permit Holder: Stephen Barr

Approved Personnel
(Please stroke out any personnel to be removed)

Additional Personnel
(Please list additional personnel here)

Jenna Kelly I will apply
Sherry Xu

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	HIV-1, E. Coli (HB101), SIV, Adeno-associated virus (replication incompetent), lentivirus (HIV based)	
Approved Cells	Human (primary): blood. Human (established): HOS, U2OS, 143B, HeLa, Jurkat, U937, 293T, SupT1. Non-human primate (established): cos-1, Vero, cos-7, Phoenix cells, (Lentiviral) HUT78 (T cells),	Untransformed fibroblast cells: 1) Callicebus moloch 2) Pan troglodyte 3) Gorilla gorilla 4) Macaca mulatta 5) Erythrocebus palas 6) Macaca nemestrina
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid: Healthy volunteers	
Approved GMO	Plasmids: pcDNA, pFLAG, pCS2, pLKO.1. Vectors: NEO-loxP-3xFlag, deltaR9, VSVg, ppt, LTR, Gag, Env, accessory genes, pTetOn, pTRE2hyg-HIV protease, (tet-responsive) pLPCX (Retroviral), pLRCX	
Approved use of Animals		
Approved Toxin(s)		

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Classification: 3

Date of last Biohazardous Agents Registry Form: Nov 11, 2008

Signature of Permit Holder [signature]

BioSafety Officer(s): [signature] June 26/09

Chair,Biohazards Subcommittee: [signature]

Summary of research:

Description of new specimens:
Untransformed fibroblast cells from six different non-human primate species (see below).

Purpose: Interferon has been shown to inhibit acute and chronic HIV replication, but little is known about the effector mechanisms of the host interferon response to HIV infection and whether HIV can circumvent these mechanisms. I have previously identified a protein called TRIM22 that is produced in high abundance during the interferon response and is capable of potently inhibiting the assembly and/or release of HIV from human cells; in other words, trapping HIV inside cells. The immediate focus of my lab will be to understand the mechanism behind this defense. Additional research projects will involve the characterization of other interferon-induced HIV restriction factors, and trying to understand how HIV bypasses these defense mechanisms in order to establish infection. Research in my lab will help us better understand the complex interactions between viruses and cells, and will hopefully lead to more effective and less toxic therapy for HIV patients.

The requested non-human primate cells will be used for the following purposes:
1) We will co-transfect TRIM22 and a plasmid encoding HIV into the non-human primate cells using Lipofectamine. The subcellular localization of TRIM22 will be determined using fluorescence microscopy. The ability of TRIM22 to block the release of HIV from the non-human primate cells will be determined by harvesting virus released into the culture media by Western blotting.
Experiments will be conducted in my Biosafety Level 3 lab.
2) We will harvest RNA from the non-human primate cells and perform reverse-transcription of the RNA into DNA. We will then PCR amplify potential antiviral genes from each of the species and use these genes for further study in cell culture with respect to their ability to block HIV release, as described in 1.
3) We will harvest DNA from the non-human primate cells for sequencing purposes in order to obtain genomic sequences pertaining to the potential antiviral genes we will study.
4) We will carry out an evolutionary comparison of the sequences and antiviral function among the different species of non-human primates. These comparisons will be carried out by sequence comparisons using computer software, fluorescence microscopy, and Western blotting.

Quantity: 1 flask containing approximately 3 million fibroblast cells for each of the following specimens:

1) Pitheciidae; Callicebinae; Callicebus moloch ("Dusky Titi Monkey")
2) Catarrhini; Hominidae; Pan troglodyte ("Chimpanzee")
3) Catarrhini; Hominidae; Gorilla gorilla ("Gorilla")
4) Cercopithecidae; Cercopithecinae; Macaca mulatta ("Rhesus Macaque Monkey")
5) Cercopithecidae; Cercopithecinae; Erythrocebus patas ("Patas Monkey")
6) Cercopithecidae;Cercopithecinae;Macaca nemestrina ("Pigtailed Monkey")

All specimens will be obtained from the Coriell Institute for Medical Research in the USA and handled under Biosafety Level 2 conditions. I also have a Biosafety Level 3 lab which I will use if Health Canada or UWO recommends this safety level instead.

Minimum Safety Guidelines
Recommended for Working with Human and Animal Cell Cultures

BIOSAFETY LEVEL

It is recommended that all cell cultures distributed by Coriell Cell Repositories be handled according to appropriate Biosafety Level guidelines established by U.S. Department of Health and Human Service, Centers for Disease Control and Prevention and the National Institutes of Health. The HHS publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition, contains details of practices and schedules of pathogens according to Biosafety Level.

Coriell Cell Repositories recommends that (unless otherwise indicated) all CCR human and animal cell lines be handled at **BioSafety Level 2**.

SUPERVISION

Administrative Responsibilities
Responsibility of Management: Management should establish a biohazards committee to institute and enforce a health and safety policy which includes a specific safety program for work involving human cell lines. The program should meet applicable federal, state, and local regulations and include safety training, maintenance of accident records, and provisions for emergency treatment.

Responsibility of the Principal Investigator: The principal investigator is responsible for the preparation of safety protocols for the research program under his or her direction. The protocols should include appropriate procedures for use, storage, decontamination, disposal, and emergency treatment. The protocols should be approved by the biohazard committee and discussed with the research staff before starting the research program.

Medical Surveillance and Screening:
Physical Examinations: Appropriate pre-employment and periodic medical examinations are desirable for persons working with human cell lines.

Work Restrictions: Persons having reduced immunologic competency should be restricted from working with these human cell lines.

Serum Collection and Banking: Serum should be collected at the time of employment to establish a baseline reference. Serum should be collected immediately after accidental injection or ingestion and at an appropriate interval thereafter. For additional information, see CDC publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition.

Laboratory Access
Access to the cell culture area should be restricted to persons working directly with the cell lines, or by specific authorization by the principal investigator or director of the laboratory.

PERSONNEL PRACTICES

Pipetting: Mechanical pipetting aids rather than mouth pipetting should be used for all pipetting procedures.

Eating, Drinking, and Smoking: Eating, drinking, and smoking should not be allowed in the same areas where cell lines are under study.

Protective Clothing: It is recognized that the criteria for protective clothing may vary according to the physical situation of the laboratory and the agents handled. Ideally, adequate protective clothing such as a fully fastened laboratory coat should be worn. This clothing should not be worn outside the work area once the work area has been entered.

PHYSICAL CONTROL PRACTICES

Recommended for all cell lines, but required for long-term lymphoid lines and their derivatives.

Ventilated Safety Cabinets or Hoods: Ventilated safety cabinets and hoods and other safety apparatus should be employed and should be tested at least annually to certify correct containment and operation. A list of specifications for satisfactory hoods and instructional materials may be obtained from the Office of Biohazards, NCI.

Housekeeping: Appropriate housekeeping procedures which suppress the formation of aerosols should be used. Work surfaces should be wiped down with an appropriate disinfectant before and after work with each cell culture and at the end of the workday.

Decontamination and Disposal: Contaminated glassware and similar materials should be appropriately decontaminated or stored for decontamination before removal from the work area for recycling or disposal. Liquid wastes should be decontaminated either chemically or by heat before being discharged to the community sanitary sewer system.

Protection of Vacuum Lines: Vacuum services, if used, should be protected with disposable absolute air filters and liquid traps. The effluent should be collected in liquid traps containing concentrated disinfectant.

Support
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- FAQs
  - Lymphoblast Cultures
  - Fibroblast Cultures
  - Fetal Bovine Serum
  - Searching
  - Cell Types
  - Culture Medium
  - ISCN Nomenclature
  - Searching Mutations
  - Quick Search
  - Safety Guidelines
  - Quality Control
  - Passage vs PDL
- Miscellaneous
  - Feedback
  - Links
  - Contact Us
  - Cell Collections

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Approved Personnel
(Please stroke out any personnel to be removed)

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	HIV-1, E. Coli (HB101), SIV, Adeno-associated virus (replication incompetent), lentivirus (HIV based)	
Approved Cells	Human (primary): blood. Human (established): HOS, U2OS, 143B, HeLa, Jurkat, U937, 293T, SupT1. Non-human primate (established): cos-1, Vero, cos-7, Phoenix cells, (Lentiviral) HUT78 (T cells).	CEM-GFP. HSC-F
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid: Healthy volunteers	
Approved GMO	Plasmids: pcDNA, pFLAG, pCS2, pLKO.1. Vectors: NEO-loxP-3xFlag, deltaR9, VSVg, ppt, LTR, Gag, Env, accessory genes, pTetOn, pTRE2hyg-HIV protease. (tet-responsive) pLPCX (Retroviral), pLRCX	
Approved use of Animals		
Approved Toxin(s)		

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** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 3

Date of last Biohazardous Agents Registry Form: Nov 11, 2008

Signature of Permit Holder

BioSafety Officer(s): [signature] April 24/09

Chair, Biohazards Subcommittee: [signature]

CEM-GFP

This is an indicator T cell line for quantifying HIV infection. Neomycin-resistant indicator cells. CEM-GFP can be used to monitor infection with HIV-1 (CXCR4, SI strains). Productive infection will generate green fluorescent protein (GFP) in the CEM-GFP cells.

I will use it to titre my HIV virus stocks after they are produced from transfection of 293T cells by calcium phosphate transfection with a plasmid encoding replication-competent HIV (see protocol on following page). HIV-infected CEM-GFP cells will be fixed with 2% paraformaldehyde for 1 hour and subjected to FACS analysis (using the London Regional Flow Cytometry Facility) to determine the percentage of cells that are positive for GFP.

A new reporter cell line to monitor HIV infection and drug susceptibility in vitro.

Gervaix A, **West D**, **Leoni LM**, **Richman DD**, **Wong-Staal F**, **Corbeil J**.

Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0679, USA.

Determination of HIV infectivity in vitro and its inhibition by antiretroviral drugs by monitoring reduction of production of p24 antigen is expensive and time consuming. Such assays also do not allow accurate quantitation of the number of infected cells over time. To develop a simple, rapid, and direct method for monitoring HIV infection, we generated a stable T-cell line (CEM) containing a plasmid encoding the green fluorescent protein (humanized S65T GFP) driven by the HIV-1 long terminal repeat. Clones were selected that displayed low constitutive background fluorescence, but a high level of GFP expression upon infection with HIV. HIV-1 infection induced a 100- to 1,000-fold increase in relative fluorescence of cells over 2 to 4 days as monitored by fluorescence microscopy, cytofluorimetry, and flow cytometry. Addition of inhibitors of reverse transcriptase, protease, and other targets at different multiplicities of infection permitted the accurate determination of drug susceptibility. This technique also permitted quantitation of infectivity of viral preparations by assessment of number of cells infected in the first round of infection. In conclusion, the CEM-GFP reporter cell line provides a simple, rapid, and direct method for monitoring HIV infectivity titers and antiretroviral drug susceptibility of syncytium-inducing strains.

Protocol for use of the CEM-GFP reporter cell line

1. Split the cell culture 1/3, 24h prior to use with viral isolates.
2. Use standard infection protocol, polybrene 2 µg/ml to enhance infection is recommended. Incubate cells at a concentration of 10^6/mL for 2h/37°C with viral isolates. Infection of 50,000 cells per well in a 96 well-plate is considered a minimun. Virus can remain in the culture and cells need not be washed. MOI as low as 0.00001 using HIV-1_{LAI} have been shown to generate a signal (see figure 1).
3. Feed cells every three days. The cells are G418 resistant (500µg/mL) but G418 should not be used during the infection assay.
4. Aliquots for GFP evaluation are taken as desired for analysis by cytofluorimetry or FACS.

Note: The reporter cell line works with HIV strains that use CXCR-4 as a receptor (Syncytium-inducing, lymphotropic isolates of HIV-1 as well as HIV-2). Both primary isolates and laboratory adapted strains have been shown to work with the reporter cell line. Passaging the cell line continuously may reduce sensitivity.

Figure 1

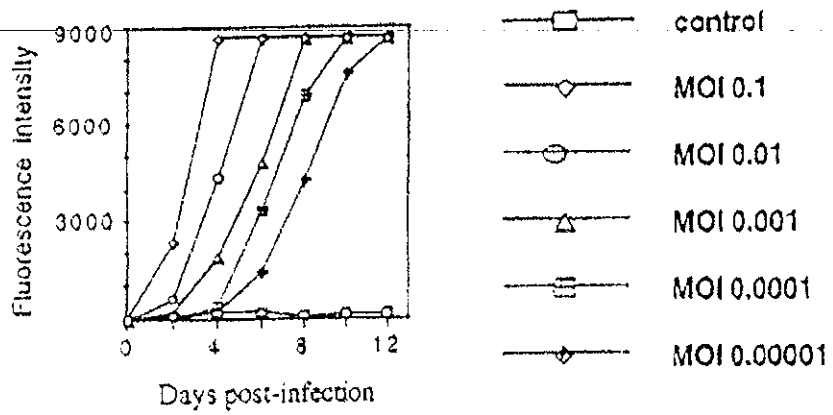

Figure 1: CEM-GFP were infected with HIV-1_{LAI} at different multiplicity of infection (MOI 0.000001 to 0.1) and intensity of fluorescence was measured by fluorimetry using a cytofluorimeter. Note that for each log reduction in inoculum an additional two days was necessary for half maximal detection (5000 arbitrary units).

HSC-F:

This cell line is a cynomolgous T cell line from Macaca fascicularis. It was transformed using Herpesvirus saimiri. It is unknown whether the cell line is productively infected with Herpesvirus. Herpesvirus saimiri has been classified as NIH Risk Group 1. I wish to culture these cells at level 2. They will be transfected with human and non-human primate TRIM22 genes. TRIM22 is a proposed antiviral gene that we have shown blocks HIV infection. Following transfection, the cells will be fixed and imaged using confocal microscopy. I also wish to co-transfect these TRIM22 genes with and without Gag-only plasmids to measure the effect on Gag-only particle release. Gag-only particles are non-infectious (level 2).

I plan to perform SIV and/or HIV infections of these HSC-F cells (or the TRIM22-transfected HSC-F) to measure the antiviral activity of TRIM22 on SIV and HIV replication. This experiment will be performed in the level 3.

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Additional Personnel
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Approved Microorganisms	HIV-1, E. Coli (HB101), SIV, Adeno-associated virus (replication ncompetent), lentivirus (HIV based)	
Approved Cells	Human (primary): blood Human (established): HOS, U2OS, 143B, HeLa, Jurkat, U937, 293T, SupT1 Non-human primate (established): cos-1, Vero, cos-7	Phoenix Cells, (Lentiviral) HUT78 (T cells) CEM (T cells), THP 1 (monocytes)
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid Healthy volunteers	
Approved GMO	Plasmids: pcDNA, pFLAG, pCS2, pLKO.1 Vectors: NEO-loxP-3xFlag, deltaR9, VSVg, ppl, LTR, Gag, Env, accessory genes	pTetOn,pTRE2hyg-HIV protease, (Tet responsive) pLPCX (Retroviral), pLXCX (Retroviral), pLNCX (Retroviral),pSIV-GFP (Retroviral), pHIV (ISCA) (Retroviral), pSIV(ISCA) (Retroviral)
Approved use of Animals		
Approved Toxin(s)		

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Date of last Biohazardous Agents Registry Form Nov 11, 2008

Signature of Permit Holder:

BioSafety Officer(s):

Chair, Biohazards Subcommittee:

Proposed Uses:

Cells:

Phoenix: For generating pseudotyped lentiviral virus. Phoenix cells are a derivative of 293T cells (human embryonic kidney), which I have already received Biosafety Approval. These cells have been modified to express Gag-Pol and an ecotropic envelope gene for the production of replication-defective virus. pLPCX, pLRCX, or pLHCX plasmids (see below) containing a Biosafety Approved gene of interest will be transfected into these cells. Once transfected, the Phoenix cells will express the replication-defective virus capable of integrating the gene of interest into the genome of the desired cell type. **All work dealing with these cells and downstream infections will be carried out in the Level 3 facility, which I have already been approved to use.**

HUT78: T lymphoblastoid cell line obtained from the NIH AIDS Reagent and Reference Program. Derived from peripheral blood of a 50 year old male patient with Sezary syndrome. Cells exhibit the features of a mature T cell line with inducer/helper phenotype. ATCC Biosafety level 1, however I will be culturing them in Biosafety level 2. We propose to transfect them with human TRIM22, HIV-1 Gag gene only and Green Fluorescent Protein (already approved by the Biosafety Committee). We will be measuring Gag protein levels and Gag protein release into the supernatant. **All work dealing with these cells and downstream infections will be carried out in the Level 2 facility, which I have already been approved to use.**

CEM: T lymphoblastoid cell line obtained from the NIH AIDS Reagent and Reference Program. ATCC Biosafety level 1, however I will be culturing them in Biosafety level 2. We propose to transfect them with human TRIM22, HIV-1 Gag gene only and Green Fluorescent Protein (already approved by the Biosafety Committee). We will be measuring Gag protein levels and Gag protein release into the supernatant. **All work dealing with these cells and downstream infections will be carried out in the Level 2 facility, which I have already been approved to use.**

THP1: These are derived from the peripheral blood of a 1 year old human male with acute monocytic leukemia. THP-1 cells have Fc and C3b receptors and lack surface and cytoplasmic immunoglobulins. There is no evidence for the presence of infectious viruses or toxic products. However, these cultures will be handled under Biosafety Level 2 containment. We propose to transfect them with human TRIM22, HIV-1 Gag gene only and Green Fluorescent Protein (already approved by the Biosafety Committee). We will be measuring Gag protein levels and Gag protein release into the supernatant. **All work dealing with these cells and downstream infections will be carried out in the Level 2 facility, which I have already been approved to use.**

Plasmids:

pTet-On, pTRE2hyg+HIV protease: These plasmids are used for the tetracycline-controlled expression of a gene of interest. The HIV-1 protease gene is cloned into pTRE2hyg, which will be under the control of a CMV promoter. This plasmid will be co-expressed with pTetOn, which expresses the reverse tet-responsive transcriptional activator from a CMV promoter. Both plasmids are from Clontech. I will co-express both plasmids in human osteosarcoma cells or Jurkat T cells (already approved for use in the Barr lab). Tight transcriptional control of the HIV-1 protease gene is required because expression of this protease gene is toxic to cells. I propose to identify human protein targets of the HIV-1 protease gene. **All work with these plasmids will be carried out under Biosafety Level 2 conditions (already approved).**

pLPCX,pLHCX, pLRCX: These plasmids are from Clontech and contain Moloney murine leukemia virus and Moloney murine sarcoma virus elements designed for retroviral gene delivery into target cells. We will clone genes of interest approved by the Biosafety Committee into these vectors and transfect them into Phoenix cells to generate the replication defective virus that will be used to infect and integrate the gene of interest into the target cells. The two genes that we propose to clone for gene delivery include human TRIM22 and human Herc5 (CEB1), which are non-viral/non-oncogenic and already approved for use by the Biosafety Committee. **All work with these plasmids (eg. virus production) will be carried out under Biosafety Level 3 conditions (already approved).**

pSIV-GFP: This is a plasmid containing Gag-Pol genes only for Simian Immunodeficiency Virus (SIV). This plasmid is non-infectious since it does not possess any of the other required genes for forming virus, nor will it be transfected with plasmid encoding these required genes. They do not possess envelope or rev genes and are therefore incapable of forming virus on their own. We propose to transfect this plasmid into human and non-human cell lines (already approved by the Biosafety Committee- African Green Monkey COS or Vero cells) and measure SIV Gag protein production and Gag protein particle release in the presence and absence of human TRIM22 (which is a restriction factor targeting Gag release). Gag particles released into the supernatant are non-infectious. **All work with this plasmid will be carried out under Biosafety Level 2 conditions.**

pHIV-1(SCA) and pSIV(HCA): These plasmids encode HIV Gag-Pol and SIV Gag-Pol genes only (respectively), except that the capsid genes (which are a part of the Gag gene) are swapped. For example, the "SCA" in the first plasmid contains HIV Gag-Pol genes except that the capsid gene of SIV is cloned in place of the HIV capsid gene; and vice versa for the second plasmid. Both plasmids are non-infectious since they do not possess any of the other required genes for forming virus, nor will they be transfected with plasmid encoding these required genes. They do not possess envelope or rev genes and are therefore incapable of forming virus on their own. We propose to transfect these plasmids into human and non-human cell lines (already approved by the Biosafety Committee- African Green Monkey COS or Vero cells) and measure SIV and HIV Gag protein production and Gag protein particle release in the presence and absence of human TRIM22 (which is a restriction factor targeting Gag release). Gag particles released into the supernatant are non-infectious. All work with these plasmids will be carried out under Biosafety Level 2 conditions.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 2008
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 are 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. 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 required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: Stephen D. Barr
SIGNATURE:
DEPARTMENT: Microbiology and Immunology
ADDRESS: DSB3006
PHONE NUMBER: Cell: 780-903-6771
EMAIL: Temporary: stephen.barr@ualberta.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 1006b, 3006a2, 6009

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the institution.

FUNDING AGENCY/AGENCIES: University of Western Ontario
GRANT TITLE(S): Start-up

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.

Currently None

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

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	Health Canada or CFIA Containment Level
HIV-1	⊗ Yes O No	O Yes ⊗ No	O Yes ⊗ No	0.01L	Human/ NIH AIDS Research Reference Reagent Program	O 1 O 2 ⊗ 3
E. coli (K12)	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	0.5L	Bacteria/ Biorad	⊗ 1 O 2 O 3
SIV	⊗ Yes ⊗ No	⊗ Yes O No	⊗ Yes O No	0.03L	Simian/ NIH AIDS Research Reference Reagent Program	O 1 O 2 ⊗ 3
Adeno-associated virus (Replication incompetent)	⊗ Yes O No	O Yes ⊗ No	O Yes ⊗ No	0.03L		⊗ 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 Peripheral Mononuclear cells (PBMC)	⊗ Yes O No	Blood	Not applicable
Rodent	O Yes ⊗ No		
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⊗ Yes O No	[illegible]	[illegible]
Rodent	O Yes ⊗ No		
Non-human primate	⊗ Yes O No	[illegible]	[illegible]
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 HC 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? ⊗ YES O NO
If no, please proceed to Section 4.0

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	Healthy Volunteers	O Yes ⊗ 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
E. coli (HB101)	pcDNA, pFLAG, pCS2, pLKO.1	Commercial: Invitrogen, Sigma, Clontech, Addgene, Openbiosystems	TRIM22, HERC5, ISG15, 3b, Tsg101, SUMO, GFP	Over-expression of protein. Make cells resistant to HIV. Produce virus

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
1.) Recombinant Adeno-associated virus 2.) Recombinant Lentivirus (HIV based)	1.) NEO-loxP-3xFlag 2.) delta8.9, VSVG, pot	1.) Dr. Zhenghe Wang (Ohio) 2.) Dr. Rick Bushman (Upenn)	1.) Neomycin, Flag 2.) TRIM22, HERC5	1.) Gene Knockout 2.) Stable Gene Expression 3.) Integrated virus into cell genome

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV ⊗ YES, please specify LTR, Gag, Pol, Env, accessory genes O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ⊗ YES, specify SIV: LTR, Gag, Pol, Env accessory genes O NO
- SV 40 Large T antigen O YES ⊗ NO
- E1A oncogene O YES ⊗ NO
- Known oncogenes O YES, please specify ______ ⊗ NO
- Other human or animal pathogen and or their toxins O YES, please specify ______ ⊗ NO

4.5 Will virus be replication defective? O YES ⊗ NO

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

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

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

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_____

9.0 Import Requirements

9.1 Will the agent be imported? O YES, please give country of origin_____ ⊗ NO
If no, please proceed to Section 10.0

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

9.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O NO

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

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

10.0 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
- 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: _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required: O 1 O 2 ⊗ 3

11.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus_____
O NO
O NOT REQUIRED

Level 3 access pending training completion (Nov 11)

12.0 Procedures to be Followed

12.1 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. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE: _____ Date: June 2, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 10 Nov. 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Nov 11/08

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: BP-UWO-0224 Expiry Date (3 years from Approval): Nov 11, 2011

Special Conditions of Approval:

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

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Stephen Barr

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM

SUMMARY OF WORK:

Type I interferons (eg. IFNα and IFNβ) possess potent antiviral activities towards HIV, but little is known about the effector mechanisms of this response and how HIV circumvents these mechanisms. I previously showed that the IFNβ-induced gene *TRIM22* restricts HIV replication in human cells and is a key mediator of the IFNβ response when expressed at natural levels. I showed that TRIM22 blocks the assembly of HIV in a cell type-specific manner. In one cell type such as HOS, TRIM22 blocks assembly by altering the intracellular trafficking of the viral structural protein Gag. In another cell type such as U2OS, TRIM22 appears to block assembly by preventing the intracellular accumulation of Gag protein. Furthermore, I demonstrated that the antiviral activity of TRIM22 is dependent on two cysteine residues (Cys15 and Cys18) that are critical for the E3 ligase activity of RING-containing proteins [Barr, S.D., et al. PLoS Pathog. 2008; 4(2):e1000007]. The general objective of my proposed research is to understand how TRIM22 blocks the assembly of HIV.

SUMMARY OF BIOHAZARD AGENT USE:

To attain the above objective, my proposed research will require the use of several biohazardous agents. These agents and how they will be used is detailed below.

VIRUSES AND HUMAN CELLS

HIV-1 (Human Immunodeficiency Virus-1): Replication-competent and replication-incompetent HIV-1 virus (Sections 1.2 and 4.3) will be used in several infection assays to test the ability of TRIM22 and various mutants of TRIM22 to block HIV-1 release. Virus will be made by transfecting one 10cm dish of human 293T cells (10mls) (Section 2.3) with a plasmid encoding replication-competent HIV-1 (strains used: R9, NL4-3, LAI) for 3 days (Section 1.2) or replication-incompetent HIV-1 (Sections 1.2 and 4.3). The concentration of all viruses produced is on the order of 10^5 to 10^6 cpm/ml. These units are based on the amount of reverse transcriptase activity present in the virus sample. Various human cell types such as those described in Section 2.2 and 2.3 will be infected in 6- or 12-well plates with various amounts of HIV-1 virus, not exceeding a concentration of 10^5 to 10^6 cpm/ml. Alternatively, the same human cells (in 12-well plates) will be transfected with a plasmid encoding replication competent HIV-1 (strains used: R9, NL4-3, LAI) and virus released into the medium will be harvested, fixed and assayed for reverse transcription activity or protein content. The concentration of virus released into the medium will be on the order of 10^5 to

10^6 cpm/ml. Infections and transfections will be carried out for 3 days. I propose to perform these procedures in a **Biosafety Level 3** room (DSB 6009).

Replication-incompetent HIV-1 vectors will be used to introduce desired genes (Section 4.2) into human cells (Section 2.2 and 2.3) for stable expression. This will result in the integration of the desired gene into the genome of these cells. The virus is generated by transfecting three plasmids into a human cell line such as 293T. The first plasmid, deltaR9 ("packaging plasmid"), contains the genes to produce the structure of the virus. This construct lacks essential components required for replication such as: it does not contain the LTR sequences (which are required for integration of the virus into the genome), it lacks the genome packaging signal (which means the virus will lack the necessary genetic blue prints to reproduce), it lacks the envelope gene, and it lacks the *opu* accessory gene. The second plasmid, VSVg ("envelope plasmid"), codes for an envelope protein from Vesicular Stomatitis Virus to allow entry into cells. This gene is provided *in trans* and will not be packaged into the genome of new virus (meaning it cannot generate new envelopes in the infected cell). This plasmid simply provides the envelope protein (which is not of HIV origin) in order to allow the virus to get into the target cell. The third plasmid, ppt ("gene delivery plasmid"), contains two LTRs and a cloning site for the insertion of the gene of choice that will be under the control of the CMV promoter. This construct will be integrated into the target cell genome.

Since this virus is replication-incompetent (ie. infected cells cannot produce virus), I propose to perform this procedure in the enclosed tissue culture room (DSB 3006b2) within my lab (DSB 3006b) under **Biosafety Level 2+ conditions** (ie. in a modified **Biosafety Level 2 room using Biosafety Level 3 practices**). The **Biosafety Level 2+** room will be fitted with a locking door, autoclave (for autoclaving all waste leaving the room), negative air flow, and any other modification deemed necessary by the Biosafety Officer/Committee. Cells containing the integrated gene will be selected for with drugs and examined for their ability to support HIV-1 or SIV infection, replication, and virus release as described above (performed in a **Biosafety Level 3** room- DSB 6009).

SIV (Simian Immunodeficiency Virus): I have previously shown that TRIM22 blocks the release of HIV-1 virus from human cells. I wish to test whether TRIM22 blocks the release of SIV, which is related to HIV-1. Plasmids encoding replication-competent SIV (strains used: SIVmac239, SIVcpzTAN) will be transfected into human or monkey cell types (Section 2.2 and 2.3) and SIV released into the medium will be assayed for reverse transcription activity or protein content. The concentration of released SIV will be on the order of 10^5 to 10^6 cpm/ml. I propose to perform these procedures in a **Biosafety Level 3** room (DSB 6009). No infections with SIV virus are planned.

Adeno-associated Virus: This virus will be used in "knock-in" assays, for the targeted insertion of a DNA tag sequence such as FLAG. The technique involves generating a DNA cassette with sequences homologous to the 5' and 3' DNA regions flanking the target locus in the genome and then to package this cassette into recombinant adeno-associated virus (replication-incompetent). I will then infect human cells (Section 2.3) in 12-well plates and select the modified cells with drugs. The result of this assay is the attachment of a protein tag to the protein of interest so that we can follow the protein levels under more natural conditions (ie. instead of over-expressing the protein in the cells). This technique (Nature Methods. 2008. 5(2):163-165) is an important tool to study endogenous proteins to which there is no effective antibody. The recombinant adeno-associated virus will be generated using the AAV Helper-Free System (Stratagene) in 24-well plates (max volumes of 1ml, titers ~10^7). No toxic or oncogenic genes will be introduced using this system. I propose to perform these procedures in a **Biosafety Level 2+** room (DSB 3006b2) (since it will already be set up as a 2+ room if approved), even though Stratagene and Health Canada only recommend a **Biosafety Level 2** room (see attached MSDS).

BACTERIA

E. coli (HB101): This bacterial strain will be used for the propagation of plasmid DNA of bacterial or human origin. The purpose is for the amplification of DNA to be used for transfecting human cells, subcloning genes (as described in Section 4.2), or for sequencing. I propose to perform these procedures in a **Biosafety Level 1** room (DSB 3006b).

GENES

Characterization of the role that TRIM22 plays in blocking HIV-1 infection will involve the cloning of several variants of the human *TRIM22* gene, and any other human genes whose protein product is demonstrated to interact with the TRIM22 protein (such as those listed in Section 4.2 and others identified from procedures proposed in my research), into mammalian expression vectors such as pcDNA, pFLAG and pCS2 (Section 4.2). Manipulation of all genes will be carried out in a Biosafety Level 1 room (DSB 3006b) These genes will be expressed in human or monkey cells and analyzed for: their capacity to support HIV-1 infection, replication, or virus release; SIV virus release; microscopic analysis; protein production.

Amendment to Biohazard Agent Registry: Dr. Stephen Barr

Proposed work under BSL2 conditions with enhanced safety precautions:

Pseudotyped HIV-1 (Human Immunodeficiency Virus-1):
Replication-incompetent HIV-1 vectors will be used to introduce desired genes (Section 4.2) into human cells (Section 2.2 and 2.3) for stable expression. This will result in the integration of the desired gene into the genome of these cells. The virus is generated by transfecting three plasmids into a human cell line such as 293T. The first plasmid, deltaR9 ("packaging plasmid"), contains the genes to produce the structure of the virus. This construct lacks essential components required for replication such as: it does not contain the LTR sequences (which are required for integration of the virus into the genome), it lacks the genome packaging signal (which means the virus will lack the necessary genetic blue prints to reproduce), it lacks the envelope gene, and it lacks the *vpu* accessory gene. The second plasmid, VSVg ("envelope plasmid"), codes for an envelope protein from Vesicular Stomatitis Virus to allow entry into cells. This gene is provided *in trans* and will not be packaged into the genome of new virus (meaning it cannot generate new envelopes in the infected cell). This plasmid simply provides the envelope protein (which is not of HIV origin) in order to allow the virus to get into the target cell. The third plasmid, ppt ("gene delivery plasmid"), contains two LTRs and a cloning site for the insertion of the gene of choice that will be under the control of the CMV promoter. This construct will be integrated into the target cell genome. Since this virus is replication-incompetent (ie. infected cells cannot produce virus), I propose to perform this procedure in the enclosed tissue culture room (DSB 3006b2) within my lab (DSB 3006b) under Biosafety Level 2 conditions with enhanced safety precautions as described below.

Adeno-associated Virus: This virus will be used in "knock-in" assays, for the targeted insertion of a DNA tag sequence such as FLAG. The technique involves generating a DNA cassette with sequences homologous to the 5' and 3' DNA regions flanking the target locus in the genome and then to package this cassette into recombinant adeno-associated virus (replication-incompetent). I will then infect human cells (Section 2.3) in 12-well plates and select the modified cells with drugs. The result of this assay is the attachment of a protein tag to the protein of interest so that we can follow the protein levels under more natural conditions (ie. instead of over-expressing the protein in the cells). This technique (Nature Methods. 2008. 5(2):163-165) is an important tool to study endogenous proteins to which there is no effective antibody. The recombinant adeno-associated virus will be generated using the AAV Helper-Free System (Stratagene) in 24-well plates (max volumes of 1ml, titers ~10^7). No toxic or oncogenic genes will be introduced using this system. I propose to perform these procedures in my Biosafety Level 2 room (DSB 3006b2), as suggested by Stratagene and Health Canada, with the added safety measures outlined below.

The practices that will be carried out are as follows:

Routine Procedures:

-All personnel will be trained by myself (Stephen Barr) prior to working in the room. I will supervise the personnel until they are deemed competent by myself.

- When virus work is in progress, a sign shall be placed on the outside of the door alerting workers that virus work is in progress and that entering personnel should adhere to the safety guidelines outlined below.

-10L of 70% ethanol will be available in the room at all times for disinfecting purposes.

-All vacuum and CO_2 hose lines will have approved filters attached.

-Waste traps will contain proper disinfectant (10% bleach) and be set up to include a secondary trap in line with the first trap to collect any residue that travels along the vacuum hose line, thus ensuring no liquid enters the main vacuum line.

-Weekly cleaning and disinfecting of the tissue culture room will be done. This will involve activities such as disinfecting all surfaces, mopping the floor with 1% bleach solution, and refilling all disinfectant.

-Samples that are to be centrifuged will be carried out in centrifuges fitted with biological safety lids. Centrifuge speeds will not exceed 3,000rpm.

-All handling of virus will be performed in the safety hood.

-Virus stocks requiring storage will be stored in locked fridges or freezers and transported to those fridges or freezers in a sealed biological transport container.

-material coming in contact with virus will be disinfected immediately after use (no pipets, tips, dishes, etc. will lie around with live virus).

Entering the room:

-The door will remain closed at all times.

-Personnel will be required to double-glove before entering the room.

-Personnel will wear dedicated tissue culture lab coats that close from behind.

-Sufficient 10% bleach will be made fresh before any work is carried out for disinfecting spills or anything coming in contact with virus.

Exiting the room:

-Hose lines will be disinfected with bleach prior to leaving the room.

-All waste will be disinfected with bleach before leaving the room (includes media, dishes, and pipets).

-outer gloves, shoes and the door handle will be sprayed with 70% ethanol prior to exiting the room.

-All gloves and lab coats will be removed upon exiting the room.

-Personnel will be required to wash their hands with waterless soap (eg. Purell) followed by a thorough handwashing with soap in the sink just outside the room.

Additional safety recommendations by the committee are welcomed and will be adhered to.