

Appendix 2: Basic rules for safe working in microbiological work areas

The basic rules for safe working in microbiological work areas (laboratories, production areas or animal enclosures involving infection experiments) are also known as Good Microbiological Practice (GMP). Apart from GMP, this appendix also describes additional requirements for safe working in a microbiological work area.

The basic rules are applicable to all microbiological work and production areas and at all containment levels, but additional rules may apply to specific levels, organisms or work and production areas. Internal procedures regarding safe working at your organisation are applicable as long as they do not conflict with the rules given below. If necessary, consult with the BSO.

CONTENT

1.	GMP guideline from the KNVM booklet	2
1.1	Work hygiene.....	2
1.2	Work clothes.....	2
1.3	Work organisation	2
2	Special risks and equipment	3
2.1	Aerosols	3
2.2	Examples of high risk devices or materials.....	3
2.3	Centrifuge	3
2.4	Work principle of a biosafety cabinet (BSC) class II.....	4
2.5	Work procedure in a class II BSC	4
2.6	Using a flame in a class II BSC	5
2.7	Particulars when using a class II BSC	5
3	Disinfection and cleaning.....	5
3.1	Disinfectants	6
3.2	Hand cleaning technique	6
4	Storage of biological agents.....	7
4.1	Security of materials and data.....	7
4.2	The storage of BA (including GMO) of containment level I or II.....	7
4.3	The storage of BA (including GMO) of containment level III.....	7
4.4	Labels and indications	7
5	Repair and maintenance	8
6	Accidents and incidents	8

1. GMP guideline from the KNVM booklet

The text in this chapter is almost literally taken from the booklet '*Veilig werken met micro-organismen, parasieten en cellen in laboratoria en andere werkruimtes*', 5th edition (2022), published by the KNVM (www.knvm.org).

1.1 Work hygiene

- Keep the work or production area clean and tidy.
- Prevent vermin.
- Keep personal items outside the work or production area.
- Do not eat, drink or smoke in the work or production area. Do not take any food into these areas.
- Do not keep animals or plants in the work or production area that are not part of the activities.
- Never pipette using your mouth.
- Avoid contact between hands and face as much as possible. Do not apply any makeup or insert contact lenses when in the work area. Avoid loose hair (tie or use a hair cap).
- Do not wear any jewellery, watches etc. in the work area.
- Make sure you have clean hands and short nails.
- Thoroughly clean hands at regular intervals, and at least after completing an experiment and before leaving the room. Use soap and water and a hand disinfectant if necessary. See the chapter 'Hand cleaning technique' in this appendix.
- Disinfect surfaces immediately after contamination and work surfaces after finishing work.
- At the end of each working day, disinfect all work surfaces, including the computer keyboard or tablet screen.
- Avoid using mobile telephones and computer equipment that will be taken outside the work or production area again.

1.2 Work clothes

- Wear closed work clothes, such as a lab coat, of the right size. Long sleeves with a cuff that encloses the wrists properly are recommended.
- Leave the work clothes behind in the work or production area.
- Replace work clothes regularly. Clean used work clothes in good time, if necessary after disinfecting them first, and in the meanwhile store them properly until cleaning.
- Immediately replace soiled work clothes with clean ones.
- Clothing that has been soiled through spillage is disinfected or sterilised before washing.

1.3 Work organisation

- Keep doors and windows shut during the activities.
- First, prepare all activities carefully to avoid unnecessary handling of microorganisms and movement of people, equipment and materials. Prevent cross-contamination.
- Label all materials in which microorganisms are grown and stored with your name, the contents and the date.
- Store microorganisms safely (see point 4).

2 Special risks and equipment

Aerosols and the use of certain devices or materials pose special risks.

2.1 Aerosols

Aerosols pose a special risk due to the potential unnoticed inhalation of microorganisms and the increased chance of infection when inhaling certain microorganisms.

- Handle with care to avoid generating and spreading aerosols.
- Avoid as much as possible any actions that may generate aerosols. If unavoidable, carry out these actions in an adequately contained space with air exhaust, such as a biosafety cabinet (BSC) of at least class II.
- See also the chapters below about using a BSC and a centrifuge.
- When using a pump or vacuum line, it is essential to protect it against released aerosols. For this, see the specific procedure within your organisation.

2.2 Examples of high risk devices or materials

- Water bath (can easily become contaminated and then is itself a dangerous source of infection).
- Vortex and shaking platform (never with open tubes!).
- Pipette (use the pipette responsibly, follow local specifications).
- Injection needle (high risk, avoid, or use adequate protective equipment).
- Glass (can break).
- Centrifuge (see the centrifuge chapter in this appendix).
- Biosafety cabinet (BSC): Although a BSC is an important means of avoiding infection, the wrong use of a BSC may create an increased risk and a false feeling of safety. See the chapters about BSCs in this appendix.

The risk can often be avoided by using another device or other material. Instead of using a water bath, one can use e.g. a thermostatic heating block. Plastic is safer than glass. For details, see the specific procedures within your organisation.

2.3 Centrifuge

Centrifuges may cause laboratory infections by breaking of a centrifuge tube, or by improperly closed or leaking centrifuge tubes.

- Preferably use self-contained centrifuges (with a sealable rotor and/or sealable buckets).
- Use properly sealable and preferably unbreakable tubes or holders and check them before use for cracks or other imperfections.
- Use centrifuge tubes that fit properly in the holder. Tubes that are too small or too big may cause stress in the material, resulting in possible breakage or leakage.
- Before use, check that the centrifuge closes properly (rubber seals).
- Never fill the centrifuge tubes so that the liquid can touch the lid while centrifuging. With angle rotors, the nominal volume is always less due to the sloping position. If necessary, first test with water and indicate the maximum filling height.
- With high rotation speeds, assure proper rotor balance.
- After centrifuging infectious material, open the holders or tubes in a BSC.
- If leakage or breakage is suspected after centrifuging, the organisation's specific procedures before opening the rotor or centrifuge must be followed. If leakage or breakage is found after opening, then specific organisational procedures are also applicable. If necessary, consult with the BSO.

2.4 Work principle of a biosafety cabinet (BSC) class II

When working with biological agents, a class II biosafety cabinet (BSC) offers both product and personal protection. A class I BSC and a cross-flow cabinet only offer product protection, making them unsuitable for use with activities involving biological agents.

Figure 1 shows the working principle of a class II BSC in cross-section. 'Dirty' (non-sterile) air from the work area ('inflow') is immediately drawn underneath the work surface at the work opening. There are two rows of extraction openings on the work surface, one along the front and one along the back. The extracted air is moved through the back of the BSC to the plenum at the top of the BSC, containing an air ventilator. From there, the air is distributed upwards (to the central exhaust of the work area) and downwards towards the work surface of the BSC ('downflow'), in both cases through an HEPA filter.

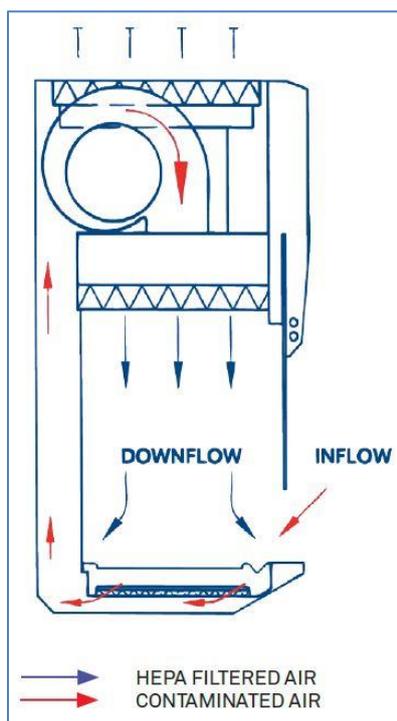


Figure 1. Working principle of a class II BSC (source: Clean Air by Baker brochure).

2.5 Work procedure in a class II BSC

- First, collect the materials required for the experiment.
- Remove the closure panel or slide the window to the 'working position' (this may differ depending on the model) and activate the BSC.
- Ten minutes after activating the BSC, wipe off the work surface and the grilles with 70% ethanol or another suitable disinfectant (in accordance with local procedures).
- Check the operation of the BSC and put the required materials in the BSC, *after* wiping these materials with 70% ethanol.
- Always keep the grilles on the front and back of the work surface free, as well as the work surface under the hands (place materials as much as possible to the left and right).
- Interrupt the airflow as little as possible while carrying out the activities in the cabinet (slow arm movements).
- Prevent disrupting the airflows in the BSC by not walking next to it or opening doors in the room while carrying out the activities.

- During the activities, collect the waste in a container/bag in the BSC, containing a layer of effective disinfectant.
- On finishing the activities, close the waste bag, disinfect the outside and put it in the refuse bin.
- On finishing the activities, disinfect the work surface and the grilles on the front and back of the work surface.
- On ending the activities, allow the fan of the BSC to run for at least another five minutes.
- Switch off the BSC and if necessary, close the front opening.
- Fill in the log of the BSC (depending on local procedures).

2.6 Using a flame in a class II BSC

Using a flame in a BSC causes an enormous disruption of the airflow, so that the effective operation of the BSC can no longer be guaranteed. This is even the case with a small flame, e.g. a pilot light of a Bunsen burner with a foot pedal. For this reason, using a flame in a BSC is strongly discouraged.

If a flame is necessary for certain activities, then it is better to place a Bunsen burner outside the BSC. If it is absolutely necessary to use a flame in the BSC, then a disruption of the safe operation of the BSC and (temporary) loss of containment should be taken into account. A risk analysis is required in this case. Consult with the BSO.

2.7 Particulars when using a class II BSC

- If the BSC ventilation fails (e.g. due to a power cut), the work opening must be closed as quickly as possible. Any materials that are open at that moment and that contain living organisms must be closed first.
- Disinfecting a BSC after contamination ('spill'):
 - Leave the BSC on.
 - Put on gloves, and treat the contaminated surfaces with a suitable disinfectant (partly depending on the organism, see local procedures and/or consult your BSO).
 - Observe the right time for the disinfectant to have an effect and then remove the contaminated material as infected waste.
 - Leave the BSC operational for another 10 minutes.
 - Consult with the BSO in case of large contaminations. If necessary, decontaminate the BSC using gas decontamination.
- To guarantee proper operation, BSCs are tested by a certified company on an annual basis. The removal and replacement of HEPA filters must also be done by an accredited company. After major maintenance, moving the cabinet, replacing filters or big alterations in the room, the BSC's operation must be validated.
- Before carrying out maintenance activities, a risk analysis must be performed to determine whether the BSC first needs to be gas-decontaminated. Consult with the BSO.
- After a large contamination, the BSC must be gas-decontaminated with a suitable disinfectant. Consult with the BSO.
- Keep the BSC inspection reports in accordance with your organisation's quality system.

3 Disinfection and cleaning

Dirt attracts microorganisms and is a potential source of infection. It is therefore essential to keep the work or production area clean and tidy. Apart from keeping clean, disinfection is required depending on the work procedures. After the activities, laboratory staff disinfect the work surfaces and equipment used. For more details, see the specific disinfection procedures within your organisation.

Household cleaning and perhaps also disinfection of rooms where work is done with biological agents can be done by a cleaning firm's employees. In this case, it is important that the cleaner has first received

clear instructions or followed training, has sufficient vaccinations and knows who his/her contact person is (usually the informant). The laboratory and production areas at the USPB site are marked according to risk level: green, yellow or red. For this, see [KAM rule 20 Access to lab and production areas](#).

Specific procedures apply to containment level 3 (red).

3.1 Disinfectants

When working in microbiological work areas, it is essential to use disinfectants.

Disinfect materials that have been in contact with living microorganisms using a validated method and a validated disinfectant. Among other things, this means that it has been demonstrated that (in the concentration used and with the method used) the disinfectant is effective against the applicable microorganisms. In the case of a spill, also pay attention to possible inhibiting substances that may be present in the culture or suspension and that could prevent the working of the disinfectant.

In short, it holds that most chlorine-containing agents are cheap, fast, work effectively and are effective against almost all microorganisms except spores. However, chlorine is corrosive. Alcohols have a narrower range of action, and are not effective against some enveloped viruses, bacterial spores and viruses without a membrane (naked viruses).

When using a disinfectant, also make sure that it has been approved in accordance with Dutch legislation. This can be verified using the approval database and on the website of the Dutch 'College voor de Toelating van Gewasbeschermingsmiddelen en biociden' (CTGB) (www.ctgb.nl). Biocides may also be permitted in the Netherlands on the basis of a permission elsewhere in the European Union. Consult the European ECHA database (<https://echa.europa.eu/nl/information-on-chemicals/biocidal-products>) if the Dutch database with approved biocides does not provide any results.

3.2 Hand cleaning technique

Along with wearing gloves, washing the hands after carrying out activities is an important measure in preventing microorganisms from spreading via an employee who has carried out work with biological agents. To be effective, it is important to wash the hands carefully. See the actions below and the illustration at the sink (if applicable).

In general, it is sufficient to wash the hands carefully with soap and water. Depending on the organism, the specific activities or local requirements, you can also disinfect the hands with a liquid hand sanitizer, usually after washing with soap and water. A hand sanitizer is not a replacement for washing the hands. Before use, verify that the disinfectant is suitable in combination with the agent used and whether it has approval for use as a hand sanitizer on the Dutch market in the approval database of the Ctgb (www.ctgb.nl).

Actions:

- Open the tap (using elbow or foot operation) and ensure a copious flow of water at a temperature that is pleasant for the hands.
- Thoroughly wet the hands and then apply a layer of liquid soap from a dispenser (preferably with elbow or foot control).
- Now rub the hands over each other thoroughly for 10 seconds. Rub the fingertips, thumbs and spaces between the fingers and wrists properly.
- Rinse the hands carefully with water.
- Dry the hands, wrists and between the fingers properly with a disposable towel.

- Shut the tap with the elbow or with the disposable towel.
- Throw the towel in the designated container.

4 Storage of biological agents

Rules may be laid down in specific procedures within your organisation for the storage of biological agents (BA, including GMO) in laboratory and/or production areas. This appendix contains the basic rules in accordance with laws and regulations.

4.1 Security of materials and data

Within the framework of biosecurity, it is desirable to store materials (in particular pathogens) that could be used by malicious parties for undesirable purposes under secure conditions. This means that this material must be stored in a locked space that only authorised staff can access.

Security within the framework of biosecurity also applies to the data about the biological materials. This means that only authorised staff may have access to the names of the pathogens and the storage locations. Detailed information about biosecurity can be found on the website of ['Bureau Biosecurity'](#).

4.2 The storage of BA (including GMO) of containment level I or II

The rules below are applicable to the storage of GMO originating from containment level I or II. These rules are also applicable to non-GMO level 1 and 2 BA, unless stated otherwise.

- Storage is done in designated and suitable spaces (for GMO, this must among other things form part of the defined GMO area).
- Storage is in a locked space under conditions where no multiplication or transfer of genetic material can take place.
- The packaging consists of a closed, unbreakable, leak-tight, double holder. A double holder is not mandatory for non-GMO BA that is classified at level 1 or 2.
- If GMO and non-GMO are stored in the same location, then they must be packaged separately.
- The GMO registration and storage location must be known to the BSO. This is not mandatory for non-GMO BA.

4.3 The storage of BA (including GMO) of containment level III

The following rules are applicable to the storage of BA (including GMO) originating from containment level 3 (or III), *on top of the above rules for level I and II*:

- Storage must exclusively take place in a room with containment level 3 / III.
- A closed, unbreakable, leak-tight *double* holder is always mandatory for BA (including GMO) of risk category 3.
- The registration and storage location of all BA (including GMO) of risk category 3 / III must be known to the BSO.

4.4 Labels and indications

The following is applicable to labels or indications on BA packaging (including GMO):

- The outside holder in which GMO (of all containment levels) is packaged is clearly identifiable as a unit by means of labels marked GMO.
- The packaging of microorganisms that are classified in risk category 2 or 3 must also be labelled with a biohazard symbol.
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- It is often impossible (e.g. too little room) or undesirable (biosecurity) to apply content information on the packaging. However, by using the information on at least the outer holder, in combination with a proper registration/administration, at least the following must be traceable:
 - The origin or source of the material.
 - The contents of the material (description of the BA and type of content).
 - The owner of the material (who bears the responsibility for it), preferably a name of a person and the relevant department.

5 Repair and maintenance

In principle, microbiological work areas are only accessible to trained staff and not to maintenance or technical staff. In principle, access to such personnel is only possible after release. Neither is it possible to simply remove equipment from microbiological work areas; this may only be done after disinfection. In

the case of essential maintenance or repairs in microbiological work areas or to devices in these areas, [KAM rule 16 'Risk Declaration'](#) must be followed.

6 Accidents and incidents

- If an accident or incident occurs, the specific organisation rules are followed.
- The organisation must have specific procedures for clearing up and decontaminating a spill, for actions to be taken after the release (spread) of pathogens and GMOs, for treating (injection) accidents and for dealing with (a suspicion of) a work-related infection.
- For the rules concerning reports of accidents and incidents, see KAM-13-GMO and KAM-13 non-GMO. If necessary, consult with the BSO.