Biosafety protocols, techniques and tools

This book serves as a reminder of the rules, techniques and tools you will need to work safely and hygienically in the HKU Biolab, as learned in the Biolab Safety Course.

- HKU Biolab rules (EN)
- Using the autoclave (NL/ENG)
- Safety course recap (theory, ENG)
- Creating a sterile environment using the Bunsen burner (EN)

HKU Biolab rules (EN)

HKU BIOLAB RULES (GMLP*)

** Good Microbiological Laboratory Practice | january 10, 2021 | contact: kas.houthuijs@hku.nl | 0615252354*



1. You are responsible for reporting spills and damages immediately to a lab technician for safe handling. Only do work you are comfortable doing and are trained to do. When in doubt: ASK!



2. No food or drink in the lab, also no smoking, no applying make-up or contact lenses allowed in the lab. Avoid hand-to-face and hand-to-mouth contact. No mouth pipetting allowed (only mechanical pipetting).



3. Wash and desinfect your hands before and after working in the lab. Keep nails short and remove jewellery before washing. **Wear a lab coat** and where necessary: wear protective gloves and/or protective eyewear.



4. Personal objects live outside the lab Keep jackets, loose pieces of clothing, pens, notebooks and phones and laptops separate from microbiological work, use the lockers.



5. Don't leave running pressure cookers or gas flames out of your sight! Ensure pressure cookers have enough water before you begin. Never release steam for faster cool-down.



6. Avoid aerosol formation by applying proper flaming technique for instruments (wet part with culture is flamed last). Move liquid culture in closed containers to avoid spills.



7. Label all bottles and plates with culture as well as all bottles with unused media with 1) initials 2) date 3) medium, and 4) organism



8. Clean up! Autoclave biological waste and contaminated materials. Clean used surfaces & objects with 70% ethanol or isopropyl alcohol, or freshly prepared 10% bleach solution.



9. Only organisms classified as BSL-1 purchased by lab technicians are allowed in the lab. You are not permitted to take any living culture outside the lab.

Using the autoclave (NL/ENG)

DE AUTOCLAAF GEBRUIKEN

- Haal voor gebruik de binnenpan uit de autoclaaf en beoordeel de hoeveelheid water, dat moet ong. 3,5 liter zijn, of tot de onderkant van de pootjes waarop de binnenpan rust.

- Zet de te steriliseren objecten in de binnenpan. Draai de doppen van flessen losjes op de hals en max 75% vol (om lucht te kunnen laten ontsnappen). Haal evt. parafilm tape eraf voordat je steriliseert.

- Zet de binnenpan terug en sluit het deksel gelijkmatig met de vleugelmoeren.

- Stel de gewenste sterilisatietijd in, wacht 5 seconden en schakel de autoclaaf uit en weer in om de nieuwe tijd te bevestigen.

- De cyclus loopt nu. Als de watertemperatuur 100 graden is klinkt een serie piepjes. Het sterilisatieproces start, de tijd loopt af.

- Nadat de tijd om is klinkt een alarm. Schakel de pan uit. Als de druk gedaald is, open je de pan. PAS OP, ALLES IS HEET!

- Na gebruik is het belangrijk om de binnenpan en de inleg helemaal droog te maken, anders gaan deze onderdelen roesten.

USING THE AUTOCLAVE

- Before use, remove the inner pan from the autoclave and assess the amount of water, ideally about 3.5 litres, or to the bottom of the legs on which the inner pan rests.

- Place the objects to be sterilized in the inner pan. Tighten the caps of bottles loosely on the neck and max 75% full (to allow air to escape). Remove the paraffin film before placing Petri dishes in the autoclave.

- Replace the inner pot and close the lid evenly with the wing nuts.

- Set the desired sterilization time, wait 5 seconds and switch the autoclave off and on again to confirm the new time.

- The cycle is now running. When the water temperature reaches 100 degrees, a series of beeps will sound. The sterilization process starts, the time counts down.

- After the time is up, an alarm will sound. Turn off the pan. When the pressure has dropped, open the pan. BEWARE, EVERYTHING IS HOT!

- After use, it is important to dry the inner pan and the insert completely, otherwise these parts will rust.

Safety course recap (theory, ENG)

Biolab Safety Course Recap

In the Biolab safety course you learned about the risks involving laboratory work with microbes, you learned about the international standard of 'good laboratory practices' for BSL1 level laboratories and have practiced working hygienically in the lab. Let's be honest: *It was a lot!!* Hence this recap and the open invitation to always ask the lab manager for reminders, help or explanations, we will be there to guide you while working.

Understanding the three main risks

In the Biolab we work with living microorganisms (also known as microbes), organisms too small to be seen with the naked eye which can only be observed through a microscope. Examples of such organisms are bacteria, fungi, yeasts or algae. Microbes are all around us and are also abundant within our bodies where they play crucial roles in processes such as digestion and immunity. Even though the microorganisms the Biolab works with are not dangerous for public health ('non-pathogenic'), it is still essential that students and teachers adhere to biosafety protocols, for three main reasons:

- **Protect yourself and others**
  Non-pathogenic microorganisms lab-grown in large quantities can still contaminate workspaces, materials, and other projects. Some individuals may have weakened immune systems or allergies that could make them more susceptible to even non-pathogenic microorganisms. Adhering to biosafety protocols minimizes the risk of accidental exposure and potential health issues.
- **Protect the environment**
  Proper disposal and handling of microorganisms grown in the lab prevent them from being released into the environment, where they could potentially disrupt local ecosystems or interact with other microorganisms in unforeseen ways.
- **Protect your work**
  Proper biosafety measures help maintain a clean and controlled environment, growing only the organisms that you intend to and ensuring that your work is not compromised by unintended microbial growth.

HKU Biolab Rules (also see the icons in the lab)

1. **You are responsible for reporting**
   Biolab safety is all of our responsibilities, so it is important to be open to giving and receiving feedback during working in the Biolab together. We help each other by pointing out mistakes and you help instructors by reporting it if something is broken or spilt and by

letting instructors know if something is unclear. Always feel free to ask for help if you are unsure or uncomfortable, it's what we are there for.

2. **No food or drinks in the lab**
   Due to risk number one (protect yourself and others) it's not allowed to eat or drink in the lab space, or to apply make-up or contact lenses. For the same reason try to refrain from hand-to-face or -mouth contact to avoid microbe transfer.
3. **Personal protection**
   When entering the lab, put on a lab coat in your size. When leaving the lab, hang the lab coat back. This way we prevent organisms spilled on the coat to leave the lab. Please tie your long hair back and wear low heeled, closed shoes.
4. **Wash your hands, wear a lab coat**
   Always wash your hands prior to and after working in the lab. Before washing, remove jewelry and if you come regularly keep your nails short. In the lab space there is a hand disinfectant pole you can use in between washing if needed.
5. **Personal objects live outside the lab**
   Keep your phones, pens, laptops etcetera outside of lab or the area where microbial work is done, as organisms can travel in and out of the lab on them. As mentioned during the course, a lab journal is allowed to stay in the lab if you come regularly. Taking pictures of your work or process is allowed, but only after notifying the instructor, who will let you know what is a safe moment and manner to do so.
6. **Don't leave running pressure cookers or flames unattended**
   Pressure cookers and autoclaves build up a lot of heat and pressure. Don't work right next to them as hot steam can exit through the ventilation valve, and make sure there is always someone in the lab when the machine is running to keep an eye out. Check the water level before starting a pressure cooker. When you are working with a bunsen burner, always sit down while working (so you can not accidentally lean over the flame) and never leave your flame unattended. If the flame needs to stay on longer (while curing agar plates e.g.) notify all the others working in the lab about it.
7. **Avoid aerosol formation**
   When disinfecting a grafting needle with culture in a flame (flaming), flame the part with the culture last to avoid aerosol formation. Make sure you know the proper flaming technique. Carry liquid cultures around only in bottles that are capped, to avoid spills.
8. **Label all bottles and plates**
   Label bottles and plates with culture or with unused media with 1) your initials, 2) the date, 3) the medium used and 4) the organism. There are special labelling tape and laboratory markers available that will last through an autoclave cycle.
9. **Clean up!**
   Autoclave biological waste and contaminated materials. You can use an autoclave bag and holder as your trashcan while working. Clean used surfaces and objects with 70% ethanol or isopropyl alcohol or a freshly prepared 10% bleach solution. SPILL PROTOCOL:
10. **Only organisms classified as BSL-1**
    HKU Biolab is a Biosafety Level 1 lab, so only organisms classified as BSL-1 and purchased by the lab instructors are allowed. You are not permitted to take any living culture out of the lab. If you are interested in a certain microorganism that is not available in the lab, check with the instructor to see if there is a BSL1 culture available for purchase.

Spill protocol

Get familiar with the procedures in case of a *spill accident with microorganisms*:
Spray the spilled area with plenty of 70% alcohol. Wipe it up with a paper towel and throw it away in an autoclave bag.
Cover the spilled area with a paper towel, spray the towel generously with 70% alcohol and let it sit for 5 minutes. Then wipe it and throw it away in the autoclave bag. Dry the area with a paper towel and throw it away in the autoclave bag.
Sterilize everything in the autoclave and then throw it away with the normal waste. Wash your hands thoroughly with soap and water. In case of a large spill or an area that is difficult to clean: Contact the responsible lab instructor.

When you drop a bottle of *nutrients* on the floor, it is equally important to clean it well (using soap and water) as microorganisms will quickly multiply on the nutrients that are left on the floor.

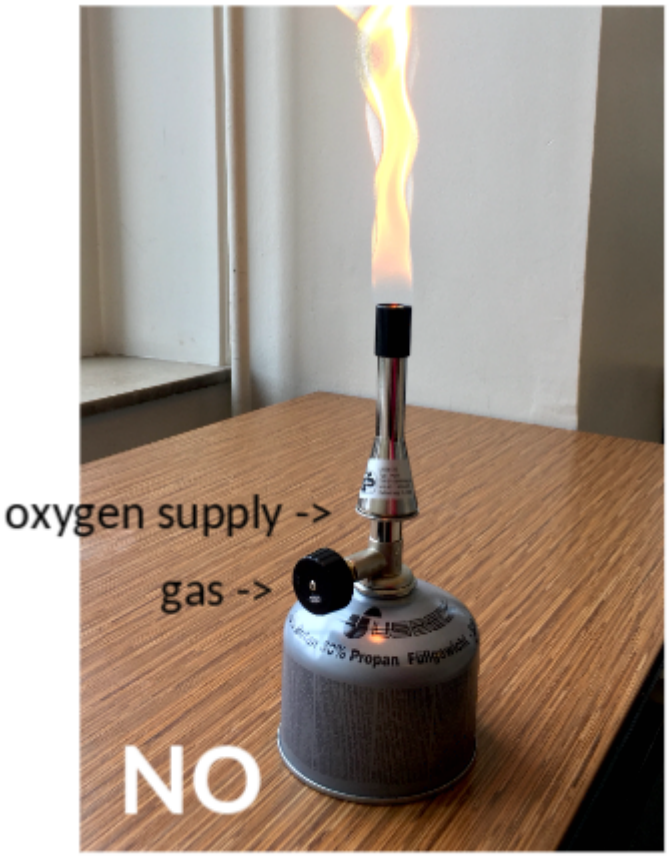
Creating a sterile environment using the Bunsen burner (EN)

Creating a sterile working environment, using the Bunsen burner

Goal: *To give “your” microorganism a head start on "foreign" microorganisms present in the air, on the table or on instruments you use.*

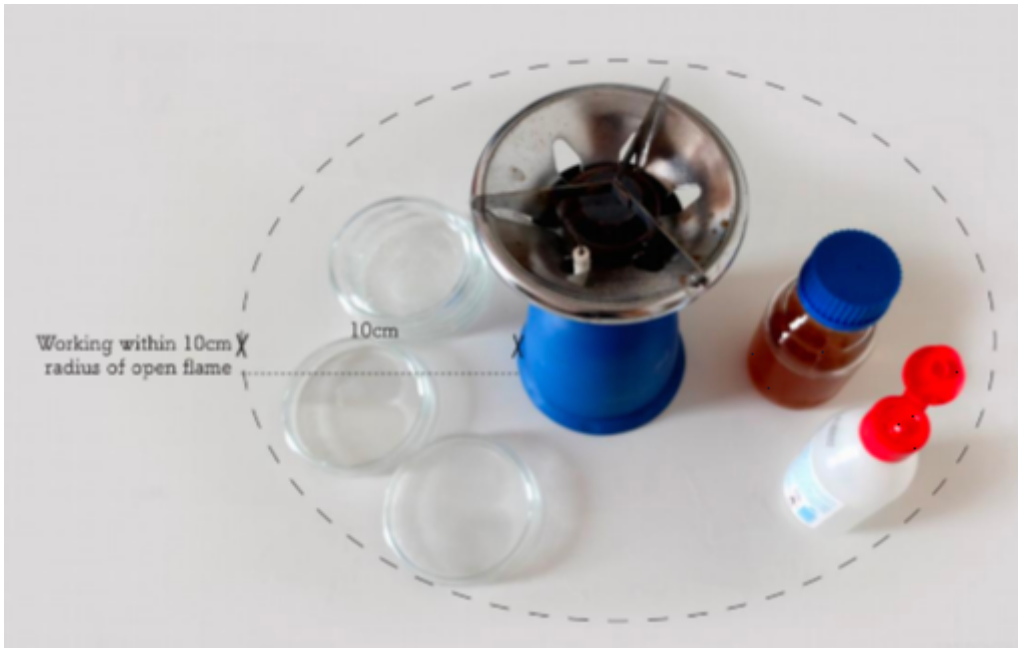
Creating a sterile bubble with a blue 'rustling' flame:

- Close windows and doors and let everyone know you will be inoculating and lighting the flame.
- No talking, no walking around.
- Sit down while working with the flame, to avoid accidentally leaning into the flame.
- Clean the table with 70% alcohol BEFORE you light the flame.
- Open the gas valve and quickly light the yellow flame. Then open oxygen supply ring until you get a noisy blue 'rustling' flame:





- Work within 10 cm radius of flame on all sides:



- Don't wear gloves or synthetic face masks (can glue to skin when hot).
- Point tip of alcohol bottle away from flame at all times!
- Open petri dishes as little as possible, open petri dishes 'oyster style' towards the flame.
- Pass neck of bottle through the flame before and after each pour to sterilize the neck.
- Work fast but don't rush, get comfortable and at all times stay seated.
- Don’t move the gas burner when it's on.
- When finished, close the oxygen supply until you get a yellow flame, then close the gas supply.