

**LOCAL JOINT HEALTH AND SAFETY COMMITTEE
DEPARTMENT OF BIOMEDICAL SCIENCES**

STANDARD OPERATING PROCEDURE

for Rm: 3638 Bldg: 39

1. **Name of SOP: USE OF BIOSAFETY LEVEL 2 LAMINAR FLOW HOOD (BIOLOGICAL SAFETY CABINET) for SAFE HANDLING OF HUMAN CANCER CELLS**

Effective Date: July 2002

Author: John Burger July, 2002, revised by Esther Semple June 2011

Purpose: to promote the safe handling (and disposal) of human cancer cells in a laminar flow hood in a Biosafety Level 2 laboratory

Approvals Required: Roger Moorehead (faculty), Esther Semple (lab manager), Local JHSC, EHS

2. **Definitions:**

Biosafety Level 2: Designation applied to facilities required when exposure to identified or unidentified moderate-risk agents associated with human disease of varying severity may occur. Use of a biosafety cabinet (Level 2) is required. Primary hazards to personnel working with biohazardous agents may include accidental autoinoculation, ingestion, and skin or mucous membrane exposure to infectious materials.

UV: Ultraviolet light. Used for decontamination of cabinet surface. The UV lamp must not be on while hood is in use by personnel. Exposure to UV light can cause serious damage to skin and eyes.

Disinfectant bleach: A solution of 5.25 % sodium hypochlorite (household bleach) diluted 1:10 with water, e.g. 50 mL of household bleach in 450 mL of water. Sodium hypochlorite is a strong oxidizer. Oxidation reactions are corrosive, solutions can burn skin and cause eye damage. **To avoid release of dangerous gases, bleach must never be mixed with acids, ammonia or hydrogen peroxide.**

3. **Requirements:**

Fulfills OHSA; University of Guelph, Safety Policy

4. **Description of the Task:**

A) Preparation

- 1) before entering the level 2 area, prepare a checklist of materials to be used
- 2) Put on **Personal Protective Equipment**: lab coat and disposable latex gloves.
- 3) check air grilles for obstructions
- 4) switch on blower
- 5) allow air to purge workspace for 5 minutes
- 6) turn OFF UV lamp – turn on fluorescent light.

B) Disinfection

- 1) spray or swab interior surfaces of cabinet with 70% ethanol and allow to air dry
- 2) the surfaces of all materials and containers placed into the cabinet should be wiped/sprayed with 70% ethanol to reduce the introduction of mould spores or other contaminants to the cabinet environment

C) Assemble material: Be sure you understand the principle of clean air flow for the cabinet you are using.

- 1) introduce only material required to perform procedure - extra supplies should be stored outside the cabinet
- 2) place material such that clean and contaminated items do not meet.
- 3) place a 250-500 mL beaker in the hood containing about 100 mL of disinfectant bleach, which will be used to decontaminate liquids (e.g. culture media, discarded cell suspensions etc.).

D) Purge (pre-use)

Allow air purge period (3-5 minutes) with no activity inside laminar flow hood (leave blower on)

E) Perform procedures

- 1) introduce hands into workspace – delay manipulation of materials for about one minute – *this allows the hood to stabilize and to remove surface microbial contaminants from the users hands and arms* - work carefully -minimizing sweeping arm movements which might disrupt the air curtain and compromise the containment provided by the hood
- 2) avoid removing hands from work space until procedures are complete and all materials are secured (covered and capped)
- 3) the front grille must not be blocked with research notes, discarded wrappers etc. – all operations should be performed on the work surface at least 10 cm. from the inside edge of the grille

F) Decontamination

- 1) all containers and equipment should be surface decontaminated with 70% ethanol and removed from the cabinet when work is completed
- 2) immediately after use, place contaminated serological pipettes into a container of disinfectant bleach
- 3) the final surface decontamination should include a wipe-down of the work surface and the hood's sides and back and interior of the glass shield

G) Purge (post use)

Allow air purge period with no activity inside (leave blower on)

H) Shutdown

Turn off blower and fluorescent lamp, close sash and turn on UV lamp as required.

NOTE: Sash must be down (closed) when UV light is on. UV operation for longer than one hour is unnecessary and wasteful.

I) Personal procedures

Remove protective clothing and dispose of gloves etc. into biohazard waste container, then wash hands

J) Terminal disinfection

Put on fresh gloves; remove contaminated materials to biohazard bag

K) Close door to lab.

Door to Level 2 laboratory should be kept closed at all times .

5. Contingency Plan and Reporting:**Procedures to be followed in the event of Biosafety Level 2 Spill**

- 1) Alert people in immediate area of spill.
- 2) Put on protective equipment. This may include a laboratory coat with long sleeves, disposable gloves, disposable shoe covers, safety goggles, mask or full-face shield.
- 3) Cover spill with paper towels or other absorbent materials.
- 4) Carefully pour a freshly prepared solution of bleach disinfectant around the edges of the spill and then into the spill. Avoid splashing. Allow 20-minutes contact.
- 5) After the spill has been absorbed, clean up the spill area with fresh towels soaked in disinfectant.
- 6) Place towels in an approved transparent biohazard bag and decontaminate in an autoclave.

6. Waste management and Environmental Responsibility:

- 1) Fill the small biohazard bag outside of the laminar flow hood with solid waste, (e.g. serological pipettes, flasks, gloves, bacterial cultures on agar etc.). When full but still closable, seal with a "twist tie" and place into a larger biohazard bag labelled on both sides using a magic marker with Rm 3638, Biomedical Sciences and name of supervisor. (*This is easier to do when the bag is empty*)
- 2) Do not place any biohazardous liquids into the bags. Liquids must be decontaminated with an appropriate volume of bleach disinfectant solution for 10-15 minutes, and can then be poured down the drain.
- 3) When the larger biohazard bag is full, seal it with a tie and take to sterilization suite (see Ed Reyes)

7. REFERENCES:

Occupational Health and Safety Act
Laboratory Biosafety Guidelines, 2nd ed. Health Canada
<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>

8. DISTRIBUTION OF COPIES:

Dr. Roger Moorehead, Faculty Supervisor

Esther Semple, Laboratory Manager

Technicians, graduate students, project students and summer students working in the Level 2 biohazard laboratory

Environmental Health and Safety

Local JHSC, Department of Biomedical Sciences

Written By: Esther Semple Lab Manager

Approved By: _____ Faculty Supervisor (signature)

Date: _____