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 C. Yong Kang
SIGNATURE C.Y.Kang
DEPARTMENT Microbiology and Immunology
ADDRESS 1400 Western Road, Rm 129, SDRI, London, ON, N6G2V4
PHONE NUMBER 519-661-3226
EMAIL CYKang@UWO.ca

Location of experimental work to be carried out: Building(s) SDRI Room(s) 129, 124

*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: Sumagen Canada Inc
GRANT TITLE(S): Development of Hepatitis C Virus Vaccine

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:

GyoungNyoun Kim
Hwayong An
Kunyu Wu
*Chad Michalski
Elizabeth Baranskowska
Raj Singh

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

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒ YES ○ 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
Vesicular stomatitis Virus (adapted)	○ Yes ☒ No	☒ Yes ○ No	☒ Yes ○ No	0.1 L		○ 1 ☒ 2 ○ 3
Adenovirus	☒ Yes ○ No	○ Yes ☒ No	○ Yes ☒ No	2 L		○ 1 ☒ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ○ NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	○ Yes ☒ No		Not applicable
Rodent	○ Yes ☒ No		
Non-human primate	○ Yes ☒ No		
Other (specify)	○ 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 ○ No	A549, HepG2	ATCC
Rodent	☒ Yes ○ No	baby hamster Kidney Chinese hamster ovary	ATCC
Non-human primate	☒ Yes ○ No	Vero	ATCC
Other (specify)	○ 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 ☒ 1 ☒ 2 ○ 3

3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		O Yes O No		O1 O2 O3
Human Blood (fraction) or other Body Fluid		O Yes O No		O1 O2 O3
Human Organs or Tissues (unpreserved)		O Yes O No		O1 O2 O3
Human Organs or Tissues (preserved)		O Yes O No		O1 O2 O3

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
DH5α XL10 Gold	Transcription Vector (pTV)	Dr. Andrew Ball, University of Texas	VSV genome and HCV genes	HCV genes were inserted into the VSV genome at G gene and L gene junction

* 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
Vesicular Stomatitis Virus (Lab. adapted) Adeno Virus	Indiana & New Jersey serotypes Serotype 5	Lab. adapted virus - Dr. Kang Lab. Microbix Biosystems	HCV genes HIV Gag gene	HCV genes are inserted into VSV genome. HIV Gag gene replaces Ad5 E1 gene

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ⊗NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ⊗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? ☒YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used mouse

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals ☒YES, specify: mouse 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 C.J. Kang x

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 ☒ 2 O 3

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

☒ YES, permit # if on-campus_____
O NO
O NOT REQUIRED

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 x C.J. Kang Date: June 16, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: S'M, K. Leslie
Date: 4 Nov, 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: J. Stanley.
Date: Oct 31/08

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

Approval Number: BIO-UWO-0064 Expiry Date (3 years from Approval): Nov 04, 2011

Special Conditions of Approval:

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

Vesicular stomatitis virus as an HCV protein expression vector

The best ways to prime CD8+ CTL are plasmid DNA vaccine or defective viral or bacterial vectors. The requirements as viral vaccine vectors are a broad range of hosts and good expression of gene of interests. VSV infects most of the mammalian cells and expresses viral proteins up to 60% of total proteins in infected cells. In nature, VSV infects pigs, cattle, and horses, and causes vesicular disease around the mouth and foot. Although human infection by VSV has been reported, it occurs very rarely and it does not cause any serious symptoms.

Although VSV is a rapidly replicating virus, eventually humoral and cellular immune responses against VSV will be elicited in the animal host, like any other viral vectors (Yewdell, J. W. et al. J Exp Med 163:1529-1938, 1986; Puddington, L. et al. J Virol 60:708-717, 1986; Kalinke, U. et al Immunity 5:639-652, 1996). Animals infected with VSV develop immune responses in one or two weeks and starts to neutralize the VSV (Kalinke, U. et al Immunity 5:639-652, 1996), which makes the foreign gene delivery via VSV incomplete. This hinders the efficacy of boost immunizations for vaccination with the same vector. VSV is neutralized by serotype specific antibodies against viral surface glycoprotein G. Two different serotypes of VSV, VSV-Indiana (VSV_{Ind}) and VSV-New Jersey (VSV_{NJ}) show 50% amino acid identity in the glycoprotein (Gallione, C. J., and Rose, J. K. J. Virol. 46:162-169, 1983). Antibodies raised against one serotype of VSV do not neutralize the other serotype of VSV (Cartwright, B., and Brown, F. J. Gen. Virol. 16:391-398, 1972). Therefore, others have used VSV_{Ind} as a vaccine vector in which the glycoprotein was replaced with that of VSV_{NJ} to minimize the problems arising from this immune response against the viral vectors (Rose, N. F. et al. J. Virol. 74:10903-10910, 2000; Rose, N. F. et al. Cell 106:539-549, 2001).

Although the VSV_{Ind} with G protein of VSV_{NJ} serotype is useful in evading the humoral immune response, it will not prevent the cellular immune response which can be triggered by nucleocapsid or matrix proteins. The cellular immune responses against VSV proteins other than the G protein may result in incomplete immune responses against the antigen of interest. Therefore, generation of additional recombinant VSV from

another serotype can increase the efficacy of using VSV as a live viral vaccine vector. Recently, we developed a reverse genetics system to recover VSV_{NJ} from cDNA for the first time (Kim, G. N. and Kang, C. Y., Virology, 357:41-53, 2007).The recombinant VSV_{NJ} is an effective viral vector together with VSV_{Ind} for the expression of foreign genes, which can be used to minimize problems associated with preexisting immune responses against VSV itself. In addition to the VSV_{NJ} vector system, we generated our own full length clone of VSV_{Ind}.

Generation of rVSV Expressing HCV proteins

We have generated the recombinant VSVs from cDNA by the reverse genetics method, both $rVSV_{Ind}$ and $rVSV_{NJ}$ expressing hepatitis C virus genes (core, glycoprotein E1 and E2, P7, NS3, NS4, NS5a, and NS5b). The HCV genes are inserted into the gene junction between VSV G gene and L gene, which positions the HCV genes at fifth in order from the first gene of VSV, N. The parental VSVs for these recombinant VSVs are laboratory adapted strains of VSV, VSV_{Ind} heat resistant strain and VSV_{NJ} Hazlehurst strain, which have been kept and passed in Dr, C. Yong Kang's laboratory.

Recovery of the recombinant VSV from cDNA

BHK-T7 cells, BHK21 cells constitutively expressing T7 RNA polymerase were co-transfected with the plasmids encoding VSV nucleocapsid protein N, phosphoprotein P, polymerase L, and a plasmid encoding full length VSV genome with the HCV genes. In 2-5 days after transfection, the culture media is harvested when cells show cytopathic effects (CPE). The presence of recovered rVSVs is confirmed by plaque assay, and the viruses are purified by plaque picking and are amplified for the stock virus preparation by infecting BHK21 cells.

Cell infection with wild type VSV and rVSV

In order to make vial stock of VSV, we infect BHK21 cells (about $1.4X10^7$ cells in 10cm diameter culture dish) with multiplicity of infection (MOI) of 0.1, which is $1.4X10^6$

plaque forming unit. The viruses are harvested at 16 hours after infection and are stored at -80 °C deep freezer.

In order to determine the expression of gene of interest (HCV proteins) from the rVSV, BHK21 cells are infected with an MOI of 6 for 6 hrs, and the cell lysates are prepared in a lysis buffer containing NP-40. The cell lysates are kept at -80 °C until the protein expression is determined by the Western blot analysis.

Note: All VSVs in Dr. C. Yong Kang's Lab, SDRI, Rm 129, are handled according to the biosafety guidelines and procedures for containment level 2

Martha's comments (Kang lab)

-------- Original Message --------
Subject: Re: VSV Kang study in rodents
Date: Mon, 26 Jan 2009 12:29:03 -0500
From: Martha Harding <mhardin8@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
References: <497DCA5E.8010305@uwo.ca>

Hi Jennifer,
While I was at Yale, I heard of a study through Yale Biosafety colleagues that Dr. John Rose's lab group performed to help with the development of biosafety protocols for VSV vaccine experiments in mice. Following the Rose lab's typical vaccination paradigm (ie. ~10^6 pfu VSV-Ind), immunocompetent mice shed the virus for a maximum of 5 days. I presume that this was following a intranasal vaccination, but don't know for sure (higher replication should occur w/ in route compared to sc or im)
Hence, Yale developed the policy that all entering the room could not have contact w/ ruminants, horses & pigs for 7 days.
There's nothing in print that I know of (nor can find on pubmed), but hope that this information helps,
Martha.

Comments from Yale Biosafety

-------- Original Message --------
Subject:RE: VSV work
Date: Thu, 29 Jan 2009 09:40:52 -0500
From: Fontes, Benjamin <benjamin.fontes@yale.edu>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: Fontes, Benjamin <benjamin.fontes@yale.edu>

Jennifer:

I don't have an "exact" copy of Dr. Harding's SOP (her form was handwritten), but it would be similar to the attached "stuff." She worked with Jon Reuter and sometimes with Dr. Brandsma (fringe collaboration).

I've attached Dr. Reuter's rDNA approval letters to show you some language that we've used.

I've also attached a HIV cell culture protocol which is close to HCV. Dr. Brandsma's VSV cell culture (generic) protocol is attached. Finally, the BSL2+ animal protocol is attached.

In regard to shedding, I'll dig up Dr. Roberts' email with her data. Her boss said zero shedding. CT has a State Waste Reg here that says that once the animal is infected with a BSL2, it is BSL2 waste for life. So, this placed significant restriction on us. However, the shedding data from Dr. Roberts, which tested for virus from all mucous membranes (top and bottom) for 10 days and found a positive result in one animal up to day 9. With that data, we were able to "limit" the USDA restriction for contact with hooved animals for 10 days (after that, they were free to play with their horses).

I hope this is helpful. Ben

Dr. John Reuter
Comparative Medicine
LSOG 117

Dear Dr. Reuter:

Under the direction of the Yale Biological Safety Committee, the Biosafety Office has reviewed your rDNA application, rDNA protocol #05-22, entitled, "Neurotropic CMV During Immune Suppression and Vaccine Development," and found it to be in conformity with the NIH Guidelines. The protocol was approved at Biosafety Level 2+ containment, with the following additional precautions:

- Experiments in animals require a start up meeting with your staff, Biosafety and representatives from the Yale Animal Resources Center (YARC). Please contact Ms. Randi Palmisano at 785-4722 to schedule a meeting if needed.
- Lab staff will provide advance notice (when at all possible) of waste generation to YARC so that immediate decontamination can be accomplished.
- Any wastes (cages, carcasses, sharps, gloves/non-sharps materials, etc.) that cannot be immediately decontaminated (after hours or unplanned) will remain in the animal room, under appropriate containment (sealed and bagged) labeled with the PI name, agent & date generated, until YARC can remove them from the room and place such wastes directly in the autoclave for decontamination.
- Lab and YARC staff handling lab strains of VSV or VSV inoculated animals will not be allowed to work with (work/home) farm species.

- BL2+ containment (BL3 work practices in a BL2 laboratory) for recombinant experiments with lab strains of Vesicular Stomatitis Virus containment with the following additional precautions:
    - The facility should be self-contained, that is, all equipment needed for the experiment must be located in your laboratory;
    - All work must be done in a biosafety cabinet;
    - Vacuum lines must be protected by filters;
    - Minimize or eliminate sharps, such as needles or glass Pasteur pipettes, from your experiments;
    - Use containment accessories (sealed tubes and safety canisters or rotors with sealed O-rings) for centrifugation;
    - A lab coat or back-fastening gown and gloves must be worn; and
    - If a worker suspects that they have become ill due to an exposure to a recombinant virus, they should report the symptoms to the PI; and
    - Researchers using live VSV or VSV recombinant viruses should avoid contact with cattle, horses and swine.

- Use containment accessories (sealed tubes and safety canisters or rotors with sealed O-rings) for centrifugation;
- A lab coat or back-fastening gown and gloves must be worn; and
- If a worker suspects that they have become ill due to an exposure to a recombinant virus, they should report the symptoms to the PI; and
- Researchers using live VSV or VSV recombinant viruses should avoid contact with cattle, horses and swine.

Enclosed is an approved registration document for your files. The Biosafety Officer will visit your laboratory periodically to monitor your facility and procedures, and to answer any questions you may have regarding safety.

Should you wish to add personnel to your project, change the scope or location of your work, you must notify the Biosafety Office. It is the responsibility of the Principal Investigator to train new personnel before they begin work.

If you have any questions concerning the above, please contact the Biosafety Office, 737-5009.

Sincerely,

Renewal Date: March 31, 2008

Benjamin Fontes
Biosafety Officer

Approval Date: March 3, 2006

CC: Randi Palmisano

Dr. John Reuter
Comparative Medicine
LSOG 117

Dear Dr. Reuter:

Under the direction of the Yale Biological Safety Committee, the Biosafety Office has reviewed your rDNA application, rDNA protocol #06-11, entitled, "VSV and Adenovirus Vaccination against CMV" and found it to be in conformity with the NIH Guidelines. The protocol was approved as follows:

Recombinant Adenovirus/mCMV:
Biosafety level 2 containment for experiments involving replication defective adenoviral vectors and subsequent challenge with mCMV. Contact the Yale Animal Resources Center to schedule a start-up meeting for your new experiments.

VSV Lab Strains:
Biosafety Level 2+ containment for work with lab strains of VSV, with the following additional precautions:

- Experiments in animals require a start up meeting with your staff, Biosafety and representatives from the Yale Animal Resources Center (YARC). Please contact Ms. Randi Palmisano at 785-4722 to schedule a meeting if needed.
- Lab staff will provide advance notice (when at all possible) of waste generation to YARC so that immediate decontamination can be accomplished.
- Any wastes (cages, carcasses, sharps, gloves/non-sharps materials, etc.) that cannot be immediately decontaminated (after hours or unplanned) will remain in the animal room, under appropriate containment (sealed and bagged) labeled with the PI name, agent & date generated, until YARC can remove them from the room and place such wastes directly in the autoclave for decontamination.
- Lab and YARC staff handling lab strains of VSV or VSV inoculated animals will not be allowed to work with (work/home) farm species.

- BL2+ containment (BL3 work practices in a BL2 laboratory) for recombinant experiments with lab strains of Vesicular Stomatitis Virus containment with the following additional precautions:
    - The facility should be self-contained, that is, all equipment needed for the experiment must be located in your laboratory;
    - All work must be done in a biosafety cabinet;
    - Vacuum lines must be protected by filters;
    - Minimize or eliminate sharps, such as needles or glass Pasteur pipettes, from your experiments;

Enclosed is an approved registration document for your files. The Biosafety Officer will visit your laboratory periodically to monitor your facility and procedures, and to answer any questions you may have regarding safety.

Should you wish to add personnel to your project, change the scope or location of your work, you must notify the Biosafety Office. It is the responsibility of the Principal Investigator to train new personnel before they begin work.

If you have any questions concerning the above, please contact the Biosafety Office, 737-5009.

Sincerely,

Benjamin Fontes
Biosafety Officer

Approval Date: June 30, 2005

CC: Randi Palmisano

YALE ANIMAL RESOURCES CENTER
BL2+ PROCEDURES

The YARC will follow established regulatory policies. The outline listed below is the standard policy for animals infected with **BL2+** agents. Biological safety may advise additional procedures on a case by case basis.

Labeling

- animal room door must be labeled with a biohazard symbol and a BL2 sign with all information completed on the sign.
- cages must be labeled with a "Biosafety Level 2+ Agent in Use" cage card(which has the biohazard symbol in the upper left corner). All information must be completed by the P.I. and the YARC supervisor for identification of special handling by YARC.

Protective clothing

- solid front, long sleeve gown with gloves pulled over the gown sleeve cuff will be worn.
  - Recommend double glove
    - Gloves should be removed, hands washed thoroughly prior to exiting room.
  - Remove contaminated gown in animal room and deposit in appropriate container.
    - Extra gowns will be available in room for change out

  a mask and eye protection must be worn if a Class II cabinet is not available
- Biological Safety will advise if additional personal protection is required (i.e. respirator).

Housing

- MI (static) cages required, once animals are infected cages should only be opened in the hood.

Engineering Controls

- A Class II safety cabinet is required for the handling of (small) animals and experimental manipulations. A case by case assessment will be made by Biosafety for larger animals or unique circumstances.
- Air pressure differential of the animal room must be adjusted from positive to negative pressure. A case by case assessment will be made by Biosafety for unique circumstances

Work space

NA Decontaminate the Class II cabinet with Clidox (1:18:1 solution is adequate but QA may require 1:5:1) before and after use.

- other work surfaces can be decontaminated with a 10% clorox or Clidox (1:18:1) solution
- room sanitizing agents must be a tuberculocidal (i.e., Quat TB or LPH)

Work Practices

- Husbandry & experimental practices/procedures shall be conducted in such manner to minimize the potential release of aerosols into the environment (i.e. do not vacuum any BL2+ waste or materials, caution when handling to prevent spills)

  chemical decontamination of the cage **EXTERIOR**, within the **Class II cabinet in the** animal room, required prior to removal from the Class II cabinet

  Personnel must wash their hands after removing gloves and upon exiting the animal room.

Decontamination

Caging

- in most instances, there will be a designated cart or rack in the animal room for soiled and labeled BL2/BL2+ cages. Filter bonnet must remain on the soiled cage to minimize potential for contamination
- Biological Safety requires BL2+ caging to autoclaved intact (w/ bedding in cage) prior to washing. **Caging will remain in the animal room until YARC can immediately decontaminate (autoclave)**
- cages can not be power-sprayed prior to placement in rackwasher or tunnelwasher.
- caging must be washed in rackwasher or tunnelwasher at 180^0f cycle for a minimum of 3 minutes.

Waste

- excreta, bedding, carcasses and gloves are bio-bagged in animal room within the Class II cabinet. A case by case assessment will be made by Biosafety for larger animals or unique circumstances.
- BL2+ cages and carcasses should be autoclaved immediately. Please label the bagged material with origin and agent..
  - If animals are found deceased on a weekend, the live animals should be transferred to a clean cage and the carcass and **original** cage left in the room on the BL2+ rack in the room until autoclaving can be accomplished. **Both cages shall be properly labeled.**
- all waste is autoclaved for 30 minutes at 250^0f at 15 p.s.i.
- upon completion of autoclaving the bagged waste is placed in the biomedical regulated waste stream. **It is critical that waste be properly disposed of in the biomedical regulated waste stream.**

Emergency Response

- secure animal(s)
- apply first aid- forced bleed and wash; flush mucous membranes
- contact Employee Health 2-0071 and OEHS 5-3550 or 111 after hrs.

Medical Surveillance

- Case by case assessment of the agent and personal health history must be conducted by the Employee Health Department and Biosafety. Sera storage, consultations, sampling or immunizations may be recommended.

Prepared by R. Palmisano, Regulatory & Safety Services
Reviewed by B. Fontes, Biosafety
Date: 12/93, 2/97, 7/01, 10/03; 2/06

Cell Culture SOP for VSV/HPV Experiments
PI: Dr. Janet Brandsma
BML BB 33
Dr. Susan Compton's Cell Culture Facility

The following SOP has been prepared from OEHS Biosafety Guidelines for performing cell culture experiments and the manipulation of tissues from animals infected with HPV or VSV lab strains.

Personal Protective Equipment (PPE)

- Protective clothing worn will be dedicated to BML BB 33. PPE worn for BL2+ work will not be worn in other areas and removed before leaving the laboratory.
- A lab coat or solid-front gown, with a knit or grip cuff.
- Double gloves shall be worn for all work within the biological safety cabinet (BSC). Gloves will extend over the sleeve of the lab coat.
- PPE will be removed before leaving the laboratory.
- Hands shall be washed with soap and water after removing PPE and before leaving the laboratory.

Engineering Controls

- All work will be performed within a Class II Biological Safety Cabinet.
- Plastic will be used in place of glass where feasible.
- Vacuum systems will be protected by hydrophobic or HEPA filters.
- Items will be disinfected with 10% bleach and 70% ethanol for 10 minutes after use for terminal decontamination.
- A sealed rotor or aerosol canister will be used for centrifugation. Rotors or canisters will be loaded and unloaded within the biological safety cabinet.
- Items will be transported according to Yale OEHS policy as noted below:

Transport of Biohazards

On Campus Transport (between labs or buildings):

- ✓ Must have two leak proof containers, including the following:
  - -a sealed primary container
  - -a sealed secondary container
  - -absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
  - -a biohazard sticker on outside of the secondary container with agent name, lab address and phone number
- ✓ Utilize plastic containers whenever feasible, avoid glass.
- ✓ Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
- ✓ If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.

Labels

Equipment housing biohazardous agents will be labeled with the universal biohazard symbol and agent name.

Spills and Exposure Incidents

- All researchers will be familiar with the applicable exposure response procedures as developed by OEHS before initiating their experiments.
- The attached OEHS Biosafety Spill and Incident Response Guide will be utilized for incident response by all laboratory members.

Standard Operating Procedures to study human dendritic cells after in vitro HIV-1 infection under BL2.5/BL3 conditions

Tasks performed:

- Transfer of the virus from the freezer to the tissue culture room and in vitro infection of human dendritic cells.
- Harvest cell culture supernatants and perform ELISA.
- Stain cells with antibodies and fix cells for further analyses using flow cytometry or confocal microscopy.
- Grow HIV-1 on PHA/IL-2 stimulated PBMCs and concentrate the virus by ultracentrifugation.

Summary:
To study the interactions between human dendritic cells (DCs) and HIV-1, we will isolate DCs from healthy human subjects. These DCs will be exposed/infected to either replication deficient HIV-1 (BL2.5 conditions) or replication competent HIV-1 (BL3 conditions). After HIV-1 exposure/infection the DCs will be prepared for analysis by flow cytometry and/or microscopy, a process in which the cells are fixed before transfer from the BL2.5/BL3 facilities. Below the procedures in both the BL3 and the BL2.5 facilities are outlined in detail, also when they overlap.

Facilities:
The BL3 facility is located in LEPH on the 5th floor
Designated tissue culture room: LEPH 512B
Freezers (-20°C and -80°C): LEPH 512F
ELISA washer and reader: LEPH 513
Ultracentrifuge: LEPH 513
Autoclaves: LEPH 512A and 512C

The BL2.5 facility is located in BCMM 348B
Designated tissue culture room: BCMM 348B
(is equipped with 2 biosafety cabinets, freezers (-20°C and -80°C), fridge, bench top centrifuge, 2 CO_2 incubators and water bath)
Autoclave: BCMM 348 (outside BCMM 348B)

Procedures BL3:

Entering the LEPH BL3 facility

When entering the LEPH BL3 facility on the 5th floor, the researcher will sign in and put on one pair of gloves before entering the laboratories. From the sign in room, the researcher will enter the back corridor to get to 512B.

Work in the biosafety cabinet (BSC) in LEPH 512B

In LEPH 512B, the researcher will put on protective clothing, goggles or face shield, back-fastening gown, and a second pair of gloves (double gloves) before initiating any work. Before initiating work in the biosafety cabinet (BSC), the BSC will be turned on and blower allowed to run for at least 5 minutes. Work surfaces will be wiped off with a towel soaked in 10% bleach and allowed to dry for a minimum of 10 minutes followed by 70% ethanol that is wiped off to remove bleach residue. Outer gloves will be changed before leaving the BSC, so that clean gloves are always worn outside the BSC. Solid waste is discarded in leak proof red buckets with lids. Liquid waste is collected in a flask with bleach (final concentration 10%), and allowed to decontaminate for a minimum of 30 minutes before liquid is discarded down the drain. Pipette tips etc can be collected inside the BSC in a bucket lined with a biohazard bag. When work is finished the bag is sealed with tape, sprayed with 70% ethanol and discarded in a leak proof red bucket. Serological pipettes will be collected inside the BSC in a bucket with 10% bleach with a 30 minute contact time; bleach is pipetted up in the serological pipette, Pipettor removed and bleach allowed to run through the serological pipette into the waste bucket. After work is finished, decontaminated serological pipettes are discarded in a leak proof red bucket. Anything removed from the BSC will be sprayed with 10% bleach and may be followed up with 70% ethanol to remove bleach residue. Culture flasks can be sprayed with 70% ethanol instead of bleach if desired.
No sharps (glass, needles etc) will be used in the work with HIV-1.
When work in the BSC is finished, the work surfaces will be wiped off with a towel soaked in 10% bleach and allowed to dry for a minimum of 10 minutes followed by 70% ethanol that is allowed to dry (minimum 5-10 minutes).

Transfer of virus from the freezer in LEPH 512F to BSC in LEPH 512B

The virus sample will be taken out of the -80°C freezer in LEPH 512F and quickly thawed in a 37°C water bath in LEPH 512B. After thawing, the virus will immediately be added to dendritic cells in tissue culture plates or tubes.

In vitro HIV-1 infection of human dendritic cells in BSC in LEPH 512B

Dendritic cells isolated from healthy donors and cultured under BL2 conditions in SHM-C424 will be brought in to LEPH 512B for in vitro HIV-1 infection in 24 well tissue culture plates or FACS tubes with lids. The plates or tubes will be transported inside a leakproof secondary container labeled with a biohazard sticker, the name of the agent, the PI name, and a contact phone number.

Dendritic cells will be exposed to infectious HIV-1 equivalent to 0.01-1μg/mL HIV-1 p24 in the BSC in the BL3 lab LEPH 512B. HIV-1 is expected to infect a fraction (0-40% depending on the donor from which the dendritic cells were isolated) of the dendritic cells under these conditions The virus will be co-cultured with these cells in 24 well tissue culture plates or FACS tubes with lids for varying time periods, ranging from minutes to days, in a 5% CO2 incubator in the BL3 lab LEPH 512B.

Harvest of cell culture supernatants from HIV-1 infected dendritic cells

To collect cell-free supernatants from the dendritic cell cultures, the cells will be centrifuged to pellet the cells. Centrifugation of the cells will be performed in FACS tubes with lids, in the bench top centrifuge in LEPH 512B. This centrifuge has lids to cover the buckets, which will ensure secondary containment. The lids of the buckets will only be opened inside of the BSC. In the BSC, supernatants will be collected by aspirating the cell culture supernatants from the tubes and transferring them to sterile cryo tubes. Supernatants will be analyzed immediately or stored in the -80°C freezer in LEPH 512F for future analysis.

ELISA

ELISA will be performed in the BSC to determine the concentrations of various proteins in the cell culture supernatants, collected as described above. The initial step of the ELISA requires treatment with a detergent (Triton X-100, final concentration 0.5%) that inactivates the virus. In addition, when the ELISA is completed (before addition of the substrate), the sample has been washed 6-8 times and the microtiter plate does not contain virus, only protein bound to antibodies. The microtiter plate with a lid, will be transferred to LEPH 513 for analysis using the plate reader located in LEPH 513. After the reading, the work area surrounding the reader will be wiped off with a towel soaked in 10% bleach and allowed to dry for a minimum of 10 minutes followed by 70% ethanol that is allowed to dry (minimum 5-10 minutes).

Staining of HIV-1 infected dendritic cells

In the BSC in LEPH 512B, the dendritic cells will be stained with antibodies and prepared for analysis by flow cytometry (FACS). Briefly, dendritic cells are washed in PBS by centrifugation in the bench top centrifuge in LEPH 512B. This centrifuge has lids to cover the buckets, which will ensure secondary containment. The lids of the buckets will only be opened inside of the BSC. The PBS is aspirated and monoclonal antibodies are added to the cells and incubated for 30 minutes at 4°C in the fridge in LEPH 512B. The dendritic cells are washed again to remove any unbound antibodies. Finally, the dendritic cells (and the virus) will be fixed with 2% paraformaldehyde (PFA) for 10 minutes, after which the virus is dead. The samples will be removed from the BL3 in FACS tubes with lids which are enclosed in a secondary container. PFA is stored at -80°C in LEPH 512F, in a 10% stock solution and will be diluted with PBS in the BSC in LEPH 512B.

To perform immunofluorescence microscopy on the HIV exposed/infected dendritic cells, the cells will be allowed to adhere on coverslips for 30 minutes at room temperature inside the BSC in LEPH 512B and subsequently fixed with 4% PFA for 30 minutes. The coverslips are washed with PBS by adding PBS to the coverslip and aspirating it off. The coverslips with fixed dendritic cells will be stored in 24 well plates with lids containing 0.5 mL PBS/well. The 24 well plates containing the fixed coverslips will be transported from the BL3 lab in a secondary container.

Expansion of HIV-1on PHA/IL-2 activated PBMCs

Day –3 or 4	SHM-C424: Isolate PBMCs by Ficoll separation from 2 healthy blood donors and stimulate the PBMCs with PHA (2,5μg/mL, Sigma) and IL-2 (75μg /mL, Chiron) at a cell density of 1-2x10^6 cells/mL.
Day 0	Transfer cells to LEPH 512B. After 3-4 days of PHA/IL-2 stimulation: pool cells from both donors, 5x10^6 cells/mL complete medium with IL-2. Add 5x10^6 cells (1mL) per 15 mL Falcon tube and 1mL HIV-1 (1μg p24; NIH AIDS Research and Reference Reagent Program, stored in -80 freezer LEPH 512F). Incubate cells with virus for 4-6 hours in 37°C in LEPH 512B. Transfer cells and virus from tubes to T125 flasks and add 13 mL pre-warmed complete medium with IL-2 to a final volume of 15 mL/flask. SHM-C424: Prepare PBMCs from 2 additional donors and stimulate with PHA (2,5μg/mL, Sigma) and IL-2 (75μg /mL, Chiron) at a cell density of 1-2x10^6 cells/mL.

Day 3-4 LEPH 512B: Feed HIV-1 cultures with new PHA/IL-2 stimulated PBMCs, approx. 15-20x10^6 cells per flask. Expand each baby flask (15mL) to T75 flasks and a final volume of 50mL per flask. HIV-1 is now growing on activated PBMCs from 4 different donors.

Check flasks continuously to see if the cells are dying of superinfection.

Day 7 Harvest the virus. Transfer the cells and cell culture supernatants from the flasks to 50 mL Falcon tubes. Spin down cells by centrifugation in the bench top centrifuge in LEPH 512B. The lids of the centrifuge buckets will only be opened inside of the BSC. Transfer the supernatant (HIV-1 stock) to sterile cryo tubes. Store in -80°C freezer in LEPH 512F or concentrate by ultracentrifugation (see below). Quantify virus titer by p24 ELISA.

Ultracentrifugation of virus

HIV will be ultracentrifuged to obtain concentrated HIV-1 and to minimize the presence of bystander factors that can cause maturation of the dendritic cells. HIV stock will be transferred from LEPH 512B to LEPH 513 in a sealed secondary container. The rotor of the ultracentrifuge (LEPH 513) is transferred to the biosafety cabinet in LEPH 513. Disposable tubes that fit the rotor are placed in the rotor and the HIV stock obtained as described above is transferred to the disposable tubes. The lid of the rotor is secured and the closed rotor is sprayed with 70% ethanol and transferred to the ultracentrifuge. To pellet the virus, the supernatants are centrifuged at 138,000g at 4°C in the ultracentrifuge. After the spin, the rotor is again moved from the centrifuge to the BSC before it is opened. Supernatants are aspirated and the virus pellets are resuspended in complete medium to obtain virus stock 10-100 times more concentrated. The concentrated HIV-1 stocks are aliquoted in sterile cryo tubes and stored in the -80°C freezer in LEPH 512F.

Exiting the LEPH BL3 facility

When work is finished, the outer gloves and back-fastening gown are discarded in a leak proof red bucket with lid before leaving the LEPH 512B. Any fixed samples that are to be removed from the facility are sprayed with 70% ethanol and placed in a secondary, closed container for transport, which is also sprayed with 70% ethanol. The researcher then exits the facility through LEPH 510C where the final gloves are removed and discarded in a red bucket with lid located next to the sink designated for hand washing. After hand washing, the towels are discarded in the regular trash. The researcher should sign out before leaving the BL3 facility on the 5th floor.

Procedures BL2.5:

Entering BCMM 348B

When entering BCMM 348B, the researcher will put on one pair of gloves, a disposable overall, disposable shoe covers and a face shield before stepping over the dressing/washing area by the sink (marked on the floor with red tape).

Work in the biosafety cabinet (BSC) in BCMM 348B
Before initiating work, the researcher puts on a second pair of gloves. The BSC will be turned on and blower allowed to run for at least 5 minutes. Work surfaces will be wiped off with a towel soaked in 10% bleach and allowed to dry for a minimum of 10 minutes followed by 70% ethanol that is wiped off to remove bleach residue. Outer gloves will be changed before leaving the BSC, so that clean gloves are always worn outside the BSC. Solid waste is discarded in leak proof red buckets with lids. Liquid waste is collected in a flask with bleach (final concentration 10%), and allowed to decontaminate for a minimum of 30 minutes before liquid is discarded down the drain. Pipette tips etc can be collected inside the BSC in a bucket lined with a biohazard bag. When work is finished the bag is sealed with tape, sprayed with 70% ethanol and discarded in a leak proof red bucket. Serological pipettes will be collected inside the BSC in a bucket with 10% bleach with a 30 minute contact time; bleach is pipetted up in the serological pipette, Pipettor removed and bleach allowed to run through the serological pipette into the waste bucket. After work is finished, decontaminated serological pipettes are discarded in a leak proof red bucket. Anything removed from the BSC will be sprayed with 10% bleach and may be followed up with 70% ethanol to remove bleach residue. Culture flasks can be sprayed with 70% ethanol instead of bleach if desired.
No sharps (glass, needles etc) will be used in the work with HIV-1.
When work in the BSC is finished, the work surfaces will be wiped off with a towel soaked in 10% bleach and allowed to dry for a minimum of 10 minutes followed by 70% ethanol that is allowed to dry (minimum 5-10 minutes).

Replication deficient HIV-1
Alexa488 labelled HIV-1ADA and HIV-1MN will be provided by Dr. Jeff Lifson, director AIDS Vaccine Program, NCI, Frederick, MD. The labeling process renders the virus replication incompetent (Morcock et al, J Virol. 2005 Feb;79(3):1533-42 "Elimination of retroviral infectivity by N-ethylmaleimide with preservation of functional envelope glycoproteins").

In vitro HIV-1 infection of human dendritic cells in BSC in BCMM 348B
Dendritic cells isolated from healthy donors and cultured under BL2 conditions in SHM-C424 will be brought in to BCMM 348B for in vitro HIV-1 infection in 24 well tissue culture plates or FACS tubes with lids. The plates or tubes will be transported inside a leakproof secondary container labeled with a biohazard sticker, the name of the agent, the PI name, and a contact phone number.

Dendritic cells will be exposed to replication incompetent HIV-1 equivalent to 0.01-1μg/mL HIV-1 p24 in the BSC in the BL2.5 lab BCMM 348B. HIV-1 is expected to infect a fraction (0-40% depending on the donor from which the dendritic cells were isolated) of the dendritic cells under these conditions The virus will be co-cultured with these cells in 24 well tissue culture plates or FACS tubes with lids for varying time periods, ranging from minutes to days, in a 5% CO2 incubator in the BL2.5 lab BCMM 348B.

Staining of HIV-1 infected dendritic cells
In the BSC in BCMM 348B, the dendritic cells will be stained with antibodies and prepared for analysis by flow cytometry (FACS). Briefly, dendritic cells are washed in PBS by centrifugation in the bench top centrifuge in BCMM 348B. This centrifuge has lids to cover the buckets, which will ensure secondary containment. The lids of the buckets will only be opened inside of the BSC. The PBS is aspirated and monoclonal

antibodies are added to the cells and incubated for 30 minutes at 4°C in the fridge in BCMM 348B. The dendritic cells are washed again to remove any unbound antibodies. Finally, the dendritic cells (and the virus) will be fixed with 2% paraformaldehyde (PFA) for 10 minutes, after which the virus is dead. The samples will be removed from the BL2.5 in FACS tubes with lids which are enclosed in a secondary container. PFA is stored at -80°C in BCMM 348B, in a 10% stock solution and will be diluted with PBS in the BSC in BCMM 348B.
To perform immunofluorescence microscopy on the HIV exposed/infected dendritic cells, the cells will be allowed to adhere on coverslips for 30 minutes at room temperature inside the BSC in BCMM 348B and subsequently fixed with 4% PFA for 30 minutes. The coverslips are washed with PBS by adding PBS to the coverslip and aspirating it off. The coverslips with fixed dendritic cells will be stored in 24 well plates with lids containing 0.5 mL PBS/well. The 24 well plates containing the fixed coverslips will be transported from the BL2.5 lab in a secondary container.

Exiting BCMM 348B

Any fixed samples that are to be removed from the facility are sprayed with 70% ethanol and placed in a secondary, closed container for transport, which is also sprayed with 70% ethanol. When work is finished, the outer gloves are discarded inside the BSC. The shoe covers, overall and gloves are discarded in a leak proof red bucket with lid by the dressing/washing area by the sink. After hand washing, the towels are discarded in the regular trash.

MEMORANDUM

DEPARTMENT OF OCCUPATIONAL HEALTH AND SAFETY

THE UNIVERSITY OF WESTERN ONTARIO

TO: Dr. Y. Kang, Dean, Faculty of Science

FROM: Gillian Norton, Biosafety Officer

DATE: 28 March 1994

SUBJECT: Research using VSV

Agriculture Canada has approved your request to use VSV at MRC Level 2. I have enclosed copies of my letter to them stating the conditions of use and the strains of the virus used plus a copy of their reply. Please let me know if you anticipate any changes in these conditions in the future. I have also sent you a copy of the requirements for Level 2 laboratories at the University. Please would you ensure that all these requirements are in place in your laboratory in SDRI. The laboratory will be inspected by me for compliance to these standards from time to time.

With reference to the necessity to obtain permission to transfer your VSV stocks (and other viruses) from Ottawa to Western, I believe that you did all the necessary paperwork at the time you relocated. However, I do not have copies of the letter of authorization in my records. Please could you call me to discuss this.

c.c. Dr. V. Morris, Chair, Biohazard Sub-Committee.

Agriculture Canada

Food Production and Inspection Branch

Direction générale, Production et inspection des aliments

Your file Votre référence

Our file Notre référence

2nd Floor West
59 Camelot Drive
Nepean, ON K1A 0Y9
(613) 952-8000
(613) 952-8884 fx

University of Western Ontario

March 24, 1994

Gillian Norton
Biosafety Officer
University of Western Ontario
Somerville House
London ON N6A 3K7

SUBJECT: Vesicular Stomatitis Virus

Dear Ms. Norton:

Your facsimile dated March 22, 1994 was received. We acknowledge your request for Dr. Y. Kang to use VSV for in vitro studies in laboratory containment facilities at MRC level 2. Authorization is given for the use of the strains of VSV referenced in your letter under these conditions.

In reference to the last paragraph of your letter, if the original viral stocks were brought into Canada under the authority of an import permit then it is necessary for Dr. Kang to obtain permission from the Animal Health Division of Agriculture and Agri-Food Canada prior to transferring this material to any other person or laboratory in Canada. This is clearly stated in a condition printed on the original import permit. A new import permit would not be issued, the letter of authorization to move the material is the only document required.

Sincerely,

B.S. Samagh
Associate Director
Veterinary Biologics
& Biotechnology
Animal Health Division

PG

PS: We would appreciate you referring to the above mentioned file number(s) when referring to the above mentioned subject(s)

The UNIVERSITY of WESTERN ONTARIO
Department of Occupational Health & Safety

22nd March, 1994

Dr. Greenwood
Animal Health Division
Agriculture Canada
2255 Carling Ave
Ottawa, Ont.

Dear Dr Greenwood,

As I explained in our telephone conversation earlier this morning, I am writing on behalf of a faculty member at the University of Western Ontario, Dr. Y. Kang, who has requested permission to use VSV at MRC Level 2 rather than Level 3 as stated in the MRC Guidelines.

Dr Kang will be using VSV strains Indiana and New Jersey but may also use Ogden and Hazelhurst. All experimental work is in vitro and there are no animal experiments. After culturing, the virus is harvested and DI particles (partial genome, non-infectious) are extracted and various molecular genetic procedures are carried out. The DI particles are not re-introduced into the virus.

Dr Kang informs me that he has the frozen stocks of these viruses in his lab which he transferred from the University of Ottawa to Western in 1992. Does he require a updated permit for the use of this virus at Western? If he does, please would you sent the appropriate forms to me at the above address.

Thank-you very much for your help,

Yours truly,

Gillian Norton

Gillian Norton, Biosafety Officer

Somerville House • London, Ontario • Canada • N6A 5B7 • Telephone: (519) 661-2056 • Fax: (519) 661-3930

Health and Welfare Canada | Santé et Bien-être social Canada
Health Protection Branch | Direction générale de la protection de la santé

MAR 2 0 1992

Office of Biosafety
Laboratory Centre for Disease Control
OTTAWA, Ontario K1A 0L2
Phone: (613) 957-1779
FAX#: (613) 941-0596

WHO Collaborating Centre
for Biosafety Technology
and Consultive Services

March 17, 1992

Ms. Gillian Norton
Occupational Health and Safety
Somerville House, Room 116
University of Western Ontario
1151 Richmond Street
London, Ontario
N6A 5B8

Dear Ms. Norton:

As per our telephone conversation, please find attached information on Hantaan Virus and Vesicular Stomatitis Virus.

There have been documented laboratory-associated infections with Hantaan (six reported to date--see attached) and work with this agent should be carried out under strict Level 3 containment facilities and operations. Any animal inoculations involving rodents must only be conducted with special caution (Biosafety Level 4). Additionally, animal inoculations involving Vesicular Stomatitis Virus is restricted by Agriculture Canada.

I trust that this information is of assistance to you.

Yours sincerely,

Maureen Best

Maureen Best
Head, Biosafety Services

MB:es

Attach.

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Vesicular stomatitis virus***

SYNONYM OR CROSS REFERENCE VSV, Vesicular stomatitis fever

CHARACTERISTICS: *Rhabdoviridae*; envelope with 10 nm spikes, bullet-shaped virion 70 x 175 nm, negative sense ssRNA

SECTION II - HEALTH HAZARD

PATHOGENICITY: Influenza-like symptoms; headache, fever, retrobulbar pain on motion of eyes, malaise, nausea, pain in the limbs and back; possible vesicular lesions in mouth, lips and hands; leukopenia; recovery may be prolonged and death is unknown

EPIDEMIOLOGY: North and South America. The Indiana and New Jersey strains are the two most prominent types in N. America. Related viruses in genus present in Asia, Africa; primarily affecting people handling dairy cattle and secondarily beef cattle

HOST RANGE: Wide host range, most mammals to insects. Cattle, horses, pigs, deer, humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Probably arthropod-borne via the bite of an infective sandfly, mosquito or blackfly; by direct contact with infected animals (vesicular fluid, saliva) or their environment; exposure to infectious aerosols has resulted in many laboratory-acquired infections

INCUBATION PERIOD: Up to 6 days, usually 3-4 days

COMMUNICABILITY: Virus is infective in blood for at least 24 hours before and after onset of fever

SECTION III - DISSEMINATION

RESERVOIR: Cattle, horses, swine

ZOONOSIS: Yes by direct contact with infected animals or by insect vector

VECTORS: Possibly sandflies, mosquitoes, blackflies. Virus has been cultured from vectors, and transmission has been demonstrated experimentally.

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: N/A

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde

PHYSICAL INACTIVATION: Inactivated on exposure to UV, heat and lipid solvents

SURVIVAL OUTSIDE HOST: Inactivated in sunlight and does not survive for long periods out of host unless protected in cool dark areas

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by virus isolation, serology

FIRST AID/TREATMENT: Symptomatic treatment (self-limiting disease)

IMMUNIZATION: Not available for use in humans

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Documented hazard to personnel handling infected livestock, tissues and virulent isolates; between 40 and 46 reported laboratory associated infections before 1980; seroconversion and illness rates are high

SOURCES/SPECIMENS: Vesicular fluid, tissues, and blood of infected animals; blood and throat secretions of humans

PRIMARY HAZARDS: Exposure to infectious aerosols and droplets; direct contact with skin and mucous membranes; accidental parenteral inoculation

SPECIAL HAZARDS: Handling infected livestock is a documented hazard

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Conditions under which this organism (not indigenous to Canada) is used and maintained for animal inoculations are established by Agriculture Canada; Biosafety level 3 practices, containment equipment and facilities for the manipulation of virulent isolates; laboratory adapted strains of demonstrated low virulence pose a lower risk

PROTECTIVE CLOTHING: Gloves and respiratory protection are recommended for the handling of infected animals and tissues

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

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

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

STORAGE: In sealed containers that are appropriately labeled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: February, 2001

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

[Material Safety Data Sheets - Index]

Last Updated: 2001-09-27 ▲ Important Notices

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Adenovirus types 1, 2, 3, 4, 5 and 7***

SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

CHARACTERISTICS: *Adenoviridae*; non-enveloped, icosahedral virions, 70-90 nm diameter, doubled-stranded, linear DNA genome.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is associated with adenovirus infection

EPIDEMIOLOGY: Worldwide; seasonal in temperate regions, with highest incidences in the fall, winter and early spring; in tropical areas, infections are common in the wet and colder weather; annual incidence is particularly high in children; adenovirus types 4 and 7 are common among military recruits (ARD)

HOST RANGE: Humans

INFECTIOUS DOSE: >150 plaque forming units when given intranasally

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal-oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available; cidofovir has shown promise in the treatment of adenoviral ocular infections.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

PHYSICAL INACTIVATION: Sensitive to heat >56°C; unusually stable to chemical or physical agents and adverse pH conditions

SURVIVAL OUTSIDE HOST: Resistance to chemical and physical agents allows for prolonged survival outside of the body. Adenovirus type 3 survived up to 10 days on paper under ambient conditions; adenovirus type 2 survived from 3-8 weeks on environmental surfaces at room temperature

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

PROPHYLAXIS: None available

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Ten cases documented up to 1988

SOURCES/SPECIMENS: Respiratory secretions

PRIMARY HAZARDS: Ingestion; droplet exposure of the mucous membrane

SPECIAL HAZARDS: Contact with feces from infected animals

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

PROTECTIVE CLOTHING: Laboratory coat; gloves when skin contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

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

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

[Material Safety Data Sheets - Index]

Last Updated: 2001-01-23 ▲ Important Notices