SAFETY PROCEDURES AND CONTAINMENT LEVELS (Based on: *Biosafety In Microbiological And Biomedical Laboratories*, Dec 2009 Fifth Edition, CDC And NIH)

There are four Biosafety Levels (BSLs) or containment levels which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BSLs described in this manual should be differentiated from Hazard Groups, as described in the NIH Guidelines and the World Health Organization Laboratory Biosafety Manual. Hazard groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The hazard group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

Biosafety Level 1

Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. BSL-1 represents a basic level of containment that relies on standard microbiological practices.

BSL-1 is suitable for use with biological agents in Hazard Group 1.

It is essential that the guidelines given below are adhered to when working with these organisms.

1) The laboratory should be easy to clean. Bench surfaces should be impervious to water and resistant to acids, alkalis, solvents and disinfectants.

2) Effective disinfectants should be available for immediate use in the event of spillage.

3) If the laboratory is mechanically ventilated, it is preferable to maintain an inward airflow while work is in progress by extracting room air to atmosphere.

4) All procedures should be performed so as to minimise the release of organisms and the production of aerosols. Flasks of cultured micro-organisms should be sealed with a foam or non-absorbent cotton wool bung where possible.

5) Cultures grown in flasks should generally not exceed 25% of their container volume.

6) The laboratory door should be closed when work is in progress.

7) There is a regulatory requirement that laboratory coats or gowns be worn in the laboratory and removed when leaving the laboratory suite.

8) Personal protective equipment, including protective clothing, must be:
   (a) stored in a well-defined place;
   (b) checked and cleaned at suitable intervals;
   (c) repaired or replaced before further use when discovered to be defective.

9) Personal protective equipment which may be contaminated by biological agents must be:
   (a) removed on leaving the working area;
   (b) kept apart from uncontaminated clothing;
   (c) decontaminated and cleaned or, if necessary, destroyed.

10) Eating, chewing, drinking, taking medication, smoking, storing food and applying cosmetics is forbidden.

11) Mouth pipetting is forbidden.

12) The laboratory should contain a basin or sink that can be used for hand washing.

13) Hands should be decontaminated immediately when contamination is suspected and before leaving the laboratory.

14) Bench tops should be cleaned or disinfected as appropriate after use.

15) Used glassware and other materials awaiting disinfection should be stored in a safe manner. Pipettes, for example, if placed in disinfectant, should be totally immersed.

16) Contaminated materials whether for recycling or disposal, should be stored and transported in robust and leak-proof containers without spillage.

17) All waste material, if not to be incinerated, should be disposed of safely by routes as designated on the flow diagrams displayed in laboratories.

18) Accidents and incidents should be immediately reported to and recorded by the person responsible for the work or other delegated person. Spillages should be recorded in a book maintained in each research group dedicated for this purpose. The record should detail the location of the spillage, the organism involved, and the method of decontamination.

Biosafety Level 2

Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

It is ESSENTIAL that the procedures outlined below are adhered to when working with these organisms.

1) Access to the laboratory is to be restricted to authorised persons.

2) There must be specified disinfection procedures.

3) If the laboratory is mechanically ventilated, it must be maintained at an air pressure negative to atmosphere while work is in progress (see paragraph 16 below).

4) Bench surfaces must be impervious to water, easy to clean and resistant to acids, alkalis, solvents and disinfectants.

5) There must be safe storage of biological agents.

6) Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet, isolator or be otherwise suitably contained. Flasks of cultured micro-organisms should be sealed with a bung where possible.

7) There must be access to an incinerator for the disposal of infected animal carcasses (if animals are used).

8) Personal protective equipment, including protective clothing, must be:
   (a) stored in a well-defined place;
   (b) checked and cleaned at suitable intervals;
   (c) repaired or replaced before further use when discovered to be defective.

9) Personal protective equipment which may be contaminated by biological agents must be:
   (a) removed on leaving the working area;
   (b) kept apart from uncontaminated clothing;

(c) decontaminated and cleaned or, if necessary, destroyed

10) The laboratory door should be closed when work is in progress.

11) Laboratory coats or gowns, which should be side or back fastening, must be worn and removed when leaving the laboratory suite. Separate storage (for example, pegs) apart from that provided for personal clothing should be provided in the laboratory suite.

12) Eating, chewing, drinking, smoking, taking medication, storing food and application of cosmetics in the laboratory is forbidden.

13) Mouth pipetting is forbidden.

14) Bench surfaces should be regularly decontaminated according to the pattern of the work.

15) When undertaking procedures that are likely to give rise to infectious aerosols, a microbiological safety cabinet (Class I, BS 5726: 1992, or unit with equivalent/better protection factor or performance) should be used. Some other types of equipment may provide adequate containment in their own right but this should be verified.

16) In most laboratories operating at Containment Level 2 where there is mechanical ventilation simply to provide a comfortable working environment, it may not be practical to maintain an effective inward flow or air. Maintaining an inward flow of air is necessary only when work is in progress.

17) The laboratory should contain a washbasin located near the laboratory exit. Taps should be of a type that can be operated without being touched by hand.

18) Hands should be decontaminated immediately when contamination is suspected, after handling infective materials and before leaving the laboratory. When gloves are worn, these should be washed or preferably changed before handling items likely to be touched by others not wearing gloves, for example, telephones, paperwork, computer keyboards, etc. Where practicable, equipment controls should be protected by a removable flexible cover that can be disinfected.

19) An autoclave for the sterilisation of waste materials should be readily accessible in the same building as the laboratory.

20) Materials for autoclaving must be transported to the autoclave in robust containers without spillage.

21) There should be means for the safe collection, storage and disposal of contaminated waste.

22) Contaminated waste should be suitably labelled before removal for incineration.

23) 'Access to an incinerator' may be taken to mean an incinerator at another site but whether local or distant, carcasses for incineration must be transported in secure containers. If stored temporarily in a chest freezer, a record must be made in the appropriate logbook.

24) Used laboratory glassware and other materials awaiting sterilisation before recycling should be stored in a safe manner. Pipettes, if placed in disinfectant, should be totally immersed.

25) Accidents and incidents should be immediately reported to the Health and Safety representative using the standard University accident/incident form. The record should detail the location of spillage, the organism involved and the method of decontamination. If GMO work, then spillages should be recorded in a dedicated book held in each research group.

Biosafety Level 3

Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a Biological Safety Cabinet.

It is ESSENTIAL that the procedures outlined below are adhered to when working with these organisms under these Biosafety conditions.

These procedures are <u>in addition</u> to those outlined for Biosafety Levels 1 and 2.

1) The laboratory should be separated from the areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom or basic laboratory.

2) Entry for personnel must be through a vestibule (i.e. double-door entry).

3) Access to the laboratory area must be designed to prevent entrance of arthropods and other vermin.

4) Access doors must be self-closing.

5) The surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings in these surfaces (e.g. for service pipes) should be sealed to facilitate decontamination of the room(s).

6) The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination.

7) Windows must be closed, sealed and break-resistant.

8) A foot- or elbow-operated or automatically controlled water source at the hand-wash basin should be provided near to each exit door.

9) There must be a ventilation system that establishes a directional air flow from access spaces into the laboratory room. Staff must at all times ensure that proper directional air flow into the laboratory room is maintained.

10) The building ventilation system must be so constructed that air from the containment laboratory is not recirculated to other areas within the building. Air may be HEPA filtered, reconditioned and recirculated within that laboratory. Exhaust air from the laboratory (other than from biological safety cabinets) must be discharged to the outside of the building, so that it is dispersed away from occupied buildings and air intakes. It is recommended that this air is discharged through high-efficiency particulate air (HEPA) filters.

11) Biological safety cabinets should be sited away from walking areas and out of cross-currents from doors and ventilation systems.

12) The exhaust air from Class II biological safety cabinets, which will have been passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system. All HEPA filters must be installed in a manner that permits gaseous decontamination and testing.

13) An autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. If infectious wastes have to be transported out of the containment laboratory for disposal, they must be transported in sealed, unbreakable and leak-proof containers according to national or international regulations, as appropriate.

14) Anti-backflow devices must be fitted to the water supply.

15) Effluents should be decontaminated before being discharged to the sanitary sewer