

VII. Classification and Containment Levels

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A. General Introduction to Classification Systems

Microorganisms have been classified according to degree of risk in terms of infectivity, pathogenicity and currently available treatment options. The classification is as follows: Additional information is also available in [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL).

1. Class 1 - Agents of no or minimal hazard under ordinary conditions of handling. Biosafety Level 1 facilities and practices are used for handling.
2. Class 2 - Agents of potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means, but which are contained by ordinary laboratory techniques. Biosafety Level 2 facilities and practices are used in the laboratory.
3. Class 3 - Agents involving special hazard or agents derived from outside the United States which require a federal permit to be imported. This class includes pathogens which require special containment. Biosafety Level 3 facilities and practices are used in the laboratory.

There is no facility available at the University of Washington for work with the following two classes of microorganisms.

4. Class 4 (BL-4) - Agents of extreme hazard or which may cause serious epidemic diseases.
5. Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

B. Classification Of Etiologic Agents On The Basis Of Hazard

The following link to NIH Guidelines classifies etiological agents on the basis of hazard. Please contact EH&S at 206-543-7278 or rbso@u.washington.edu for additional information.

http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm

C. Cell Culture Systems

1. Introduction

It is prudent to adopt procedures for cell culture which interfere minimally with the execution of experiments, but which will afford maximum protection to personnel against the most likely hazard associated with these cultures.

The potential hazard of in vitro cell research is the presence of an unknown viral agent. Viruses or viral genome are carried in different ways by cells and unmasked as infectious entities by a variety of operations. The potential risk from releasing this infectious agent must be kept in mind when planning the manipulation of the cells.

Most cultured cells are known to harbor viruses either adventitiously (ie., C-type particles) or deliberately in the cases of SV40 transformed rodent and human cell lines or human lymphoid cell lines, which are transformed by Epstein-Barr virus.

Primary and permanent cell lines from normal mouse, hamster, rat, etc. are handled at biosafety level 1 but a biological safety cabinet is not mandatory.

Manipulations of the following materials are handled at biosafety level 2 and a biological safety cabinet must be used.

Culture of all human and primate cell culture.

Isolates from tissues susceptible to or likely to harbor mammalian oncogenic viruses.

Cells transformed by herpes or Epstein-Barr virus

All established or permanent cultures of human lymphocytes should be handled on the assumption that they harbor the Epstein-Barr virus.

Refer to sections IV., VIII. and IX. for information on biosafety levels and practices.

Under no condition should individuals handle lymphoid cells of the line derived from themselves, or a first-degree relative. Handling such lymphoid cell lines eliminates the important protection provided by histocompatibility barriers.

2. Classification of Cell and Tissue Culture on the Basis of Hazards

It is difficult to establish general rules, but probably long-term culture of cells enhances the risk of rescuing an oncogenic agent; whereas an autonomous infectious virus, perhaps carried as a persistent indigenous infection, is likely to be released on short-term manipulation of freshly isolated cells, within two or three weeks.

The risk of releasing a virus infectious to humans is less the further the manipulated cells are from the human cells on the evolutionary scale. The following general cell types and manipulations are used to classify cell culture risks.

Type of cell to be cultured:

- a. human tissue and cells
- b. non-human primate tissue and cells
- c. non-primate mammalian tissue and cells
- d. non-mammalian tissue and cells (birds, fish, reptiles, amphibians, insects, plants)

Manipulations:

- a. short term culture (2-3 weeks)
- b. long term culture
- c. deliberate fusion (co-cultivation) between cells and agents (such as Sendai virus or polyethylene glycol) that could lead to the rescue of an oncogenic virus or the formation of recombinant between C-type particles with altered species specificity
- d. passage in animals of the same species as the cells (homograft) or different species (xenograft)

The latter operation in particular carries the risk of releasing a xenotropic virus and the danger very likely would be increased if the animal were immunodeficient (eg., nude mice) or immunosuppressed.

It is recognized that in the case of fusion (co-cultivation) with other cell types, there are apparent inconsistencies in the following table. Each experiment of fusion (co-cultivation) must be looked at from both cell types used, and the more stringent containment levels applied.

The lowest containment level recommended is biosafety level 2.

D. Classification Of Cell Culture Procedures According to Containment Level

1. Human Tissues And Cells

All culture of human tissue and cells require BL-2 containment with the use of biological safety cabinets for each category of experiment unless the virus known or suspected to be involved requires a higher containment level according to the classification for viruses.

1. Biosafety Level 2 Containment:
 - a. Short term culture
 - b. Long term culture including continuous culture of cell
 - c. Fusion (co-cultivation) with:**
 other human cells
 non-mammalian cells
 - d. Animal passage in non-mammalian hosts
2. Biosafety Level 3 Containment:
 - a. Fusion (co-cultivation with** cells of non-human primates
 - b. Animal passage in:
 non-human primates
 - c. Culture of cell lines known to contain infectious primate retroviruses or EBV.
3. Not permitted: Passage in human beings

**This category includes attempts to unmask human tumor viruses by induction with chemical agents. In cocultivation or fusion, the potential hazard is the release of a virus whose genome has been integrated or in the formation of recombinant of C-type particles with increased pathogenicity.

2. Tissues And Cells From Non-Human Primates

The potential risk is the release of herpesvirus simiae (B virus) from monkey cells during short term cultivation. If cultures are known to be free of this virus, BL-2 containment can be used. After having utilized BL-3 containment for the first 3 weeks of primary cultivation, the danger from B virus should have passed.

1. Biosafety Level 2 (BL-2) Containment:
 - a. Long term culture including continuous culture of cell lines
 - b. Fusion (co-cultivation) with:
 - cells of other non-human primates
 - non-mammalian cells
 - c. Passage in living animals:
 - non-human primates
 - non-primate mammals
 - non-mammalian hosts

2. Biosafety Level 3 (BL-3) Containment:
 - a. Short term culture
 - b. Fusion (co-cultivation) with:
 - human cells
 - cells of non-primate mammals
 - c. Culture of cell lines known to contain retroviruses

3. Not Permitted: Passage in human beings

3. Non-Primate Mammalian Tissues And Cells

BL-2 practices should be followed for each category of experiment unless the virus known or suspected to be involved requires a higher containment level according to the classification of viruses.

1. Biosafety Level 2 (BL-2) Containment:
 - a. Short term culture
 - b. Long term culture including continuous culture of cells
 - c. Fusion (co-cultivation) with:
cells of other non-human primates
 - d. Passage in living animals:
non-human primates
non-primate mammals
non-mammalian hosts
2. Not permitted: Passage in human beings

4. Non-Mammalian Tissues And Cells

BL-2 practices should be followed for each category of experiment unless the virus known or suspected to be involved requires a higher containment level according to the classification of viruses.

1. Biosafety Level 2 (BL-2) Containment:
 - a. Short term culture
 - b. Long term culture including continuous culture of cells
 - c. Fusion (co-cultivation) with:
cells of other non-human primates
non-mammalian cells
 - d. Passage in living animals:
non-human primates
non-primate mammals
non-mammalian hosts
2. Not permitted: Passage in human beings

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