The University *of* Western Ontario

BIOLOGICAL AGENTS REGISTRY FORM

Approved Biohazards Subcommittee: October 14, 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. Gregor Reid**
DEPARTMENT:	**Microbiology and Immunology**
ADDRESS:	**Lawson, 268 Grosvenor St., London, On, N6H 4V2**
PHONE NUMBER:	**519-646-6100 x65256**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**gregor@uwo.ca**

Location of experimental work to be carried out :

Building : **Lawson Health Research Institute** Room(s): **F3-127, F3-127a, F3-13**

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: **Western Heads East, Gates Foundation**

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
Dr. Jeremy Burton	**jeremy.burton@lawsonresearch.com**	**March 6, 2012**
Shannon Mifflin	**shannon.mifflin@sjhc.london.on.ca**	**August 6, 2010**
Megan Enos	**menos@uwo.ca**	**February 27, 2012**

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.

Reid Lab Involvement in under-nutrition and the human microbiota study

Collection of the samples from under-nourished pregnant women and those women receiving probiotic yogurt (Yogurt made on-site in Africa contains Lactobacillus rhamnosus GR-1 and two standard yogurt starter culture organisms, Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus) will be done in Mwanza, Tanzania at the Bugando Medical Centre by Dr. Charles Majinge. Processing and storage of the samples will be overseen by a member from the Reid Lab and all samples will be frozen and shipped back to the lab where they will be stored in a -80oC freezer until processed. Vaginal swabs, stool (approximately 2g), oral swabs and breast milk (approximately 10mLs from women who can provide it) will be the samples collected from the women as well as stool (approximately 2g) and oral samples from the newborns. This study includes all volunteers who give signed consent and who meet the only criteria of being under-nourished as defined by a clinical examination, BMI and daily diet, thus, some samples involved may be HIV positive as it is not an exclusion criterion.

Once the samples are back in London, the DNA will be extracted using various techniques, dependent on the sample, and extracted DNA will be sequenced using 16S rDNA Illumina Sequencing technology to analyze and compare the microbiota profiles. Recently, the Reid lab has developed the sequencing methodologies needed to identify not only the genera or phyla, but the bacterial species and abundances in various sites to aid in the sample analysis conducted in this study. Thus, we are confident that we will identify any profile differences between the group subsets. The data we receive from the samples collected in Mwanza, will then be compared to samples of obese pregnant women and their babies collected in London, Ontario, to determine if the microbiota changes between the two aberrant states and whether or not this can be transmitted to the newborns. DNA from the samples will be stored at -80C until the study and all analysis is completed. After this, they will be disposed of in Stericycle bins for removal and destruction.

From this study we hope to gain a greater understanding of the role that our microbiota takes under abnormal conditions. Many studies have looked at the microbiota of obese individuals but this will be the first study to look at the microbiota of individuals who are undernourished. In addition, it is known that newborns of individuals, who are obese or undernourished, tend to be more prone to these conditions and other abnormalities later in life. Accordingly, by including studies on the infant, we hope to determine whether or not the microbiota is passed on from the mother, thus leading to these observed long term effects. In addition, by including the individuals who receive probiotic yogurt we hope to see the microbiota change to one that more resembles a "normal" state, providing evidence that a simple food intervention could lead to improved maternal and newborn health.

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

Summary of the effect of under-nutrition on the human microbiota

Objectives: The objective of the proposed study is to describe, by high-throughput sequencing, the gut and vaginal microbiomes of healthy and malnourished pregnant women, and identify changes due to dietary intervention. This study will provide the first ever microbiome profiles of malnourished pregnant women and will aid in further understanding how the diet affects the human microbiome.

Background: It has been well documented that every individual provides a home to ten times more microbial cells then human cells. These microorganisms inhabit all orifices and are particularly abundant in the gastrointestinal tract where 100 trillion of more than 1000 species reside. Fortunately, the gut microbiota is beneficial in many processes, including food degradation and immune modulation. Because of this, the composition of the gut microbiome has been of interest and, with the development of high-throughput sequencing, determining its exact composition has become feasible. It is now known that the microbiome profile between individuals varies significantly, however the core microbiome, dominated by the Bacteroidetes and Firmicutes phyla, can be stable. This gut microbiota expresses 3.3 million genes, approximately 150 times more than the human genome. This represents a powerful encoding system that may drive a person to ingest certain foods. If this is the case, the microbial profiles will not change much when the diet is altered significantly. This would partly explain why people who go on various slimming diets tend to revert easily back to a desire for the foods that made them obese, in essence because the microbiota has become dependent on these foods. On the other hand, if a nutritious dietary intervention significantly alters the gut bacterial profile leading to improved well-being and nutrient uptake in under-nourished subjects, this will suggest that the microbiota are less responsible for the drive for specific foods, and it will also help identify key organisms that improve how food is processed.
In addition, it is known that the vaginal microbiota originate mostly from the gut. It has not yet been determined to what extent the diet alters the gut microbiota, which could in turn, cause a change in the vaginal microbiome. As the latter is important in reproductive health and defense against diseases, including sexually transmitted ones common in African women, it will be important to show an effect on this microbiome due to eating probiotic nutrient supplemented yogurt. In addition, under-nutrition is widespread in Africa and results in damaged gut permeability, the risk of other infections and poor uptake of nutrients. If the daily yogurt intake leads to improved BMI and signs of improved nutritional status, it will imply that the yogurt and/or the bacteria in it have helped repair the gut epithelial barrier and in turn nutrient uptake. Understanding the link between diet, gut and vaginal microbiomes, and nutritional status could then lead to further studies determining how this repair took place.

Study Design: Samples from nourished and under-nourished individuals will be collected and prepared for bioinformatic analysis to better explain how microbial profiles change over time and with dietary intervention. To achieve this, stool, oral, breast milk and vaginal samples of 10 obese pregnant women, 10 under-nourished women, and 10 healthy normal women will be collected. Samples will be processed and analyzed using Illumina sequencing. In addition, the samples from malnourished women given micronutrient supplemented probiotic yogurt daily for 6 months will be characterized to determine if the microbial community profiles change within an individual and across the group. Whole gene list analysis for approximately 20 of the most commonly found organisms will be performed on samples from the two subject groups that show the greatest differences in microbiota.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species _____
What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

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
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
						☐ 1 ☐ 2 ☐ 2+ ☐ 3
						☐ 1 ☐ 2 ☐ 2+ ☐ 3
						☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

HIV (possible)?
"Microbiota"?

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	☐ 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:

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	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
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 PHAC or CFIA containment level required ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

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	**breast milk/stool**	☐ Yes ☒ Unknown	**potentially HIV**	☐1 ☐2 ☒2+ ☐3
Human Blood (fraction) or other Body Fluid	**vaginal fluid/saliva**	☐ Yes ☒ Unknown	**potentially HIV**	☐1 ☐2 ☒2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: **HIV+ African woman are NOT excluded from this study. There is the potential for samples to be HIV+ but we are NOT isolating or sequencing for HIV.**
MSDS Attached

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
- 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: _____

HIV (possible) ?

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 List the location(s) for the animal experimentation and housing.

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

7.6 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:

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 LD_{50} (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 **Tanzania** ☐ 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 # **pending** ☒ 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 _____ *Date:* 26 [redacted] 2012

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: March 26, 2012
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

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:
Please see Appendix A.

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/workplacehealth.html

An X in the check box indicates you agree with the above statement.. ☒
Enter Your Name [signature] *Date:* 28 MAR 2012

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

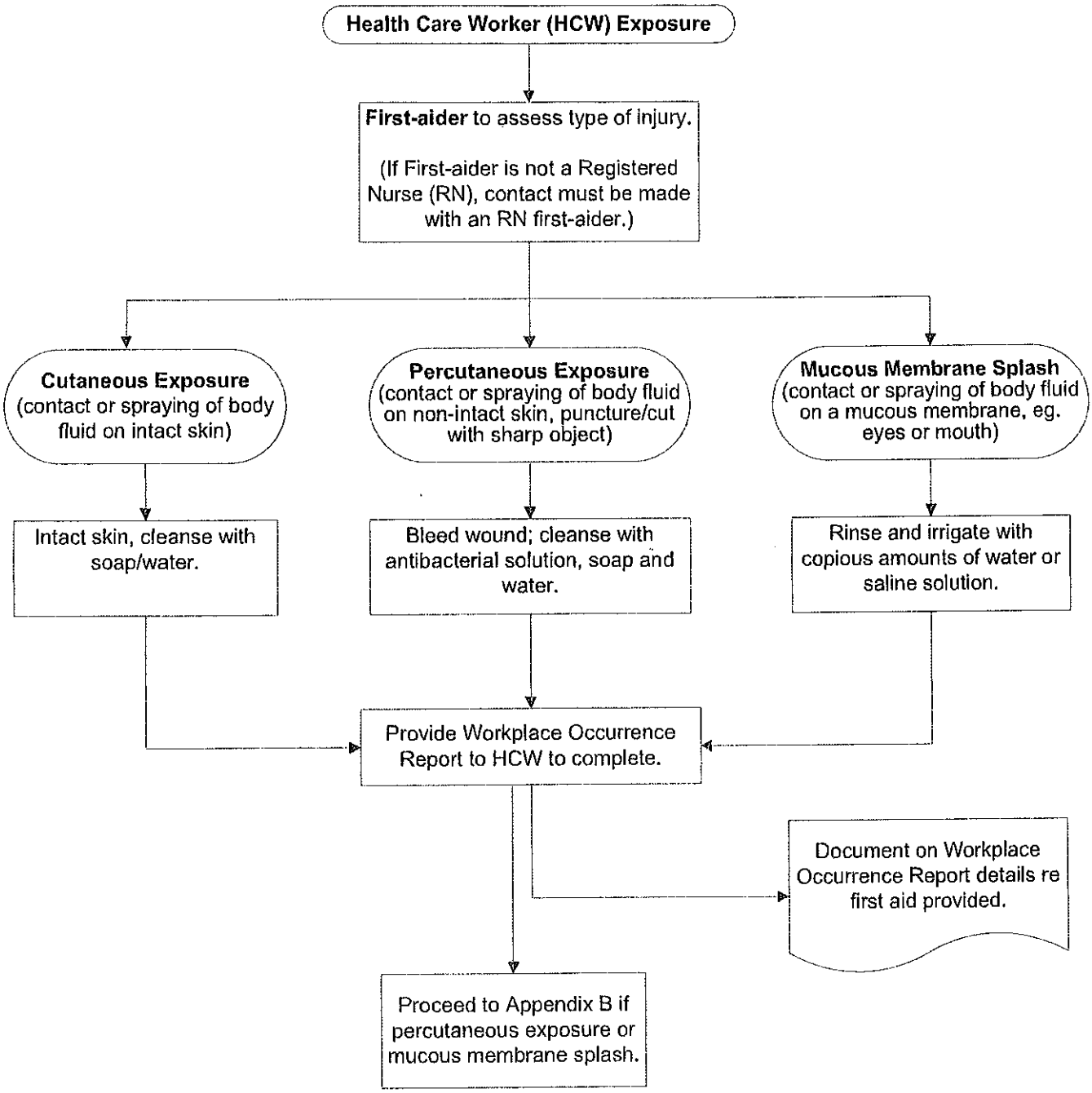
3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: [signature]
Date: March 26, 2012

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

FIRST AID PROCESS FOR EXPOSURE TO BLOOD-BORNE PATHOGEN (BBP)

Appendix A



Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

HUMAN IMMUNODEFICIENCY VIRUS

PATHOGEN SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: Human immunodeficiency virus (HIV).

SYNONYM OR CROSS REFERENCE: HIV, acquired immune deficiency syndrome, AIDS [1-20]. Was previously known as lymphadenopathy-associated virus, human T-lymphotropic virus type III (HTLV-III), immunodeficiency-associated virus, and AIDS-associated retrovirus [1-20].

CHARACTERISTICS: HIV is a member of the *Retroviridae* family, genus *Lentivirus* [14, 15]. HIV is an icosahedral, enveloped virus, of approximately 100 to 110 nm in diameter, and has a single-stranded, linear, positive-sense RNA genome [14, 16]. HIV has two recognised strains: HIV-1 and HIV-2 [11, 16, 17]. Upon entry into the host cell, retroviral RNA is converted to DNA by a virally encoded reverse transcriptase enzyme, the DNA transcript is integrated into the host's chromosomal DNA [14].

SECTION II - HAZARD IDENTIFICATION

PATHOGENICITY/TOXICITY: AIDS is characterised by symptoms and infections caused by the breakdown of the immune system (by destruction or functional impairment of CD4 receptors) due to HIV infection [10, 12]. HIV can infect many cell types, mainly lymphocytes, but also macrophages, and microglia in the brain, and other neurological cells, resulting in profound asthenia, dementia and damage to the peripheral nervous system [12]. Due to immunodeficiency, patients succumb to various fungi, parasites, bacteria, and/or viruses and are prone to certain tumours [10, 12]. Globally, *Mycobacterium tuberculosis* is the most common cause of death of HIV-infected individuals. The clinical features of HIV infection vary depending on the stage of the disease [6]. Acute infection is accompanied by non-specific "flu-like" and "mononucleosis-like" symptoms such as myalgia, arthralgia, diarrhoea, nausea, vomiting, headache, hepatosplenomegaly, weight loss, and neurological symptoms [6, 16, 21]. Early-stage disease refers to the period of clinical latency between the time of the primary infection and the development of symptoms indicative of advanced immunodeficiency. Typically, when the patient's CD4+ T-cell count falls below 500 cells/µL, syndromes indicative of depressed cell mediated immunity can appear. Examples include orophayngeal and recurrent vulvovaginal candidiasis, bacillary angiomatosis, recurrent or multidermatomal herpes zoster, listeriosis, infections due to *Rhodococcus equi*, pelvic inflammatory disease, oral hairy leukoplakia associated with Epstein-Barr virus, cervical dysplasia, long lasting diarrhoea, idiopathic thrombocytopenic purpura, and peripheral neuropathy [21]. Late-stage disease refers to the period when the patient's CD4+ T-cell count falls below 200 cells/µL [10, 21]. The loss of the integrity of cell-mediated immune responses allows ubiquitous environmental organisms with limited virulence to become life threatening pathogens [6]. Examples of conditions (as set out by the US Centers for Disease Control and Prevention) include candidiasis of bronchi, trachea, lungs or oesophagus, invasive cervical cancer, coccidioidomycosis, cryptococcosis, cryptosporidiosis, cytomegalovirus disease (other than liver, spleen, or nodes), cytomegalovirus retinitis (with loss of vision), HIV-related encephalopathy, herpes simplex, histoplasmosis, isosporiasis, Kaposi's sarcoma, Burkitt's lymphoma, immunoblastic lymphoma, primary lymphoma of the brain, *Mycobacterium avium* complex, *Mycobacterium tuberculosis*, *Pneumocystis jirovecii* pneumonia, recurrent pneumonia, progressive multifocal leukoencephalopathy, recurrent salmonella septicaemia, toxoplasmosis of the brain, and wasting syndrome due to HIV [21].

EPIDEMIOLOGY: HIV is a major global problem with approximately 25 million HIV-related deaths and another 40.3 (36 to 45.3) million infected individuals worldwide [1, 2]. AIDS was first described in 1981. The new retrovirus (HIV-1) was found in tissues from AIDS patients in 1983 and the causative relationship

between HIV and AIDS was established in 1984 [3, 12]. HIV-2 was discovered in 1986 and is the least pathogenic form of HIV, displaying low rates of transmission and rarely causing AIDS [4]. The majority of people with HIV live in the developing world (approximately 95% of the individuals infected worldwide). Sub-Saharan Africa is by far the worst-affected area in the world [10]. This region has slightly more than 10% of the world's population but is home to more than 60% of the total population living with HIV/AIDS [10].

Globally, infants who acquire the disease from their mothers constitute about 11% of all HIV infections [10]. Ten percent of infections worldwide are associated with injection drug use; 5 to 10% are transmitted by sex between men; and 5 to 10% occur in health care settings [10]. The predominant means of infection is sex between men and women, which accounts for nearly two thirds of new infections, and 85% of existing infections worldwide [10, 17]. About 50% of all new HIV infections worldwide occur in individuals younger than 25 years old [10].

HOST RANGE: Humans [3-6, 8, 10-13, 15-17, 20-23].

INFECTIOUS DOSE: Unknown.

MODES OF TRANSMISSION: HIV is transmitted either by exposure of the virus to oral, rectal, or vaginal mucosa during sexual activity; by intravascular inoculation through transfusion of contaminated blood products; by using contaminated equipment during injection drug use; or from mother to infant during pregnancy, delivery or breastfeeding [6, 16]. There are no obvious differences in disease manifestations in individuals infected by mucosal versus blood-borne routes [6]. Sexual transmission accounts for more than 90% of HIV infections worldwide [6, 15].

INCUBATION PERIOD: Variable. Commonly the time from infection to the development of detectable antibodies is generally 1 to 3 months; however, the time from HIV infection to diagnosis of AIDS had an observed range of less than 1 year to 15 years or longer [11].

COMMUNICABILITY: The highest levels of per-act risk for HIV transmission from person-to-person are: blood transfusion from an infected donor, needle sharing by infected injection-drug users, receptive anal intercourse, and percutaneous needle injuries [6, 11, 12, 20]. Insertive anal intercourse, penile-vaginal exposures, and oral sex represent substantially less per-act risk [6, 11, 23]. HIV can also be passed from mother to child *in utero* (vertical) as well as during childbirth, and from breast milk [6, 11]. HIV has also been documented to have been transmitted by bite injuries [22].The period of communicability begins early after HIV infection and is thought to last throughout the life of the infected individual [11]. Infectiousness is related to viral load.

SECTION III - DISSEMINATION

RESERVOIR: Humans [6, 8, 10-12, 16, 17, 22].

ZOONOSIS: None, although current evidence suggests that HIV-1 and HIV-2 entered into the human population through multiple zoonotic infections from simian immunodeficiency virus-infected non-human primates [17].

VECTORS: No laboratory or epidemiological evidence suggests that biting insects have transmitted HIV infection [11, 16].

SECTION IV – STABILITY AND VIABILITY

DRUG SUSCEPTIBILITY: Antiretroviral agents from 5 drug classes are currently available to treat HIV infection, namely: the nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), proteinase inhibitors (PIs), and fusion inhibitors [10, 15].

SUSCEPTIBILITY TO DISINFECTANTS: HIV is susceptible to fresh 2% glutaraldehyde, 2% Jodopax (detergent and iodine), hypochlorite, iodine, phenolics, and to a lesser extent 70% ethanol, NaOH and isopropanol [7, 9, 18].

PHYSICAL INACTIVATION: HIV is inactivated by ultraviolet (UV) light; however, the level of the

inactivation is heavily influenced by the proximity of the UV source to the sample and the concentration of protein in the sample environment. HIV is easily inactivated in a cell free medium; however, in cell associated samples and blood samples complete inactivation requires much longer exposures to the UV source [9]. HIV is also inactivated at pH higher or lower than the optimal level of 7.1 [18]. A temperature of 60°C for 30 minutes will likely inactivate HIV; however, higher temperatures and incubations may be required depending on the initial titre of the virus [18].

SURVIVAL OUTSIDE HOST: HIV can remain viable in blood in syringes at room temperature for 42 days, and in blood and cerebrospinal fluid from autopsies for up to 11 days [1, 2]. Although drying in the environment is known to cause a rapid reduction in HIV concentration, under experimental conditions, Cell-free HIV dried onto a glass coverslip in 10% serum can survive for longer than 7 days, depending on the initial titre [19].

SECTION V - FIRST AID / MEDICAL

SURVEILLANCE: HIV is diagnosed by tests that assess whether an individual's immune system has produced an HIV-specific immune response [16]. Common tests include the indirect binding assay, antibody capture assay, the double antigen sandwich, ELISA, immunofluorescence, Western blotting, line immunoassays, and PCR, as well as viral isolation [16].

FIRST AID/TREATMENT: AIDS must be managed as a chronic disease. Antiretroviral treatment is complex, involving a combination of drugs and resistance will appear rapidly if only a single drug is used [11]. The 5 available classes of antiretroviral drugs, NRTIs, NtRTIs, NNRTIs, PIs and fusion inhibitors, can be combined to provide highly active antiretroviral therapy (HAART). For many (but not all) patients, HAART converts an inexorably fatal disease into a chronic disease with a fairly good prognosis [8, 13].

IMMUNIZATION: None.

PROPHYLAXIS: HIV postexposure prophylaxis regimens are based on the nature of the exposure. The majority of HIV exposures will warrant a two drug regimen, using 2 NRTIs or 1 NRTI and 1 NtRTI. Combinations include: zidovudine (ZDV) and lamivudine (3CT) or emtricitabine (FTC); stavudine (d4T) and 3TC or FTC; and tenofovir (TDF) and 3TC or FTC [15].

The addition of a third or fourth drug should be considered for exposures that pose an increased risk of transmission. The preferred drugs in this case are proteinase inhibitors such as lopinavir/ritonavir (LPV/RTV) [15, 16].

SECTION VI - LABORATORY HAZARD

LABORATORY-ACQUIRED INFECTIONS: Although there have been many reported cases of HIV infection through occupational transmission, the numbers of laboratory acquired infections are low. As of 2001, there have been a total of 57 cases of documented occupationally acquired HIV among U.S. health care workers [24].

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, pleural fluid, pericardial fluid, amniotic fluid, other specimens containing visible blood, breast milk, unscreened or inadequately treated blood products, and infected human tissues [13, 15, 20].

Faeces, nasal secretions, sputum, sweat, vomitus, saliva, tears, and urine, are not considered potentially infectious unless they are visibly bloody [11, 13].

PRIMARY HAZARDS: Needlestick, contaminated sharp objects, and/or direct contact of non-intact skin or mucous membranes with HIV-infected specimens/tissues [15, 16].

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and/or splashing infected materials. HIV should be presumed to be in/on all equipment and devices coming in direct contact with infected materials [23].

SECTION VII - EXPOSURE CONTROLS / PERSONAL PROTECTION

RISK GROUP CLASSIFICATION: Risk Group 3 [26].

CONTAINMENT REQUIREMENTS: Containment Level 2 facilities and equipment for work involving clinical specimens and non-culture procedures. Containment Level 3 facilities, equipment, and operational practices for all work culturing HIV and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV [25].

PROTECTIVE CLOTHING: Solid-front gowns with tight-fitting wrists, gloves, and respiratory protection should be worn over laboratory clothing when infectious materials are directly handled [25].

OTHER PRECAUTIONS: All activities with infectious material should be conducted in a biological safety cabinet (BSC) or other appropriate primary containment device in combination with personal protective equipment. Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are unloaded in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings. Additional precautions should be considered with work involving animals or large scale activities [25].

SECTION VIII - HANDLING AND STORAGE

SPILLS: Allow aerosols to settle and, while wearing protective clothing, gently cover the spill with paper towels and apply 1% sodium hypochlorite starting at the perimeter, working inwards towards the centre. Allow sufficient contact time before clean up [25] .

DISPOSAL: Decontaminate all materials for disposal by steam sterilisation, chemical disinfection, and/or incineration [25].

STORAGE: Infectious material should be stored in sealed, leak-proof containers that are appropriately labelled [25].

SECTION IX - REGULATORY AND OTHER INFORMATION

REGULATORY INFORMATION: The import, transport, and use of pathogens in Canada is regulated under many regulatory bodies, including the Public Health Agency of Canada, Health Canada, Canadian Food Inspection Agency, Environment Canada, and Transport Canada. Users are responsible for ensuring they are compliant with all relevant acts, regulations, guidelines, and standards.

UPDATED: September 2011.

PREPARED BY: Pathogen Regulation Directorate, Public Health Agency of Canada.

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.

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Date Modified: 2011-09-08

Hi Jennifer,

Please find attached our application for the continuation of this registry form.

While we currently do not have an import permit, as the exact location of where the samples will be coming from is not yet determined, Jeff Tucker has completed the Level 2 forms for Health Canada and they are ready to apply for the import permit once the samples are collected and ready. This is why this form says pending.

I hope this is satisfactory.

Thanks,
Shannon

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Under the authority of the Human Pathogens Importation Regulations

Sous le régime du Règlement sur l'importation des agents anthropopathogènes

Importer-Name, address and postal code - Importateur-Nom, adresse et code postal

St. Joseph's Health Centre
268 Grosvenor Street
London, ON
N6A 4V2
Attn: Kate Crowley

Facsimile-Télécopieur: 519-646-6031

Telephone no.- No. de téléphone: 519-646-6100 ext. 65120

Supplier-Name and address - Fournisseur-Nom et adresse

National Institute for Medical Research
Mwanza Research Centre
D 12126 / 6386, Isamilo Road
PO Box 1462
Mwanza, Tanzania, East Africa

Name(s) of Port(s) of Entry- To Clear Customs at Port(s) of entry
Nom(s) du(s) point(s) d'entrée -Dédouanement au(x) point(s) d'entrée

Various ports

Description of Pathogen(s)-For the importation of- Description du(s) agent(s) anthropopathogène(s)-Pour l'importation de

Vaginal dacron swabs from female with Bacterial Vaginosis positive for HIV.

On the following terms and conditions as marked:-Selon les conditions indiquées:

1.	Work involving any of the imported material shall be limited to *in vitro* laboratory studies.	■ Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire *in vitro*.
2.	Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.	■ Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.
3.	All animals exposed to infection by any of the imported material shall be so exposed and held only in isolated insect-and rodent-proof facilities	☐ Les animaux exposés à l'infection par la matière importée doivent y être exposés et être gardés uniquement dans des installations isolées à l'abri des insectes et des rongeurs
4.	All equipment, animal pens, cages, bedding, waste and other articles under the importer's control, that come in direct or indirect contact with any of the imported material, shall be sterilized by autoclaving or incinerated	■ L'équipement, les enclos pour animaux, les cages, les litières, les déchets et tout autre article sous la responsabilité de l'importateur qui viennent en contact direct ou indirect avec la matière importée doivent être stérilisés par autoclavage ou incinérés.
5.	Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.	■ Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.
6.	No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR	■ La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis AUCUNE AGENT ANTHROPOPATHOGÈNE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR
7.	On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.	■
8.	Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.	■
9.	NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	☐
10.	The Director must approve all new work with the imported material involving construction of recombinants that requires an increase of containment from level 2	☐ Tous nouveaux travaux de manipulation génétique (recombiné) avec la matière importée qui demandera que le niveau 2 de confinement soit augmenté exigera l'approbation du Directeur.
11.	No culturing of Risk Group 3 or 4 pathogens shall be done	■ Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise

This study was previously level 2+ per PHAC import permit ^ 2009

12. **This permit is valid only for:** / **Le présent permis n'est valide que pour:**

☐ a) a single entry into Canada or / une seule entrée au Canada ou

■ b) importations at intervals of / les importations effectuées à intervalles de during the period beginning on / au cours de la période commençant le FEBRUARY 1 2 2009 and ending on / et se terminant le FEBRUARY 1 2 2010

Authorization-Signature of Director
Autorisation-Signature du Directeur

Marianne Heisz

Date FEBRUARY 1 4, 2009

Note: Transporting and otherwise dealing with imported material are subject to federal, provincial and municipal laws (if any), to the extent that, those laws apply in respect of that material.

Nota : Les opérations relatives à la matière importée, y compris le transport, sont assujetties aux lois fédérales, provinciales et aux règlements municipaux applicables.

Canada