Local Biohazard Risk Assessment
Requirements, Instructions and Template
For Risk Group 1, 2, and 2+ Material

Abbreviations:
PHAC  Public Health Agency of Canada
CFIA  Canadian Food Inspection Agency
ATCC  American Type Culture Collection
PHAC PSDS  Public Health Agency of Canada Pathogen Safety Data Sheet

Local Risk Assessment Requirements:

It is a requirement of the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) that each laboratory performs a detailed local risk assessment (LRA) to determine the biohazard containment level required for both facilities and operational practices to mitigate the risks associated with the biohazardous agents in use.

The local risk assessment of all work with biohazardous material (Risk Group 1 and 2 and 2+), is to be documented as part of a Queen’s University Biohazard Permit Application.

A local risk assessment will:
- identify the Risk Group of the microorganism, (or tissue that might contain this microorganism)
- describe the potential hazard associated with the microorganism, including symptoms of disease if it is pathogenic (which it is important for all lab members to know so that they will be aware of any potential lab acquired infection so that it can be diagnosed and treated appropriately. Although disease is unlikely with RG1 microorganisms, if working with an RG1 agent that is other than a cloning strain of bacteria, determine if it has been pathogenic in immune-compromised individuals.)
- indicate whether the material will be used only in vitro, or also in vivo
  o what is being done with the material and where; consider procedure’s potential for generating aerosols that might contain and spread infectious agents
  o indicate whether or not sharps will be used and the precautions associated with them
  o in vivo use of infectious materials increases the risk of exposure, so the facilities and operational practices for in vivo work must be described separately from that for in vitro work
- describe the overall risk mitigation strategy and details of this strategy including:
  o physical containment and engineering controls (i.e. lab design) This can be indicated simply by stating the which of your containment level 1 or 2 laboratories (or shared facilities) will be used for the different types of work, because the Biohazard Committee inspects all laboratories.
  o operational requirements
    ▪ containment equipment and supplies
- equipment might include e.g. Biological Safety Cabinet, centrifuge cups with aerosol resistant lids containing o-rings
- supplies might include e.g. closed, screw-capped tubes
  - appropriate personal protective equipment (PPE)
  - decontamination and disposal methods
  - medical surveillance (e.g. immunization, titre checks, first aid and medical response to accidental exposure)
  - training needs (this must be supplied as a separate statement in the application)


**At Queen’s** the Principal Investigator’s local risk assessment is to be documented and appended to the Biohazard Permit Application along with any applicable risk assessments from reputable sources (e.g. PHAC PSDS) and lab specific procedures/SOPs.

- In general, more detail is required for material and activities that pose a greater risk.
- The local risk assessment and associated documents are reviewed and approved by the Biohazard Committee.
- After approval, these documents become an integral part of the training of lab personnel.
- Following approval of a Biohazard Application or a Biohazard Amendment that changes the type or risk group of material used in the lab (reviewed by the Biohazard Committee), each member of the biohazard lab team is required to:
  - read the approved Biohazard application/amendment and associated documents that are posted on the TRAQ/Romeo site
  - have any questions that they might have answered by their P.I. and/or the Biosafety Officer
  - sign a Biohazard Team Member Attestation form to indicate that they understand and will abide by the requirements for working safely with the biohazardous material. The form will be submitted via email by the P.I. or Secondary Biohazard Contact to the Biosafety Officer.
- **All new personnel, as part of their lab specific training, must read the version of the local risk assessment** that has been approved by the Biohazard Committee before they are added to the list of authorized individuals on your Biohazard Permit. Ensure that this document is available in your laboratory.
- **All new laboratories must be inspected after the Biohazard Permit is approved and before work with biohazardous material begins.**

**Instructions regarding the use of the Template:**

*Italicized text is a comment, example, alternative or a question.*
In the Template below, please remove my italicized comments and highlights and remove or alter any other text that does not apply to your lab.

Examples or partial templates of local risk assessments are provided below to assist researchers in developing a local risk assessment for their laboratories.

- It is not required that this format be used and the examples are not exhaustive.
- The requirements for your Local Biohazard Risk Assessment are outlined above, and in the Queen’s Environmental Health and Safety SOP- Biosafety-04 which is available on the web site www.safety.queensu.ca.
- The material that is regulated as a biohazard through the Queen’s Biohazard Committee is described in SOP-Biosafety-05 http://www.safety.queensu.ca/pol.htm#biopol.

Although some of the information required for the local biohazard risk assessment is contained in the biohazard permit application form, and in your biohazard inventory and risk group table, the local risk assessment is very helpful for the Biohazard Committee and for your personnel because the narrative form clarifies what is being done and demonstrates that the researchers have thought about the safety hazards and how to mitigate the risks. In some cases the procedures being used affect the risk and this is much clearer in the local risk assessment than from looking at the form.

If a Queen’s Biosafety SOP has been written for the biohazard, equipment, or procedure that you will be using then it should be mentioned in your local risk assessment, indicating that you and your personnel are aware of the contents of the SOP and will follow it http://www.safety.queensu.ca/pol.htm. Also, where appropriate, indicate deviations from the SOP (and briefly justify the change) or indicate where practices will be followed that are recommended but not required in the SOP. Please do not attach Queen’s EH&S SOPs.
**P.I. name** Biohazard Containment Level 1 and 2 Local Risk Assessment

**General Program Overview:**
Start with one or two sentences or a short paragraph describing the research project goals and general approach.

**Biohazardous Material:**
Then describe, first in general terms, what types of biohazardous material are used and the common techniques, and then more specifically with paragraphs for each type of biohazard e.g. 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 ml 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 things or techniques are needed eg. fluorescent activated cell sorting of live cells or flow cytometry analysis of fixed cells*).

*In each of the sections below, remember to describe any in vivo work.*

**Bacteria and 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 level 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, DH5□) 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 (final dilution 1:10, minimum 30 minute contact time) (*or list another appropriate disinfectant*), or autoclaved before disposal in the sewer, to ensure that the environment is not contaminated with plasmids carrying antibiotic resistance genes or other potentially harmful genes.

*For other types of level 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 if personnel are so affected then additional precautions can be taken and/or alternative duties assigned.*
Describe any in vivo work that includes intentional infection with bacteria.

Some of the yeast cells contain recombinant DNA encoding all or part of the **** protein(s).

It is acceptable to make the following note here and on your inventory: Bacteria (or yeast) 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.

Parasites:
Work with parasites is not common at Queen’s, so an example is not provided. If you will be working with parasites then a local risk assessment of the parasite and the work to be done with it, along with a description of mitigation measures must be provided.

Viruses or viral vectors:
Describe the virus being used, its hazard, work to be done, and the precautions taken. Discuss the risks and precautions to mitigate the risks of in vitro and in vivo (animal) work separately.

The local risk assessment 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 risk assessment.

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. For an example of the considerations ask the Biosafety Officer for an updated Queen’s lentivirus SOP for work at 2+ (level 2 facility with level 3 operational practices) or level 2, depending on the generation of the viral vector and the characteristics of the transgene.

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)?

What medical surveillance is required? i.e. are any immunizations recommended? What should the response be if someone was accidentally exposed to the virus? Specify first aid and medical response including antiviral chemotherapy to prevent infection if available.
Tissues or cells from animals or humans:
Xx tissues or cells from zz species are used for ** analysis. Those from zz species are from purpose-bred specific pathogen free animals. They are therefore not considered biohazardous but are treated with good general lab practices (so this doesn't actually have to be reported to the Biohazard Committee, but sometimes it is helpful to clarify in the context of an application)......

&/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 fresh human blood and tissue samples will be treated as risk group 2 because they might contain unidentified human pathogens. All personnel will read and follow Queen’s SOP-Biosafety-08 on Human Tissue, Blood or Other Bodily Fluid.

The actual risk (hazard times probability), the amount of care in containment, and the response to an accident will vary depending on the source of the human material, the volume handled, and the techniques used.

Describe 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), and if so then indicate the precautions that will be taken....consider whether aerosol resistant centrifuge cups should be used and opened only in a biological safety cabinet (or if appropriate state that no centrifuging will be done).

Volume handled at one time is important because risk increases with large volumes.

Consider the risk of what will be done with the blood or tissue. Will the blood or tissue be manipulated in the lab in ways that could generate aerosols? Describe these techniques and the precautions that will be taken to reduce or contain aerosols (e.g. proper pipetting technique, pipetting down the side of the tube with no forceful expulsion, to reduce aerosol generation; capped tubes and rotors to contain aerosols during centrifugation; aerosols allowed to settle for 10 minutes before tubes opened after centrifugation if working outside of a BSC).

It is preferable to do level 2 work inside a BSC to contain aerosols and this is recommended. However if this is not feasible then indicate why and what work is done outside of the BSC using cells or their extracts or serum.

If using a BSC consider whether or not the cells/serum/etc. have been treated in a way that would inactivate any level 2 pathogens that are, or might be, present before removal from the BSC for further work with the material eg. detergent extraction, formalin fixation, etc. If not so treated then what is done to minimize aerosol generation and contamination of the laboratory?

Cell or tissue culture:
All mammalian cell lines created or received by the lab from outside sources are found on the attached inventory list that indicates the risk group (biosafety level) 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 plasmid 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 have a biosafety level 2 as indicated in the forms (or appended table).

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 level 2 precautions. Any cell lines infected with the viruses used in the lab will be handled in a BSC using level 2 precautions.

*a specific example for some commonly used cells (if you use this wording please remember to remove any mention of cell lines and viruses that are not used in your lab):* The human cell lines HEK293, HEK293T and HeLa cells are classified as risk group 2. They are known to contain viral DNA from viruses that are pathogenic to humans. HEK293 contain adenovirus genetic material and HEK293T also contains Simian Virus 40 large T antigen (SV40T). HeLa cells contain or papilloma virus genetic material. 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 microbiological techniques will be used so as not to contaminate these cells with wild-type virus that might recombine and mobilize these viral genes into infective particles.

Following harvest, cells are used for *(indicate what work is done outside of the BSC using cells or their extracts. If working outside of the BSC, have the cells been treated in a way that would inactivate any level 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 they are 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; note that you follow the SOP (and append the SOP). This is most commonly accomplished by multiple passages in vitro following transduction. If the cell line could potentially contain HIV (ie. if it is CD4+ve), then the absence of viral production should be confirmed by PCR.*
If work must be done with unfixed RG2 cells outside of the BSC because of the nature of the techniques then indicate this, and explain how creation of aerosols and spread of contamination will be minimized. Eg. proper pipetting technique, slow expulsion at the side of the tube, not blowing bubbles to minimize aerosol generation.

If a human virally transduced cell line is to be used in vivo and if the transgene is potentially hazardous, then the absence of viral production must be confirmed if one wishes to downgrade the containment level of the mice post injection.

Recently established and not well characterized human cell lines will be handled strictly as Risk Group 2 material, just like fresh human tissue, 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, what containment level does this mean the cells should be handled at?

**Biological toxins:**

Biological toxins listed on Schedule 1 of the Human Pathogens and Toxins Act must be identified to us. Briefly describe what you will use the toxin for. An SOP for the handling of the toxin will be required, but some for common toxins have been written. Contact the Biosafety Officer for assistance with SOPs if you are reporting the new use of a toxin.

For clarity, the following information about containment facilities and practices used to mitigate risk can be provided in each of the sections above for the different types of biohazardous agents if it differs among them. Alternatively, if there are common practices that are better described together that can be done here. If this information is included in your lab specific SOPs, then just make a general statement here about Physical and Operational Containment being CL1 or CL2 or CL2+ as described in detail in the attached SOPs.

**Physical containment and engineering controls:**

Make sure that it is clear which of your containment level 1 or 2 laboratories (or shared facilities) will be used for the different types of work.

**Containment Equipment and Supplies:**

- Containment equipment used will be e.g. Biological Safety Cabinet, centrifuge cups with aerosol resistant lids containing o-rings
- Containment supplies will include e.g. closed, screw-capped tubes
Operational practices: For Risk Group 1 material, we use standard microbiological and biochemical techniques and follow all the general operational practices for microbiological biosafety level 1 as described in the Queen’s Biosafety Manual. For Risk Group 2 material we follow the CL2 requirements of the Canadian Biosafety Standards.

Personal Protective Equipment:
- describe the personal protective equipment (PPE) that will be used e.g. lab coat, closed front gown, gloves, double gloves, goggles, face shield if required for a splash hazard

Transport
If you will need to transport the biohazardous material or waste outside of a level 2 lab, then specify that it will be double contained and how (e.g. double bagged, or tupperware container with lid, or ice box with latching lid).

Decontamination and Disposal:
Briefly describe decontamination: Work surfaces are decontaminated using ______ specify appropriate disinfectant, and if different disinfectants are used for different material then clearly indicate that – see note at the end of this paragraph) at the end of each work period. 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 (bleach is cheap and effective against many pathogens, but remember that bleach corrodes stainless steel so it can create problems if used routinely and not rinsed well; bleach may be necessary if you are culturing bacteria that form spores). Note that 70% ethanol is a good disinfectant for soaking instruments (10 minute contact time), and is good as a sterile rinse after other disinfectants, but it is not as effective on surfaces because it evaporates quickly, reducing the contact time. If using a commercial disinfectant, ensure that specifies it is virucidal and tuberculocidal. Examples of some alternative disinfectants used on campus are: Backdown detergent disinfectant, a quaternary ammonium compound effective against viruses, bacteria, and fungi. BDD is available from Fisher Scientific; Virkon S a general purpose disinfectant. It is inexpensive, highly effective against a broad range of microbes, and does not have the associated problems of metal corrosion or odour. Read the manufacturer’s instructions for any disinfectant carefully and specify the appropriate dilution and contact time.

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 opened and autoclaved in ____ (location) following procedures specified in SOP-Biosafety-09 Autoclaves – Biohazard Waste Treatment. The efficacy of autoclave decontamination is determined by weekly tests with biological indicators and a log kept by _______. (or other e.g. disposed as biomedical waste by KGH, or sent for incineration via Queen’s EH&S). Material that has been in contact with bleach is not autoclaved.

Contaminated sharps are placed in an approved plastic biohazardous sharps container which disposed of through Queen’s Department of Environmental Health and Safety (or other e.g. disposed of through KGH).
Glassware (and reusable plastic items) are decontaminated using 10% freshly diluted bleach (or other specified disinfectant) prior to washing (or disposal in glass garbage).

Contaminated PPE is disposed as biohazardous waste, or decontaminated by autoclaving or chemical disinfectant prior to being sent to the laundry.

**Medical Surveillance:**
The *Canadian Biosafety Standards* specify that you must consider what medical surveillance is required for personnel working in your laboratory as part of the local 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.

At Queen’s medical surveillance most commonly includes some of the following. Edit, remove, and add information as appropriate:

- Specific immunizations (which ones are required for your lab? e.g. Hepatitis B, rabies), and serum titre testing to confirm response to the immunization.
- A plan of what first aid and medical response is to occur in case of an incident involving exposure must be written, approved, and posted in the laboratory. (eg. *If using virus/viral vectors, what should the response be if someone was accidentally exposed to the virus? Specify first aid and medical response including antiviral chemotherapy to prevent infection if applicable.*)
- Training to develop an awareness that changes in the health status of personnel can increase their personal risk from the biohazards in that laboratory. In particular, changes in health status that might affect immune responsiveness (immune-compromised) should be reported. For these individuals, some risk group 1 microorganisms which do not normally cause disease can be pathogenic and Risk group 2 microorganisms can cause much more severe disease than normal, or even death. *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 foetuses.*
  - If the organism being worked with has been attenuated or genetically altered to be less hazardous than wild-type, you should be aware of the mechanism of attenuation (if known) and any conditions that might make the attenuated organism more pathogenic for you.
  - Note that, without the need to reveal personal medical information, the occurrence of such a change should be reported to their supervisor so that, if necessary, appropriate adjustments in the operations or risk mitigation methods can be made in consultation with their personal physician and/or the Queen’s University Occupational Health Services provider or other medical experts as necessary.
  - Conditions of concern include:
    - Pregnancy (pregnant women may need to take extra precautions or be reassigned to other duties early in their pregnancy because certain microorganisms can damage the fetus and because their own immune responsiveness may be altered)
    - Immune-deficiency
    - Immune-suppressive drugs (e.g. with organ transplantation)
- Anti-inflammatory medications
- Cancer
- Treatment for cancer
- Age (the elderly; also very young children are more susceptible to infection, which is one of the reasons that they are not permitted in research laboratories)
- Other conditions as determined by your physician

- Occupational Health services for personnel working in and around Queen’s research laboratories is available through Walsh and Associates Occupational Health Services. Details and a map are located at http://www.safety.queensu.ca/walsh/. Charges will be billed to departments through the Department of Environmental Health and Safety and payment is the responsibility of the supervisor.

At Queen’s the medical surveillance required for working with human tissues, blood or other body fluids is included in SOP-Biosafety-08 so if you are using this material then refer to this SOP. All personnel have been immunized against Hepatitis B (or a waiver signed to indicate that they have been informed of the risks, know that their supervisor will pay for the immunization, and that they are declining). If the material is from unscreened individuals, they have also had their titres checked to confirm that they responded to this immunization.

Any required immunization and titre checks are at the expense of the P.I. and may be obtained through the University Occupational Health Services provider. (also if material is known to be positive for a certain pathogen then, include specific training about the pathogen and first aid and medical response including whether antiretroviral prophylaxis starting within 2 hours of the incident is not required vs. should be considered vs. is recommended)

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.

If any specific first aid or medical response is recommended because of the biohazardous material in use then indicate that this information is included in the lab specific training and ensure that it is on the Emergency Response Procedure posted in your laboratory.