

# **Guidelines for Work with BSL 2 Organisms** **(Level S2, Genetically Modified Laboratory Strains)**

(According to Federal Ordinances ESV 814.912 and SAMV 832.321 both of Aug. 25, 1999)

## **1. Location:**

Laboratory of Organic Chemistry  
ETH Hönggerberg, building HCI  
CH-8093 Zürich  
Room HCI C343

## **2. Approval of the S2 Facility and the S2 Work:**

The permission to operate this Biosafety Level 2 (BSL; S2) facility and the herein executed experiments involving genetically modified organisms was obtained (Notice A040192/2 of 30.9.04; no objections within 45 days; confirmed on 23.7.08) for culturing genetically modified *Klebsiella pneumoniae* mutants to isolate chorismic acid or isochorismic acid, or a *Salmonella typhimurium* mutant to isolate prephenic acid. In addition, work will be carried out with the wild type and genetically modified variants of *Mycobacterium marinum* (having modifications in the shikimate biosynthetic pathway) (Modification to Notice A040192/2 on 7.11.08; no objections within 45 days; confirmed on 10.3.09).

Any other planned experiments belonging to BSL 2 must be reported at least one month prior to start of the work, and must be logged in a lab notebook.

## **3. Working Procedures for the Laboratory Environment:**

### **3.1 Access and designations:**

Facilities in which biosafety level 2 (BSL 2) activities are conducted must be clearly signposted:

- Biological hazard sign
- Restricted access sign: authorized personnel only

### **3.2 Biological agents used and hazards for humans and the environment:**

The lab is designated for genetic work with recombinant pathogenic agents belonging to risk group 1 or 2 (no or little potential danger).

### **3.3 Safety rules:**

The principles of Good Microbiological Practice should be adopted.

In particular, observe the following rules for work with biological hazards under BSL 2:

- Keep windows and doors closed while working with genetically modified or potentially pathogenic microorganisms.
- Eating, drinking, smoking, and storing food in the working area is prohibited.
- Wear appropriate laboratory clothing (lab coats); lab coats have to be kept separate from street clothing.
- Wear gloves while handling BSL 2 materials.
- No pipetting by mouth.
- The creation of aerosols should be avoided, if possible (e.g. centrifuge cells only in closed containments).
- If aerosol formation or splashing is unavoidable, work must be carried out in a sterile laminar flow hood (see appendix).
- Equipment that was in contact with BSL 2 microorganisms must be autoclaved or disinfected before cleaning; all waste must be autoclaved.
- Working surfaces must be disinfected after work is completed (70% EtOH or 1% detergent).
- After completion of each working step, and before leaving the lab, hands must be carefully washed and disinfected; any contaminated water taps must be decontaminated immediately.
- The laboratory should be kept clean and benches must be free of materials that are not pertinent to the work.
- Coworkers unfamiliar with BSL 2 work have to be instructed by the project manager or the biosafety officer about the potential risks and receive theoretical and practical instruction.
- Check identity of organisms in use on a regular basis.
- Control and contain any bug infestations.

### **3.4 Legal obligation for reporting of results:**

According to law, recombinant genetic work must be thoroughly described in a bound laboratory notebook.

### **3.5 Storage and waste management:**

GMOs have to be clearly labeled for storage.

The storage can be:

- in the designated 4°C, -20°C and -80°C refrigerators/freezers in the BSL 2 laboratory.

- in a -80°C-freezer in F343. This -80°C storage (strain collection) must be especially designated with the biohazard symbol and the room will be locked during nights and weekends.

Waste containing GMOs must be appropriately sterilised before disposing with normal trash or with waste water:

- plates and disposable materials, which have been in contact with GMOs ----> autoclave in biohazard autoclaving bags in an autoclavable plastic container in room C343.
- cell culture supernatants ----> sterilise with disinfection solution (EtOH, diluted bleach) or autoclave in room C343.

### **3.6 Service and maintenance:**

For service and maintenance, the project manager must be consulted before any work begins.

## **4. In case of emergencies:**

Contact persons:

Project manager(s):	Peter Kast (F333) / Donald Hilvert (F337)	Tel. 044 632-2908 / -3176
Biosafety officer:	Regula Grüninger (F330)	Tel. 044 632-2973

### **4.1 Accidental spills of GMOs:**

Spilled GMO-containing material must immediately be sterilised with EtOH or bleach solution, wiped up with paper towels and afterwards be autoclaved.

If large volumes are spilled the affected area has to be evacuated and the project manager must be informed.

### **4.2 Skin contact:**

If, despite the security measures, skin comes in contact with potentially hazardous biological material, the affected skin area must be disinfected carefully and the project manager must be informed.

### **4.3 Injury and accident:**

Any wound must first be disinfected and dressed.

Emergency showers are located on each floor in the central corridors.

**Ambulance: 0-144 or 24 hr Help Service ETH: 888**

Medical and emergency personnel have to be informed about the possibility of an infection with biologically hazardous material. The project manager must be informed.

### **4.4 Fire:**

The evacuation and emergency plans of the institute must be strictly observed.

For the fire fighting personnel working in a BSL 2 area, no additional security measures are necessary.

Emergency showers are located on each floor in the central corridors. The locations of fire extinguishers are indicated.

**Fire Department Zurich: 0-118**

**24 hr Help Service ETH: 888** (if false alarm was given, immediately give the all-clear)

## **5. Responsibility:**

The project managers are ultimately responsible for compliance with these regulations.

### **Appendix: Working in a sterile laminar flow hood**

For BSL 2 work that may result in aerosol formation, a BSL 2-approved laminar flow hood equipped with a high-performance particle filter must be used:

- switch on the flow hood at least 10 min prior to using it.
- all devices that go into or out of the flow hood must be cleaned and sterilised (e.g. with ethanol).
- do not block air inlets or outlets of the flow hood.
- have as few materials as necessary in the flow hood to avoid turbulence.
- clean the flow hood after each use, wipe with disinfectant, and (if so equipped) switch on the UV light.

# Specific Hygiene Plan for Work in the BSL 2-Safety Zone in HCI C343 (Kast/Hilvert Laboratory)

**General information:** When specifically signaled on the outer door of HCI C343, work classified as Biosafety Level 2 (BSL 2) is being carried out in this room. Such work may encompass experiments with potential pathogens of the family of *Enterobacteriaceae* (*Salmonella*, *Escherichia coli*, *Klebsiella* spp.) or the family of *Mycobacteriaceae* (*Mycobacterium marinum*). These organisms can be inactivated by treatment with 70% ethanol or 1% detergent (soak for 1 hour), as well as with all commercially available disinfectants.

## **1. General BSL 2 Laboratory procedures (laboratory, waste management).**

Appropriate laboratory procedures and protective equipment must be used to protect personnel and to avoid release of pathogens and genetically modified microorganisms into the environment.

- Wear gloves, goggles and lab coat (buttoned) and sturdy shoes (no sandals).
- Wash hands regularly and each time before leaving the BSL 2 area.
- Wear the lab coat only in BSL 2 area and keep it separately from other clothing.
- The lab coat must be disinfected if contaminated:

Soak the coat in 70% EtOH or 1% detergent (at least 1 hour), afterwards rinse it and wash it.

- Regularly wipe (no spraying!) bench surfaces with 70% EtOH or 1% detergent.

### **- Waste management:**

- **Contaminated glassware or glassware containing liquid infectious material ("biowaste"):** place in autoclaving tray and autoclave it in room C343. The autoclaved content must be disposed of (sink) and the glassware must be washed.

- **Infectious solids ("biowaste"):** collect in an autoclaving bag in an autoclavable waste container. Label the container with "biowaste", so that the maintenance personnel will not empty it. When the bag is full, close it and autoclave it within the waste container in room C343 (wet program!). Finally, the autoclaved bag must be transferred to a regular waste bag and put in the normal trash container.

- **Non-infectious (non-biological) waste:** Collect with regular trash. Make sure that absolutely no infectious material goes into this waste! The regular trash will be emptied by the cleaning staff, and the content goes untreated to the public waste disposal.

- **Contaminations on the floor:** Any contaminating material on the floor must immediately be wiped off with paper towels and the area must be cleaned with (70%) EtOH or 1% detergent (at least 1 hour) and paper towels (see 2B below). The (decontaminated!) floor will be cleaned by the maintenance staff, according to their regular cleaning schedule.

## **2. Accidents**

### **A. Post-exposure prophylaxis**

Procedure after injuries with infectious material: For **snag or stick injuries or eye or oral mucosa contact** with potentially infectious material, special steps are necessary:

#### **Immediate measures**

- **Stop work, ask a colleague for help.**
- **Stimulate bleeding, to eliminate as much contaminating material as possible out of the stick injury.**
- **Disinfect the wound well with skin-/hand-disinfectant (see below) for 2-3 minutes.**
- **Eyes: rinse very well (!) with water. Oral mucosa: rinse well with water or with 40% EtOH, if possible.**
- Afterwards: **immediately** call the project manager to discuss further measures to take.

### **B. Procedures for decontamination**

- **Hands/body:** When you think your hands have been contaminated, first rub them with 70% EtOH, then wash hands with soap. Treat contaminated areas of your body with 70% EtOH, then wash with detergent.
- **Clothing:** Treat with 70% EtOH and, if needed, with detergent. Take the contaminated clothing off and soak it in detergent (for at least 1 hour).
- **Equipment/floor:** Soak contaminations (no spraying!), e.g. in 70% EtOH. Wipe up any contaminating material with paper towels and afterwards clean the area with (70%) EtOH or 1% detergent (at least 1 hour) and paper towels. Put all used cleaning materials in the biowaste for autoclaving.

(updated by P. Kast, August 21, 2009)