



ENHANCED CONTAINMENT CL2 + (Level 3 practices in a Level 2 lab)

The use of Containment Level 3 (CL3) practices and procedures (as listed below) in a Containment Level 2 lab (often referred to as “CL2+”), allows for research work with microorganisms to take place in an environment where the safety practices are enhanced over and above the practices required at Containment Level 2. These requirements must be incorporated to achieve CL2+ or Enhanced containment.

1.0 Pathogens: Enhanced containment is required for (but not limited to)

- Cultures of Verotoxigenic *E.coli* [e.g. Shiga toxin-producing *E.coli* (STEC) or most commonly identified *E.coli* 0157; other strains to be determined upon review (case by case)]
- Samples from humans or other reservoirs species not known or reasonably suspect of being free of HIV, HTLV, Hepatitis C Virus.
- Viral vectors with gene inserts consisting of oncogenes or genes of unknown function that may have the potential to disrupt cellular processes
- High concentrations of RG2 viruses (but not volumes; for volumes more than 10 Liters, Large-scale Level 2 containment shall apply). *Consult Biosafety officer if intention is to work with > 10 L.*
- Lentiviral vectors rated at CL2+
- Samples derived from primates within the genus *Macaca*,
CL-2+ is not appropriate for isolation and manipulation of CL3 pathogens such as *Mycobacterium tuberculosis*. Further, each decision to use enhanced containment must be made via the risk assessment process in consultation with the Biosafety Officer and the Biosafety committee.

2.0 Physical requirements:

1. A separate room/lab (tissue /cell culture small lab inside a CL2 lab works as well) housed with a Biological Safety cabinet (BSC) is required to fulfill enhanced physical containment requirement.
2. Number of BSC within this room should not exceed more than two.
3. Must be dedicated to existing CL2+ work, no other projects of lower containment must be ongoing during that time.

Exception: Other CL2 work could be conducted in the same room, if there is no active manipulation of the pathogen requiring 2+ containment. Additionally, personnel that are competent and trained in CL2+ procedures can perform level 2 containment work in the same room. However, containment devices must be devoted to CL2+ work only.

4. Room must be self-contained, in accordance to the design of the experiment i.e. also equipped with centrifuge, water bath, microscope, etc.
5. Centrifuges must be equipped with sealed safety cups (or rotors). These safety cups or sealed rotors must be unloaded only inside the biosafety cabinet.
6. The laboratory should be adequately secured. Keycard access for those registered on project or Non-reproducible keys to be used when key-locks are used as the controlled access system.
7. Sink for hand washing should be inside the CL2+ space or hand hygiene (as approved by Biosafety committee) could be performed using an alcohol-based hand sanitizer, which must be available, the exit, and other areas where hand hygiene may be indicated.
8. Two-way communication system in accordance with function [i.e. telephones, intercom systems, 2-way radios, and communicating through a window (e.g.,



notes and signs, hand signals), networked computers and scanners etc. should be provided for personnel safety in the event of an emergency and to minimize the movement of notebook/papers and personnel into & out of the containment zone.

9. Lab notebooks or portable electronic device used to capture research results must stay inside the lab or alternatively have safety measures (e.g., decontamination resistant flexible covers) and/or written SOPs in place for decontamination prior its removal from the lab.
10. Freezer should be provided within the same lab complex (i.e. accessible without traveling along a public corridor) for storing pathogen and/or infectious material of interest. This freezer or the freezer room must be lockable with limited access. The pathogen must be stored and transported in a labelled, leak proof, impact resistant, (preferably in clip-on) secondary container.

2.1 PPE: Minimum Required Personal Protective Equipment (PPE)

- Solid front, impermeable disposable gown with cuffed sleeves
- Double gloving with disposable (e.g. nitrile) gloves, double gloving is
- Safety glasses

2.2 ITEMS: The following must be available at all times inside CL2+ lab:

- Stocks of disposable lab coats/gowns
- Disinfectants in accordance to function for e.g. Accelerated Hydrogen Peroxide (AHP) or suitable disinfectants as approved by committee.

Note: If bleach is preferred then a Standard Operating Procedure (SOP) for using bleach as disinfecting agent is required.

- Small waste receptacle lined with autoclavable bag inside BSC and biohazard bin lined with autoclavable bag outside BSC but inside CL2+ space
- Assembled biological spill kits (Tongs/forceps, safety goggles, N-95 respirator (annual fit testing is required to use it), and pairs of gloves
- Roll/sheets of absorbent paper, scoop and dustpan, disinfectants, autoclavable bags. Follow the slide biological spill kit in module 3.1 Spills (online biosafety training).

3.0 Operational practices: Enhanced containment consist of Containment Level 3 operational practices therefore following must apply.

1. All manipulation must be performed inside a biosafety cabinet.
2. Handlers must wear a long-sleeved solid front disposable gown and gloves that securely seal over the protective clothing sleeve.
3. A double-gloving protocol must be used. The outer layer of gloves should be removed inside the BSC (upon work completion) and placed directly into a waste receptacle lined with an autoclavable bag.
4. The autoclavable waste bag used to collect the waste inside the BSC must be tied inside the biosafety cabinet.
5. After completion of all procedures, first the disposable gown should be doffed and then inner layer of gloves should be removed following the appropriate glove removal protocol (taking care not to contaminate the hands/ underlying clothing or the laboratory environment), and immediately placed into the biohazard bin placed inside the CL2+ space.



6. The bag of this biohazard bin must also be immediately sealed prior bringing it out of the CL2+ space for autoclaving.
7. If there is inadvertent contamination of underlying clothing, potentially contaminated items must be removed and handled as per contaminated protective clothing protocol.
8. In absence of the sink inside the CL2+ space, a sanitizer (except for alcohol resistant organisms) can be used to disinfect the hands after removal of the inner gloves.
9. The biosafety cabinet work area must always be carefully disinfected with approved disinfectant immediately after use.
10. Glass containers (e.g. tubes and pipettes) and sharps must be avoided as much as possible. However, if procedures require the use of glassware and sharps e.g. a needle and syringe, scalpels etc. the use of safer (engineered devices) sharps or sharps precautions in a written SOP must be included in the risk assessment process.
11. All waste generated in a CL2+ lab must be handled as biohazardous.
12. Emergency response templates must be displayed inside the CL2+ space.
13. Signage on the door with the timely project info must be displayed at the door.
14. Personnel must be trained in appropriate CL2+ procedures, such as proper donning & doffing Personal Protective Equipment (PPE).

Laboratory Manager or an experienced lab member/ representative working actively and/or having thorough info about the project must be appointed by PI to take care of the designated CL2+ space.