

**Safety regulations for BSL-2 laboratories  
at the Department of Chemistry,  
KBC-building  
Umeå University  
(2024-04-10)  
For CBCS instrument users**

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## GENERAL SAFETY REGULATIONS

Before you start to work at the premises you need to read the “Safety Regulations for the chemistry department” that apply to all present in all premises of the Department of Chemistry. The Safety Regulation Document provides safety information that you are obliged to be familiar with. You will find the Safety Regulation Document at:

<https://umeauniversity.sharepoint.com/sites/Chemistry/SitePages/Work-Environment.aspx>

### Responsibility

According to the Work Environment Act, the employer is ultimately responsible for the activity. Anyone who works with micro-organisms should be informed about and think about the potential risks of work. Incidents and accidents should be reported to the Head of Department, Safety representative at Department (*arbetsmiljöombud*) and Work Environmental Coordinator at the University (*arbetmiljösamordnare*). Each employee has by law a personal responsibility for themselves and a responsibility for co-workers.

### Accidents

**Personal injury:** Eyewashes and emergency showers are available in the corridor. There is also a first aid kit at the exit in the middle of 4<sup>th</sup> floor. If you don't feel well call emergency at **112** if severe injury, or health advisor at **1177**.

**In case of Fire:** Maps showing emergency exit routes are posted in the corridor next to the elevator. Find out where fire alarm buttons and fire extinguishing materials are located. Study maps and memorize emergency exit routes and the reassembly place. Each incident without injury or event involving injury, must be reported to the Head of Department and to the Safety representative at Department (*arbetsmiljöombud*). This shall be notified to the Work Environmental Coordinator at the University (*arbetmiljösamordnare*) who in turn notifies this to the “Försäkringskassa”.

**In case of an incidence with infective material:** It is important to obtain an immediate assessment, because some measures such as treatment and prophylaxis must be deployed quickly. For larger scale or in an accident in public corridors and stairwells, immediately:

- Block off.
- Wipe up the spilled material and disinfect (use gloves).
- Note the individuals involved and who have passed.

In case of an incidence with infectious risk, immediately inform the nearest supervisor, and contact infectious disease clinic (**090-785 23 17, 785 23 15**) for the assessment of further action.

### Transportation

You are not allowed to ride the elevator with the tank/tube/flasks when transporting Liquid Nitrogen, CO<sub>2</sub> or hazardous chemicals. For safe transportation, put the tank/tubes in the elevator, attach the safety chain and press the button and close the elevator door. Walk up the staircase and bring the tank/tube out safety.

# SAFETY REGULATIONS FOR BIOLOGICAL AGENTS

## Definitions

### Biological substance (AFS 2018:4)

Biological agents/influential factors of the following kinds:

- a) Microorganisms, any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material.
- b) In vitro cultured cells derived from multicellular organisms, i.e. animals and plants.
- c) inferior replicable entities, such as viruses and prions,
- d) human endoparasites and
- e) components of or substances produced from agents as per (a) to (d) when occurring in conjunction with those agents.

**GMM/GMO:** Microorganism in which genetic material is altered in a way that does not occur naturally by mating or natural recombination.

### Biosafety Levels (BSL)

Safety precautions required for permission to work with biological agents and GMM/GMO. BSL defines requirements for facilities, equipment and work routines for different risk classes. BSL range from 1-4 with BSL-1 being the lowest risk.

#### *Requirements for BSL-2 (a full table is found in AFS2018:4)*

- Work must be confined to designated areas
- Basin for hand wash, with non-touch dispenser for hand disinfection
- Floor and bench top surfaces must be resistant to acids, alkalis, solvents and disinfection.
- Observation window facing the corridor to monitor employees inside.
- Access to an autoclave
- Biosafety cabinets
- Effective protection against pests.
- Limited access to facilities

## Classification of microbiological agents (AFS 2018:4, revisions 2021:1)

The classification system is a tool to put biological agents and GMM/GMO in a given BSL and provides the basis for the regulations regarding safety precautions, permission to use and reporting to “Arbetsmiljöverket”. Microbiological agents are classified into different risk classes. A list with classification of biological agents is included in AFS 2018:4, revisions 2021:1. In case of uncertainty the higher BSL should be used.

**Risk class 1 (requires BSL-1):** (low risk) Biological agents which do not normally cause infection in humans. Risk class 1 also includes biological agents which do not cause infection but may cause other health problems, such as hypersensitivity or production of toxin.

**Risk class 2 (requires BSL-2):** (moderate risk) Biological agents which can cause infections capable of giving rise to diseases of varying severity for which prophylaxis or treatment is

available or which normally self-heal without any serious long term health problems. Risk class 2 includes viruses which have been isolated from humans and do not come in a higher risk group. It also includes biological agents which are suspected of being able to cause cancer in humans but where the likelihood of exposure leading to cancer is very small, e.g. because a combination of many different factors is needed.

## **Classification of GMM/GMO (AFS 2011:2, revisions 2018:9)**

Activities involving GMM/GMOs are categorized into **F-Activity (low risk, BSL-1)**, **L-activity (moderate risk, BSL-2)**, and **R-Activity (High risk, BSL-3 and 4)**. For GMM, the risk class (as described in AFS 2018:4) of the host, the vector and the GMM must be considered when determining the category level of the activity. The safety precautions required for using the GMM is based on the category of the activity. A table listing requirements for the different safety levels can be found in AFS2011:2, revisions 2018:9.

**F-Activity: (Low risk)** The host will not likely cause disease in humans, animals or plants. The character of the vector and the transferred genetic material will not change the phenotype of the GMM in a way that it can cause disease in humans, animals or plants or have negative impact on the environment. Only GMM with the character described above can be used at BSL-1, all other GMM should be classified as L- or higher activity.

## **BSL activities at KBC, floor C4, C2, F2 and E1**

Work by CBCS and other research groups at Dept of Chemistry includes both F and L-Activities. The activities involve microorganisms such as viruses, bacteria, parasites, GMO and GMM, including cell-lines, which are classified as risk class 1 and 2 organisms. More information of BSL-activities at Dept of Chemistry is available in "Sammanställning verksamheter - Kemins lokaler" at <https://umeauniversity.sharepoint.com/sites/BioLabShared>

## **Risk of exposure**

Infection can be transmitted through;

### **Air**

- Droplet infection: The splash of infectious materials to the eyes and lips, or by aerosol, for example opening of the tubes or centrifuge.
- Dry sweeping can fly up dust: Floors in laboratories shall be swabbed with water.

### **Contact**

- Direct contact with samples, plates or inoculation loops can transmit infections to for example, wounds.
- Indirect contact through contaminated hands from workspaces, condensation or instruments by which the infection is transmitted to the eyes or mouth.

**Since biological agents assigned to risk group 2 can be pathogenic, "good microbiological practices" should always be observed**

## Good microbiological practice

Good microbiological practice must be applied and this means that;

- No eating, drinking, applying cosmetics, using tobacco or handle food in work area (in the lab).
- Observe the cleanliness and good order.
- Wipe up spills safely and disinfect contaminated area.
- As far as possible avoid use sharp objects.
- Handle needles and sharps, which have been in contact with body fluids or used on humans or animals, safely and immediately put them in containers intended for scalpel blades, hypodermic needles and syringes waste.
- Wear protective clothing (labcoat and gloves) within but not outside the work area.
- Have emergency preparedness and have procedures for dealing with adverse events
- Never put the cover back on a needle or other sharp objects used on humans or animals or who comes in contact with infectious agents or body fluids.
- Use all protective technical equipment needed to avoid contamination.
- Manage waste in a safe manner.
- Manage cultures in closed containers or otherwise to prevent the spread. Tubes and flasks must be labelled with the initials and details of the bacterial strain and the resistance marker.
- When culturing flasks should be marked with the bacterial strain and resistance in case something happens and the suspension must be decontaminated.
- No pipetting by mouth or otherwise act in a way that microorganisms could end up in your mouth.
- Avoiding the formation and propagation of aerosols, spills and splashes.

## Specific safety rules for BSL-2 laboratories

Everyone who will work in the lab (including instrument users) must attend a safety introduction. Fill in your contact information in the sheet on the sliding doors after completion.

### *All-BSL-2 labs:*

- Use “good microbiological practice”
- Sign, date and put some information on the content on every plate, bottle, flask or tube that you leave in the room
- Wear lab-coat, gloves and protective goggles
- For work with **BSL-2 microorganisms that are easily transmitted by aerosols** see specific instructions (annex 1).

### *Cell-labs (B4-11-01, B4-13-01, B4-13-07):*

- No outer-ware is allowed inside the lab, including outdoor shoes.
- Use cell-lab shoes or shoe covers and protective gloves. Dispose of shoe-covers in the trash-bin when leaving the lab.
- Use disposable lab-coats (yellow for infection-lab and blue in the clean-lab). If you are going to use the lab frequently sign your own lab-coat and reuse unless contaminated.
- Change lab-coat and gloves when entering the clean lab.
- Avoid unnecessarily running in the cell culture room.

- The doors must be kept closed!
- Windows may not be opened at any time.
- Never bring anything back from the infection lab to the clean lab.

**External/Instrument users:**

- Are obliged to follow the routines for safety regarding personal protection and contamination risks listed in the above paragraphs.
- Must file a printed copy of the risk assessment in the risk assessment folder if bringing live microorganisms into the lab
- Must bring all necessary equipment and consumables unless otherwise agreed on.
- Must remove all waste and equipment and clean the workplace when finished.
- **Are not allowed to dispose of hazardous waste in the Biolabs** unless taking part in the lab-duties.

## Risk assessment, permissions and notification

The **risk assessment** confers the basis for selection of protective measure and required BSL in each case. How serious infection the agents can cause, infectivity and route of infection, available treatment and risk of spreading into the environment are factors that need to be addressed in the **risk assessment**.

**OBS! Risk assessments must be performed for all use of live microorganisms that is brought into Dept of Chemistry, regardless of biosafety level.**

### L-Activity and use of pathogens at BSL-2 must be reported.

- Arbetsmiljöverket must be notified of **any new** GMM/GMO use and/or non-genetically modified pathogen at BSL-2 before any work is initialized.
- Permission is required for culturing large quantities (>500L total volume).
- For GMM use forms LA (new use), for pathogens see “Anmälan om användning av smittämnen i riskklass 2” (29 § AFS 2018:4, revisions 2021:1) specified below

### Info required for notification of new microorganisms at BSL-2 (see also appendix II)

1. The employer's name and corporate registration number.
2. The name and address of the workplace.
3. The name of the person or persons allotted tasks relating to health and safety in the workplace, and particulars of their capabilities relevant to the task. Availability of advisory competence for risk assessment.
4. Description of the nature of the activity. General particulars of the infectious agents which will be used, of the maximum volume to be handled and of handling procedures.
5. Risk assessment documentation as per Sections 4-5.
6. Description of facilities and technical devices material to safety, together with protective measures and other preventive measures planned.
7. Program of medical preventive measures and controls.
8. The number of persons to be employed in the activity

Email your notification and risk assessment to [anna.u.eriksson@umu.se](mailto:anna.u.eriksson@umu.se)

Risk assessments/msds are found at <https://umeauniversity.sharepoint.com/sites/BioLabShared>

Printed versions for floor C4 are kept in room B4-19-01 (Biolab).

## **BSL-2 designated areas and equipment at floor C4, C2 and E1**

Work involving microorganisms that requires biosafety level 1 and 2 are performed in corridor C, 4<sup>th</sup> floor in rooms B4-11-01, B4-13-01, B4-19-01, B4-13-07, B4-43-07, room B2-29-07 on the 2<sup>nd</sup> floor, and E1-05-01 on the first floor. The -80 and -150 freezers used for long term storage of microorganisms and cell-lines are located in room B4.23.07.

The majority of experiments with bacteria, fungi and parasites are performed in the Bio-lab (room B4-19-01) and in room B2-29-07 where the large incubators are located. Spectrophotometric studies are performed in the NMR-lab (room E1-05-01)

Room B4-43-07 is the HTS-lab where the majority of our plate readers and liquid handlers are located. The room is equipped with exhausts funnels that should be turned on when handling organic solvents. The HTS-lab is further equipped with 2 incubators for short-term culture, of which one is connected to CO<sub>2</sub> for eukaryotic cell-culture and a cell-centrifuge.

Culture of established eukaryotic cell-lines is carried out in room B4-11-01, B4-13-01 and B4-13-07 where B4-11-01 is designated for cell-lines only. Infectious agents such as parasites, bacteria and viruses are prohibited from room B4-11-01. Incubation of infected cell cultures are done in closed plates in room B4-13-01 and B4-13-07. Microorganisms are stored in the -80 and -150 freezers located in room B4.23.07.

### **Contact information**

Information on whom to contact in case of failure of equipment (freezers, incubators, plate readers...) or any other issues while using the rooms is posted at the entrance of each room.

### **Biosafety cabinets**

Rooms B4-11-01 and B4-13-01 are equipped with one SCANLAF cabinet each and room B4-13-07 are equipped with a K-Safety KR cabinet operating on the recirculation principle. All 3 biosafety cabinets comply with EN 12469 and provide protection of the operator, the environment and the processed product against microbiological contamination. The biosafety cabinets are programmed to be sterilized by UV between 00.00-02.00, daily.

### **CO<sub>2</sub> Incubators**

CO<sub>2</sub> incubators are present in rooms B4-11-01, B4-13-01, B4-13-07 and B4-43-07, do NOT increase the CO<sub>2</sub> pressure above 1,5 bar. If you suspect a gas leakage, check the CO<sub>2</sub> meter that can be found in each room. For levels above 1000 turn off the main CO<sub>2</sub> switch, close the incubators, leave the lab and contact the person responsible for the room (contact information is posted next to the lab entrance).

### **Thawing cell-lines from Liquid Nitrogen**

Cryo tubes that have been submerged in liquid nitrogen may explode when thawed due to leakage in the tank. This imposes a risk of contamination and injury. Tubes with male cap and silicon O-rings are less likely to explode. To avoid explosion when thawing cryo tubes emerged from liquid nitrogen tank, immediately loosen the cap to make sure that there is no liquid nitrogen inside the tube.



## Booking and using instruments and equipment at floor C4 and C2

### *Instruments:*

- For booking of ImageXpress, plate readers and liquid handling equipment use resource booking at <https://booking.chem.umu.se/>
- Fill in the log, report failures or incidences
- No gloves on the computers
- Remove your plates, cuvettes, tubes etc. when finished
- Turn off the instrument when finished unless otherwise agreed on.

### *CO<sub>2</sub> incubator (B4-43-07):*

- Note when you refilled/changed water and sterilized.
- When changing the default temperature, change back when finished.

### *Biosafety Cabinet (B4-13-07):*

**Before you start: MAKE SURE THE WORKPLACE IS NOT BOOKED**

- Booking lists for biosafety cabinets are posted on the front panel
- Never bring your own lab coat, find designated lab coats in the cupboard.
- Clean the interior with 70% EtOH before use (Spray bottle by the sink)
- Spray the outside of bottles, boxes and bags with 70% EtOH before putting them inside
- Don't open tip-boxes and bags with cell-culture flasks etc. outside the biosafety cabinet.
- Collect **ALL contaminated** plastics (tips, eptubes and plates) in a small plastic bag (tips could also preferably be collected in an empty plastic bottle). When finished, seal the bag with a cable strip.
- Do NOT discard contaminated waste (gloves etc.) in the grey waste bin! They should only be used for waste that has not been in contact with any biological agents (for ex, paper towels used after washing your hands/plastics removed when opening new bottles).

### **When finished:**

- **Remove all equipment and waste and bring back with you. Do NOT leave any bottles or plastics inside the biosafety cabinet.**
- Clean all surfaces with 70% EtOH or other suitable disinfectant.
- In case you spilled any liquid during your work you need to remove the stainless-steel benchtop panels, wipe up your spill from the bottom (underneath the panels) and disinfect the surface with 70% EtOH or other suitable disinfectant.
- UV-sterilization is scheduled for 2h from 00:00-02:00 for all 3 biosafety cabinets unless otherwise notified.