

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 8, 2011
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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	Dr. C.Y. Kang
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Location of experimental work to be carried out :

Building : Dental Sciences	Room(s): 6004
Building : _____	Room(s): _____
Building : _____	Room(s): _____

***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: _____

GRANT TITLE(S): _____

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Elizabeth Banasikowska	ebanasik@uwo.ca	October 10, 2006

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Biological agent (HIV) is stored in -80 C freezer at level 3 facility. All material used for experiments is autoclaved at level 3 facility.

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

We propagate HIV-1 in cultured human T lymphocytes in the BSL3 facility. We have generated nef-, vpu- and the Env protein signal peptide replaced mutant of HIV-1 for the development of prophylactic vaccine against HIV/AIDS.

Protocols

A. Protocol for Preparation of Inactivated HIV-1 Whole-Virus Antigen

Virus Culture

1. A3.01 cells should be maintained in a humidified chamber at 37°C with 5% CO₂ at a cell density of $\sim 2 \times 10^6$ cells/ml in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 100U/ml penicillin G, 100µg/ml streptomycin, and 2mM L-glutamine.
Note: Low passage cells should be used for infection.
2. To infect, pellet cells by low-speed centrifugation at 1000xg for 10min.
3. Remove supernatant and resuspend cell pellet in viral inoculum (viral stock diluted in serum- and antibiotic-free RPMI 1640) at a multiplicity of infection of 0.01.
Note: Use as small a volume as possible to maximize virus-cell contact.
4. Allow virus to adsorb for 1hr at 37°C, mixing gently every 15 minutes.
5. Resuspend infected cell mixture in normal growth media at a cell density of 0.5×10^6 cells/ml and incubate at 37°C with 5% CO₂.
6. Following infection, cultures should be monitored daily for signs of cytopathic effect (cpe). Every 2 days post infection (dpi), cells should be split 1:2 and media refreshed. Approximately 6dpi, cpe and cell clustering should become readily apparent. At this point, and every 2 days thereafter, culture supernatants should be harvested for virus purification and monitored for p24 content. Expected p24 yields in culture supernatants should range from 0.5-1µg/ml. Beyond 8dpi, cell viability decreases rapidly, however cultures may be maintained by the addition of fresh A3.01 cells, again maintaining cell density at approximately 2×10^6 cells/ml. Continuous cultures should be maintained no longer than 20dpi.
7. To harvest, remove cells and cell debris by low-speed centrifugation at 1000xg for 20min. Collect the virus-containing supernatant.
Note: Cells may either be resuspended in fresh media and returned to continuous culture or discarded.
8. Further clarify the supernatant by passage through a 0.45µm filter.

Inactivation

9. Concentrate clarified, virus-containing supernatant ~ 1000 fold by ultracentrifugation at 80,000xg at 4°C for 2h.
10. Remove supernatant and resuspend viral pellet in 1mM aldrithiol-2 (AT-2).
11. Incubate 1h at 37°C.
12. Perform secondary inactivation by gamma irradiation at 50kGy.

Purification

13. Concentrate and purify inactivated-virus antigen by ultracentrifugation at 120,000xg at 4°C for 45min, through a 25-50% sucrose gradient.
Note: This step will both concentrate the virus as well as remove impurities and AT-2 from the preparation.

14. Collect banded virus, suspend in TNE buffer, and pellet the virus by ultracentrifugation at 120,000 x g at 4°C for 45 min.
15. Resuspend pelleted virus in PBS. Quantitate both p24 content as well as total protein content.
16. Final antigen concentration should be adjusted to 500µg/ml total protein in PBS.
17. Aliquots of antigen can be stored at -80°C.

Standard Level 3 procedures were followed during virus production, decontamination and disposal.

The University of Western Ontario Biohazardous Agents Registry Form

Description of Work

Characterization of HIV-1 replication and bioprocessing methods, including:

- Optimized transfection and infection methods
- Optimized growth conditions
- Concentration and purification of viral stocks (i.e. downstream processing)
- Viral inactivation methods
- Etc.

Signature: _____


Dr. C. Y. Kang

Date: _____



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:

One can have an accidental exposure to HIV-1 when culture flasks are broken. We will use disposable plastic culture flasks only to avoid accidents.

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific 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
<i>HIV</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	NIHAIDS	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input checked="" type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf*

Additional Comments: _____

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="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	A3.01, H9, AA-2		NIHAIDS
Rodent	<input type="checkbox"/> Yes <input type="checkbox"/> No			
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> 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 type(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

Additional Comments: level 3 required once infected

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="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

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

** Please attach a plasmid map.

***No Material Safety Data Sheet is required for the following strains of *E. coli*:

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

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.

4.3.1 Will virus be replication defective? YES NO

4.3.2 Will virus be infectious to humans or animals? YES NO

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

5.0 Will genetic sequences from the following be involved?

- ◆ HIV NO YES, specify **HIV-1 B subtype**
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens NO YES, specify
- ◆ SV 40 Large T antigen NO YES
- ◆ E1A oncogene NO YES
- ◆ Known oncogenes NO YES, specify
- ◆ Other human or animal pathogen and or their toxins NO YES, specify

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 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 7.0

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

7.1 Will live animals be used? YES NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

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

7.5 Will the agent(s) be shed by the animal:
 YES NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 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 9.0

8.2 Will live animals be used? YES NO

8.3 If YES, please specify the animal(s) used:

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

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

Commercial fetal calf serum

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES NO If no, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)

Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? YES NO

If YES, Please provide details:

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? Initiate and maintain colony, give location:

"One-time" use, give location:

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

10.7 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:

11.0 Plants

- 11.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 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.

An X in the check box indicates you agree with the above statement...
Enter Your Name C. Yong Kang **Date:** August 24, 2011

14.0 Containment Levels

14.1 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

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

YES, location and date of most recent biosafety inspection:

NO, please certify

NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0199**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? YES NO

If **YES** please describe:

15.2 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:

Decontamination with 70% Ethanol and 20% Javex. We don't use sharp items on Level 3, but if needlestick injury happened the person will be admitted to the University Hospital.

15.3 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.shs.uwo.ca/workplace/newposition.htm>

An X in the check box indicates you agree with the above statement...

Enter Your Name C. Yong Kang **Date:** August 24, 2011

15.4 Additional Comments: _____

16.0 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: