

Security instructions and general behaviour

Think before you act!

Be responsible from A to Z!

Be part of the common life in the laboratories.

Work with respect to others.

The following rules are not only indispensable for your own security but also for the comfort and working progress in this laboratory. We expect from every collaborator to attentively read this “**Charter**”, to respect all rules stated herein, and to confirm that they agree with them by signing the last page.

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Newcomers are not allowed to work in the lab before they have been introduced to the general lab functioning by a **technician**.

1 General remarks

1.1 Working space

Every person or working group gets assigned a place for storage (cupboard, drawer). It is their responsibility to tidy up the storage space and to clean it regularly. This is also expected for the tables and benches that are designated for short and/or long term use.

In all laboratories the general working spaces (and storage) are considered **as common goods**. This means that after finishing your work **every one has** to be cleaned as follows:

- **Tables, benches, fume hoods** have to be cleaned with 70% alcohol if you have worked with bacteria, then rinsed with water and if necessary a cleaning product.
- **The laminar flow hoods** have always to be cleaned with 70% alcohol before and after working, and thoroughly cleaned once a month or depending on frequency of use.

Remark: After working with **toxic products**, only paper towel should be used for cleaning. These towels should be thrown into the normal trash or the waste for hazardous substances, according to the degree of toxicity of the product used.

- If you are working with **soil, sand or glass beads** in the laminar flow hoods, it is important to clean thoroughly under the roast immediately, after finishing.
- When working with **gas** on the bench or in the laminar flow hood be especially careful of:
 - Do not leave the safety flame of the Bunsen burner on when leaving your working place!
 - Always close the gas connection after finishing your work!

After your work is finished, please **put everything (equipment and chemical products) back into its designated place** to avoid others searching for it everywhere.

Before you leave the lab for the day, please walk through all laboratories to make sure you did not forget anything (turn off equipment, close gas connection, close windows, etc).

1.2 Products

The chemical products (with exception of few) are listed in a blue folder on the table of room 205.

The products are listed in groups in alphabetical order:

- Antibiotics
- Dyes
- Minerals
- Organics
- Solvents
- Others

In the folder you will find the names, chemical formula, internal product number, their storage place and also all remarks and instructions regarding toxicity (risk phrases) and safety. Consider this information before you use a product.

You are obligated to read and follow all safety instructions when manipulating chemical products!

1.3 Material

The laboratory material is **very costly!**

Please take care of the material and do not waste it!

Every product you prepare (solutions, cultures, Petri-dishes, bags, etc.) **need to be labelled** as follows:

- Name of product, concentration and toxicity information
- Your name
- Date

Please remember that storage places (in drawers, cupboards, cold chamber, refrigerators and freezers at -20°C and -80°C) are put aside for every person or at least for every ongoing project. Put your material in the assigned storage places for your project and not anywhere else.

Every material that is not labelled properly and not stored in the right place will be eliminated.

At the end of your stage, master thesis or doctoral thesis, you are kindly asked to clean out all the material in all storage places (don't forget refrigerators, cold chamber and freezers at -20°C and -80°C).

1.4 Glassware

The glassware and other lab material have to be washed in room 204 as follows:

- **toxic chemicals should be dispensed into the dedicated containers** under the fume hood in room 225
- lab material has to be rinsed with warm water
- labels (stickers or written with marker) should be removed before washing
- material goes in the designated racks for the washing machine and placed such as to ensure a water flow into it
- the screw-caps or other caps are placed in the basket and sorted by size
- the Swinnex filters belong in a separate basket (rinse them thoroughly before use)
- the syringes belong in a separate basket (rinse them well before use)
- the glass pipettes (cotton removed) should be rinsed and placed in the soaking bath with tips upwards

In weekly rotation every collaborator is responsible for the washing of all glassware and lab materials. The schedule is on the door of room 204.

1.5 Autoclaving

Every collaborator (except interns) has to learn how to run both autoclaves, the large and the small one. To avoid confusion, **every material** to be autoclaved – **including waste** - should be indicated by adding a piece of autoclaving tape to it. All material for autoclaving is collected until 11.30 h to start the autoclave, in order to save energy. If there is only few material to be autoclaved, the small autoclave should be used (A210).

The person that opens the autoclave at the end of the programme is responsible for putting the material to its respective place, such as: putting the liquid agarose at 60°C, all solutions on the bench in lab A205, everything for drying in the incubator aside the autoclave, empty the trash bins, etc.

1.6 Equipment

The equipment and instruments of the laboratory are delicate and very expensive! You are kindly asked to take care of the instruments and consult the user manuals for necessary information.

The usage of an instrument requires the prior knowledge of its full functioning as well as its maintenance. You will be instructed by the responsible person of the instrument or by your tutor. **Some instruments are dangerous, in no case you are allowed to start an experiment by trial and error!** Some instruments such as balance, microscope and centrifuge have to be cleaned after every usage. If you notice a disturbance or breakdown of the instrument or equipment, immediately alert the responsible person.

1.7 Orders

Every time you notice that a product or material is running low on stock you are obligated to order it yourself!

Every collaborator has to be familiar with the procedures on how to place an order. If you don't know it yet, talk to your/ a responsible person; they will show you the necessary procedures for placing an order. A copy of each order (even if placed by phone or Internet) has to be archived in the folder for orders (Schneitter, magazine CAFS or other).

2 Security

2.1 Handling of hazardous substances

Before you use a chemical product, you need to read the security instructions concerning the product (look in the blue folder or catalogue from Fluka). **It is important that you carefully follow all security instructions (→ wear disposable gloves, safety glasses, work under fume hood, etc.)**

Many products used in this lab can be toxic, corrosive, carcinogen, mutagenic, teratogenic or others.

You have to know the meaning of the symbols indicating the hazard, displayed on the label of each chemical product:

Xi = irritating substance

Xn = harmful substance

☠ = toxic substance

The indications by the producers concerning ...

- risks = << **sentence R** >>
- security instructions = << **sentence S** >>
- property of substances = << **sentence F** >>

have to be read before using the products. (Sentences R, S and F can be found in the blue folder or catalogue from Fluka)

Think before act! Use your general chemical knowledge before mixing two substances (endergonic or exergonic reaction, explosive, release of gas, etc).

In case of doubt on a chemical product (its use or the precautionary measures) please ask someone!

Some examples (List not complete!):

- **Formamide** is teratogenic.
- **TEMED** (involved in polymerization of acrylamide) is destructive to human tissue, its inhalation can be fatal, extended contact can cause irritations and burns of your skin. It is a product easily inflammable.
- **Phenol** is very toxic for inhalation, ingestion, contact and can cause serious burns. It is advised to wear two pair of gloves (latex and nitril). In case of contact, rinse thoroughly with water and don't use ethanol, which will make it worse!
- **Chloroform** can irritate your skin, your mucous membranes and respiratory tracts and is carcinogen.
- **SYBR green** is carcinogen. This product is highly mutagenic. It is important to limit the use of these products to the designated laboratories and rooms.
- **Acrylamide** is neurotoxic when not polymerized. Avoid all contact with skin and inhalation (in powder form). The polyacrylamide can contain non-polymerized acrylamide and has to be handled with same care.

2.2 Security equipment

Wearing a lab coat is compulsory in the microbiology laboratories as well as the gel-electrophoresis room (paper coat that stay in this room).

In contrast, take off the lab coat before entering the coffee corner, offices, meeting room or the toilets

If required, wear **gloves, safety glasses, masks and use fume hoods** in maximum ventilation state. In case of high transpiration the latex gloves don't protect you after 15 min. Using ethanol can increase the chances of a product penetrating your gloves or your skin. It is therefore advised to change your gloves in regular intervals or when you got in contact with a toxic product. It is important to avoid touching anything when wearing contaminated gloves, lab benches, fridge doors, pipettes, etc. **Before leaving a laboratory, your gloves have to be taken off!**

It is compulsory to wear UV-safety glasses when in proximity of a UV illuminated hood (**risk of damage to your eyes**). When working on UV-table, you should wear a **UV-protective face shield**, because the safety glasses are not sufficient (risk of burned skin). Also the rest of skin should be covered (fingers, wrist, and collar).

In case of doubt, ask for all necessary instructions from the responsible person.

2.3 Storing hazardous substances

Easily inflammable and volatile chemicals are stored in the **fire-safety cabinet** and have to be put back immediately after use. Avoid storing inflammable products in the fridge if your flask is not hermetically closed. The cleaning products -usually volatile- that are used and stored in the lab can be a source of explosion. Be careful!

The **gas bottles** have to be attached to the wall with a chain. Keep in mind: If an O₂-gas bottle falls, it transforms into a rocket and can shoot through a wall!

After using a gas from a bottle, the valve has to be closed tightly. Then open the pressure reducer valve to empty the pipes and close it again.

If you want to replace a bottle of gas or move it around the lab, **do so only with the designated cart.**

2.4 Centrifuge

Before you start to centrifuge, take the following security measures and verify the following:

- is the rotor **adapted** to the centrifuge?
- is the rotor **well positioned** and straight on the axe?
- has the rotor been loaded in order to keep **perfect balance** (tubes always opposite to each other) (the balance scale is available in A212)?
- is the **lid tightly closed**?

Stay with the centrifuge until full speed has been reached.

A potential accident concerning the centrifuge can seriously harm humans, the building or equipment around it. For cleanliness and security reasons, the centrifuge has to be cleaned thoroughly after each use (the condensed water can harm the rotor because of rust damage). The rotors of the large centrifuge Beckman (A212) have to be placed back into the cupboard on the wooden grills and posed upside down.

3 Microorganisms

3.1 Handling

We rarely work with hazardous microorganisms but when working with isolates from environmental samples, some strains may be pathogenic opportunists or even strictly pathogenic.

Before and after experiments with microorganism it is important to **wash your hands** with soap, dry them properly and apply Sterillium for 30 seconds to disinfect your hands. The working surfaces have to be cleaned with **70% alcohol** before and after working.

The laminar flow hoods create a sterile working environment. To ensure sterility, the flow hood has to be started 15 minutes before working (between two experiments, the hood should not be turned off completely, but rather put on minimal aeration to ensure sterility). A laminar flow hood is only sterile when the airflow can be maintained properly. To ensure that, don't cover the grill at the front with objects. If you use environmental samples (soil, roots, etc) or very contaminated material like mold (spores!), please clean the flow hood thoroughly after use. That includes cleaning under the grill and turning on the UV for 15 minutes. All culture solutions and media (Petri dished, tubes, flasks, bottles) as well as all biologically contaminated material have to be autoclaved before disposal in waste bins or the drain.

3.2 Contamination

Mites can be an important source of contamination, especially in the incubation chambers. Their presence can be seen by the traces they leave on the culture dishes. They are a pain to get rid of. Once contaminated the incubation chamber has to be emptied, cleaned with a special product and subjected to UV treatment.

So:

When storing soil, compost, plant material and all other field material, put them into sealed plastic bags (properly labelled) to avoid cross contamination.

It is advised **to seal all Petri-dishes with parafilm** when they are stored in the cold chamber, incubation chambers or fridges. Besides, sealing them also avoids desiccation of the agar.

4 PCR room (only for Microbiology)

The laminar flow hood in the PCR room as well as all material that is found in this room (especially the pipettes) are designated for preparing the master mix for PCR or RT-PCR. Don't bring in any case bacteria or DNA samples in this room.

Write your name in the reservation list when intended to work under the laminar flow hood in the PCR room. Also you need to reserve a PCR machine.

For every new PCR program, add a file to the PCR folder as well as to the folder on Bota_Mbiol, stating your program with the respective steps.

If you modify any programs in the PCR machines, please alert other users by leaving a note on the respective machine.

5 Electrophoresis room

IT IS COMPULSORY TO WEAR A LAB COAT WHEN WORKING IN THIS ROOM: USE THE PAPER LAB COATS THAT ARE AVAILABLE IN THIS ROOM. THESE COATS SUCH AS ANY OTHER MATERIAL FROM THIS ROOM SHOULD NOT BE TAKEN OUT OF THIS ROOM.

WEARING BLUE NITRILE GLOVES IS ALSO COMPULSORY FOR ALL EXPERIMENTS IN THIS ROOM. EXCEPT WHEN TOUCHING THE COMPUTER! WEAR NEW (WHITE) LATEX GLOVES WHEN WORKING WITH THE COMPUTER.

The preparation of all samples and electrophoresis gels should be done on the designated bench space marked with **white tape**.

The melted agarose has to be cooled to (~55°C) before you pour the gel, otherwise the plastic of the support will warp and break. A 1% agarose-TBE solution is available in the incubator at 55°C. If the bottle is empty, please prepare a new stock and label the top with 55°C A221, when autoclaving. The migration buffer (TBE buffer) should be replaced after 5 migrations. Note the date of change as well as the number of times, it has been used. Also the TBE buffer 0.5X has to be prepared, if the stock is empty. The boxes with pipette tips have to be refilled after each use. The bin with the used pipette tips has to be emptied regularly into the contaminated waste bin.

Staining of gel

Reminder: Gel-red and ethidium bromide are highly toxic because they intercalate in our DNA. The GelRed solution 3X (4L) is available in the fridge. Since GelRed is a compound that intercalates into DNA, so even though the concentration is low, we cannot be sure that it does not have any carcinogenic or teratogenic effects. So consider it as a product that is **potentially dangerous**. For this reason, the use of GelRed has to be restricted to the designated bench **space marked with red tape**. To save the use of the solution, we minimize the quantity that is used (only just cover the gel). Note the number of times the solutions has been used on the lid as well as the date, when you changed a solution. Approximately staining 10 times can be done with the same solution.

Waste management:

Liquid waste (migration buffer, GelRed solution) should be disposed of in the white container with activated carbon (on top of the incubator) to absorb toxic remainders. Once the container is full, it is placed on the DGGE bench while stirring overnight. The decontaminated buffer can then be poured down the drain and the activated carbon goes in the solid contaminated waste bin. Two new bags of activated carbon have to be put in the container and placed back on top of the incubator.

Solid waste (agarose gels, acrylamide gels, gloves, paper) has to be placed in the specified containers. Once the bin is full it has to be closed with the respective lid and marked with "GELRED" and stored under the bench. When all bins are full, contact the CAFS and fix a date to remove the waste bins. Fill in the respective form for disposal of hazardous waste (in drawer of bench). The CAFS weighs the waste bins to issue the bill. Bring back empty waste bins from CAFS.

6 Coffee corner/ Food

In respect of all other users, please keep the place tidy and comfortable. The dishes have to be washed, dried and put back after each use. Also please keep the tables, coffee machine and microwave clean. Please don't hoard coffee mugs in your offices, but wash them and bring them back regularly. The container with the used coffee has to be emptied regularly.

Please take care to keep the coffee corner and kitchen clean and tidy for everyone to enjoy.

7 Agreement Signature

I declare having read all instructions and agree to respect all directions.

Neuchâtel, the (date).

First and last name

Signature

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