Risk Assessment Form

For Containment Level 2 Labs, Using Risk Group 2 Agents

A: Description of General Techniques

i. Include a general description of techniques used, i.e. volumes, types of cells/agents manipulated
The project involves the use of multiple standard laboratory techniques for the manipulation of mammalian, insect, bacterial and yeast cell cultures and the proteins, RNA and DNA produced in these cultures. These techniques include small volume sterile culture (< 10 L for bacteria and yeast cells, xxx L for mammalian and insect cells) (*volume is important because risk increases with large volume culture at one time)*; cell lysis by mechanical or chemical means; separation of protein, RNA, or DNA from the lysed cells by mechanical or chemical means; and analysis of protein location in cells by expressing recombinant proteins of interest or by fixing and staining cells with antibodies specific for proteins of interest and viewing by microscopy. Techniques more specific to this project include analysis of the activity of the **** protein and other related proteins by XXX assays using (*whatever particular special items or techniques are needed i.e.* fluorescent activated cell sorting of live cells or FACS analysis of fixed cells).

B: Decontamination/Disposal Procedures

i. Describe decontamination of work surfaces
Work surfaces are decontaminated using 70% ethanol (*or other appropriate disinfectant – specify what you use)* at the end of each work period.
ii. Describe decontamination of spills/splashes
Any spills are decontaminated with freshly prepared 10% bleach for 30 minutes prior to cleanup (*or list another disinfectant if it is more appropriate for the biohazardous material that you are using or the surface being decontaminated (remember that bleach corrodes stainless steel). Examples of disinfectants are:* Bacdown detergent disinfectant, *a quaternary ammonium compound effective against viruses, bacteria, and fungi, BDD, available through Fisher Scientific*; Virkon S *a general purpose disinfectant instead of bleach. It is inexpensive, highly effective against a broad range of microbes, and does not have the associated problems of metal corrosion or odour)*.
iii. Describe decontamination/disposal of solid and liquid laboratory waste
Disposable plastic ware that has contacted biological material is placed in an autoclave bag inside the BSC, and the bag closed with tape prior to removal from the BSC or laboratory. Prior to disposal the bag is vented and autoclaved in ____ (*location*) following procedures specified in BIO-006 Biological Waste Management and the Autoclave SOP. The efficacy of autoclave decontamination is determined by weekly tests with biological indicators and a log kept by the Research Instrumentation Technician.
iv. Describe decontamination/disposal of sharps
Contaminated sharps are placed in an approved plastic biohazardous sharps container which disposed of through Environmental/Occupational Health and Safety Department. Glassware (and reusable plastic items) are decontaminated using bleach (*or other specified disinfectant)* prior to washing (or disposal in glass garbage).

C: Immunization/Medical Surveillance

i. Immunization Required? Yes ☐ No ☐ **If yes, provide type of immunization:**
Click here to enter text.
ii. Medical surveillance has been discussed with lab workers and includes, but is not limited to a medical examination; serum screening, testing and/or storage; immunizations; and possibly other tests as determined by the risk assessment process. Yes ☐ No ☐

Use the space below to provide any additional description of medical surveillance:
As per section 2.4 of the Public Health Agency of Canada Laboratory Biosafety Guidelines, you must consider what medical surveillance is required for personnel working in your laboratory as part of the risk assessment process. This could include but is not limited to: a medical examination; serum screening, testing and/or storage; immunizations; and possibly other tests as determined by the risk assessment process. Be sure to state whether or not the micro-organisms that you use could be a particular health risk for immunocompromised individuals or pregnant women or their fetuses. An example statement: Cloning strains of E. coli are unlikely to be a health risk for immunocompromised individuals. Nevertheless anyone undergoing cancer chemotherapy or immunosuppressive therapy should tell their physician that they work with these bacteria to check their particular risk.

D: Training

i. Provide a description of laboratory specific training given by you or a designate. Ensure training records are available for internal and external audits.
All laboratory personnel will have WHMIS training and biosafety training as outlined in the document BIO-007 Safety Training for Biological Labs. All personnel have reviewed the Biosafety Manual and applicable Biohazard Standard Operating Policies and Procedures (*which ones?*). All new personnel are provided with a general lab safety orientation and lab specific training in the handling of the biohazardous and other hazardous material in the laboratory *(EOHS can provide this training upon request of the researcher)*. They are informed of the risks of working with the biohazards in this laboratory, are aware of what changes in their health status would increase their personal risk from the biohazards in this laboratory and that such changes should be reported to their supervisor so that, if necessary, appropriate adjustments in the operations or risk mitigation methods can be made. In addition, when an individual needs to learn a procedure which is new to them, they are instructed by a competent experienced person to ensure that the technique is performed correctly, that they are aware of any hazards, and that they know how to do the work safely. Signed and dated training records are kept in the lab (*or wherever you keep them).* Training records must be available to both internal auditors and external regulators.

E: Use of Tissues, Cells or Cell Cultures Not Applicable ☐

i. Provide the source of cells/tissues, are they pathogen free?
Xx tissues or cells from zz species are used for ** analysis. Those from zz species are from purpose-bred specific pathogen free animals and from tissues that should not contain large numbers of bacteria. All mammalian cell lines created or received by the lab from outside sources are found on the attached cell line list (*or if only one or two cell lines list here and describe)* that indicates the risk group for each cell line; also on this list are cell lines that contain recombinant DNA that have been created or received by the lab (*or cell lines containing recombinant DNA are too numerous to list, however they have all been created using XXX viral vector or by a plamid transfection of the YYY cell lines as parent cell lines these all contain stably integrated **** gene(s) or parts of that/those gene(s) and do not pose any greater risk than the parent cell lines OR pose a risk of.... if accidentally injected)*. These human cell lines are risk group 2 as indicated in the forms (*or appended table*).
ii. Describe handling and use procedures
Since the cells are from purpose-bred specific pathogen free animals, they are not considered biohazardous but are treated with good general lab practices. ***Or** are from the intestines and therefore contain large numbers of risk group 1 bacteria and so the*

tissues/cells are treated using containment level 1 procedures and containment (describe important lab safety practices briefly if not described elsewhere in summary) ***&/or*** are from zz species that are collected from the wild or are not specific pathogen free. This species could contain the following zoonoses that are classified as risk group ?? pathogens for humans, so the following precautions are taken ***&/or*** are from humans.

All human cell lines have the potential risk of producing unidentified human viral pathogens. Human cell lines, whether designated by the source company as RG1 or RG2 will be cultured in a BSC using containment level 2 precautions. Any cell lines infected with the viruses used in the lab will be handled in a BSC using containment level 2 precautions.

a specific example for some commonly used cells: Although HEK293 and HeLa cells are classified as risk group 2 by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) because they contain adenovirus or papilloma virus genetic material, respectively, (and SV40T for HEK293T), because these cells do not contain the complete viral genome for the respective viruses the risk of generation of these viruses by these cells is extremely low. Proper aseptic microbiological techniques will be used so as not to contaminate these cells with virus that might recombine and mobilize these viral genes into infective particles. As required by the PHAC and CFIA on import permits, HEK293 and HeLa cells will be treated as risk group 2 agents.

Following harvest, cells are used for (*indicate what work is done outside of the BSC using cells or their extracts. Have the cells been treated in a way that would inactivate any risk group 2 pathogens that are or might be present? eg. Detergent extraction, formalin fixation.. if not so treated, then what is done to minimize aerosol generation and contamination of the laboratory?*
e.g. Once the cells are killed the material will be handled using good general lab practices to reduce the generation and spread of aerosols. Any work with these cell lines that will generate significant aerosols will be done in a biological safety cabinet (BSC) until treated in such a way that any virus that might be present would be been inactivated *(specify what you will do)*.

e.g. when lentiviral vectors (even replication incompetent vectors) are used one either has to demonstrate that no virus is associated with or being produced by the cell line or one has to treat the cells or their extract in some way that would inactivate any lentivirus that might be present before removing them from level 2 containment.

Fresh human tissue, and recently established and not well characterized human cell lines should be handled strictly as Risk Group 2 material until they are treated in a way that would inactivate any unidentified human pathogens that they might contain (*including the use of aerosol resistant caps on centrifuge cups*).

Consider whether or not you can make the following statement about cell lines from your laboratory: None of the recombinant cell types, nor any that may be created during the proposed project, pose any additional or known biohazard threat beyond that of the non-recombinant or parental strain. *If you used a viral vector to create the cell line then how is it ensured that the replication incompetent vector has not recombined with viral genes in the cell or the environment to produce a recombinant infectious virus? If you have not ensured this, then considering the nature of the particular vector and the nature of the transgene that you are using, which containment level is required for handling of the cells??*

iii. For human blood and tissue samples, describe the source (i.e. from a healthy population) and the sample screening.

All fresh human blood and tissue samples *would be treated as risk group 2, but the actual risk, the level of*

care in containment and the response to an accident would vary depending on the following: the population that the samples are from and what the associated risks might be eg. Is the population a generally healthy population? Is it screened for HIV, Hep B, Hep C, etc. and are samples from positive individuals excluded? Are the patients all positive for HIV (or positive for some other human pathogen in which case consider enhanced containment 2+), so the following precautions will be taken.....consider whether aerosol resistant centrifuge cups should be used and opened only in a biological safety cabinet.

iv. Describe how spills are handled

Products such as Vital-1 are available through Fisher Scientific for cleaning spills. The product is a solid material which is shaken onto a blood spill and it forms a gel which makes cleanup very easy. It also contains crystalized chlorine which denatures materials in the blood.

F: Use of Bacteria and Fungi Not Applicable ☐

i. Describe handling and containment of bacteria or fungi.

If using level 2 bacteria or fungi then describe their risks and how they are used and contained and any medical surveillance required.

For risk group 1 bacteria (cloning strains) or yeast strains commonly used for molecular biology, write something to the effect of: Cloning strains of bacteria derived from E. coli K12 (e.g. BL21, DH5α) are classified as risk group 1. They do not carry the well recognized pathogenic mechanisms required by strains of E. coli that cause the majority of enteric infections. E. coli strains EQ1, DH5a, BLR and BL21 are considered to be non-pathogenic and unlikely to survive in host tissues and cause disease. (Chart, H., *et al.* 2000. Journal of Applied Microbiology 89, 1048-1058) Bacteria and yeast cells are cultured on the open bench using flame sterilization. Media, cells, and cell lysates are decontaminated using freshly diluted bleach (*or list another appropriate dissinfectant)* before disposal. *Comment whether aerosol generation must be considered and how it will be controlled.*

For other types of risk group 1 bacteria that might be more likely to pose a health risk to immunocompromised individuals, note this fact and indicate what medical conditions should be reported so that additional precautions can be taken alternative duties assigned.

Describe any animal work that includes intentional infection with bacteria.
Some of the yeast cells contain recombinant DNA encoding all or part of the **** protein(s). Baculovirus is used to transfer human DNA encoding part or all of a protein into insect cells. Bacteria containing recombinant DNA are too numerous to list; however, they are all BSL1 and all of the recombinant DNAs encode either all or part of wild-type or mutant **** or related proteins, or proteins thought to interact with the **** protein.

G: Use of Parasites Not Applicable ☐

i. Describe handling and use procedures.

Include a description of the parasite being used, risks involved and handling procedures.

H: Use of Viruses or Viral Vectors Not Applicable ☐

i. Describe handling and use procedures.

Describe the virus being used, its risk and the handling procedures. Discuss the risks and precautions to mitigate the risks of in vitro and in vivo (animal) work separately.

The statement might be as simple as: The only viral infectious agent being propagated and manipulated is

a baculovirus that infects only insect cells and are therefore classified as risk group 1 and handled with general lab practices as described elsewhere in this summary.

Alternatively, if viruses/viral vectors are used that infect mammalian cells (especially human cells) then they need to be described in more detail, including the biology and associated risks of the viral vector itself and also the transgene. Describe the packaging system and any special containment practices for in vitro and/or in vivo work.

If the vector system is supposed to be replication incompetent indicate how this is ensured eg. What generation is the vector system? What viral genes have been deleted? How many plasmids involved in generating the virus? Is there a deletion created in the LTR when it integrates into the genome?

What might the effect of the vector be if a person was accidentally infected?

What would the effect of the transgene be if a person was accidentally infected? Is the transgene hazardous enough that special precautions should be in place (possibilities include - no use of sharps; mandatory goggles so eyes cannot be touched)?

I: Use of Biological Toxins Not Applicable ☐

i. Describe handling and use procedures.

Biological toxins listed on Schedule 1 of the Human Pathogens and Toxins Act (http://lois-laws.justice.gc.ca/eng/acts/H-5.67/page-19.html#h-23) must be identified to us for local oversight and so that we can register their use with the Public Health Agency of Canada. The regulations under the Act have not yet been written. Until that time each toxin will be assessed individually by the Institutional Biosafety Committee.

This document was developed using similar documents from Queen's University.