


# National Public Health Laboratory

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## 1. Working Safety in the BSL-3/MLII Laboratory

## 2. Objectives & Scope

This SOP describes safe working practices for the BSL3/MLII laboratory.

## 3. Abbreviations and definitions

- BSL Biosafety Level
- BSO Biological Safety Officer
- EO Equipment Officer
- GMO Genetically modified organism
- LF Laminar flow hood
- LM Laboratory Manager
- LT Laboratory technologist
- ML Micro-organism laboratory classification level
- QM Quality Manual
- SHW Specific hospital waste
- UV Ultraviolet light
- RC Research Coordinator
- RE Responsible employee

Category-3 micro-organisms: micro-organisms which can cause serious human illness with a high probability of contagious spread under the population for which an effective prophylaxis, treatment or remedy exists.

BSL3 is the classification level of control measures required for laboratories working with category-3 micro-organisms: these measures are described in Annex V of European directive 2000/54/EG L 262/21.

MLII is the classification level of control measures for laboratories working with GMOs as determined by the Ministry of Housing, Spatial Planning and the Environment (Department for GMOs).

Responsible employee: the employee responsible for the daily supervision of the GMO activities, for preparing GMO work protocols and for ensuring that they are implemented.

For general abbreviations, definitions and terminology, refer to QM1 "General".

## 4. Tasks, responsibilities and accountabilities

For general authorizations refer to the Authorization Matrix.

Task	Authorized	Responsible
Authorisation of competences for new employees and administration of P12 form 01 "Checklist for initial training of employees at the BSL3/MLII laboratory"	BSO	BSO
Supervising the work activities in IA3-183	Person responsible for the IA3-183 lab	BSO
Supervising the work activities in IA3-181	Person responsible	BSO

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	for the IA3-181 lab	
Preparing the cleaning schedule	Appointed LT	BSO
Monthly cleaning of the BSL3/MLII laboratory	All persons mentioned in cleaning schedule	BSO
Providing access to the BSL3/MLII laboratory	BSO	BSO
Overall safety of the BSL3/MLII laboratory	BSO	BSO
Facilitating safety in the BSL3/MLII laboratory by making the necessary resources available	LM	LM
Providing information, vaccinations and check-ups on employees	BSO	LM
Regularly monitoring compliance with safety regulations in the BSL3/MLII laboratory	BSO	BSO
Supervising compliance with the classification level and requirements specified by the Department of GMOs for project related GMO activities	RE	BSO
Implementation of laboratory activities in accordance with the requirements specified for the activities	Everyone with access to BSL3/MLII	BSO
Annually changing the access code for the BSL3/MLII laboratory	TO	BSO
Administering the BSL3/MLII laboratory logbook	BSO	BSO
Equipment maintenance	EO	LM

Also refer to P12 form 01 "Checklist for initial training of employees at the BSL3/MLII laboratory."

## 5. Procedure

For the general safety rules, refer to the Biosafety Manual. Immediately stop the work when contact with a class 4 microorganism is suspected and inform the BSO.

### 5.1 General

#### 5.1.1 Entry regulations

Entry to the BSL3/MLII laboratory is secured via a combination code. The code is provided by the BSO to persons authorised to access the laboratory. The code is changed annually. Persons who are authorised to enter the BSL3/MLII laboratory receive initial training in accordance with the checklist P12 form 01 "Checklist for initial training of employees at the BSL3/MLII laboratory".


#### Activities during office hours

When activities are carried out, a minimum of two persons must be present in the BSL3/MLII laboratory. If it turns out that this is not possible, then the person concerned must report in to a colleague upon commencing his/her activities and must also report out after completing them.

#### Activities outside office hours

Activities can be carried out outside office hours (including weekends) in consultation with and after approval of the RC or RE. The person concerned must report to the Hospital Security (tel. 888) upon commencement and upon completion of the activities.

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Before commencing the activities in the laboratory, the logbook must always be filled in with the name, time and nature of the activities and organism involved.

## 5.1.2 Clothing regulations


- The clothing reserved especially for working in the laboratory (green lab coats with a solid water-repellent front) must always be worn when entering the specified areas but should never be worn outside these areas.
- It is not permitted to wear any ornaments/jewellery on the arms or hands or open shoes.
- Always wear gloves during work in the Laminar Flow hood.
- Wear a "Filtermask P550" surgical mask and safety goggles in case of a calamity in which aerosol formation or dispersion of organisms might have occurred.
- When working with chemicals that present an explosion hazard or which can release hazardous vapours, always work inside the fume cabinet in laboratory IA3-183 and wear a surgical mask and safety goggles.
- Using personal equipment (e.g. cell phones) is prohibited.

## 5.1.3 Working procedure

1. All activities involving category-3 pathogenic organisms and materials which may possibly contain category-3 pathogenic organisms (such as diagnostic material) must be carried out in the class 2 Laminar Flow hood (see 5.1.4 "Use of Laminar Flow hood (LF)").
2. Sterilize the materials a.s.a.p.
3. Avoid the use of sharp objects whenever possible.
4. Make sure the doors of the working area are closed during your work.
5. Before using them, check all centrifuge and other tubes and culture containers for any defects. Materials that are defective or in poor condition should never be used. Dispose of such materials in the blue container specifically reserved for hospital waste, in accordance with P09 Waste Segregation and Disposal.
6. Label all cultures, reagents, plates, plastics, etc. clearly with your name, date and contents. If it is not clear what it is and/or who the owner is it may be removed by the lab responsible person.
7. It is obligatory to register on the form at the shake incubator which cultures are grown and by whom.
8. When using the centrifuge, use only centrifuge tubes that can be properly closed.

Clean reusable centrifuge tubes as follows:

- Cook for 15 min in water;
  - Rinse overnight in 0.1 N HCl;
  - Rinse thoroughly with Milli-RO water;
  - Allow to air-dry;
  - Do not use any alcohol or other solvents, as these damage the tubes.
9. Never centrifuge at speeds above the maximum allowable speed (rpm) for the tubes (in case of doubt, consult the manufacturer).
  10. Seal off material with pathogenic organisms in a leak-proof fashion, disinfect with 70% ethanol, and attach a descriptive label (with name of organism, quantity, name technologist, date) before removing any such materials from the Laminar Flow hood.
  11. Store infectious materials in the BSL3/MLII laboratory. Only disinfected materials may leave the laboratory.
  12. Inactivated pathogens class 3 may only leave the BSL3/MLII laboratory if they are packed according to P620 regulations (see P33 "Sending of (infectious) biological material").
  13. Installed equipment should stay in the BSL3/MLII laboratory. Temporarily installed equipment must be thoroughly disinfected before leaving the laboratory and only with written release statement from the BSO. This must also be done with permanent removal of equipment.


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## 5.1.4 Use of the laminar flow hood (LF)

1. In principle, the LF can be used by only one person at a time. If the nature of the work demands it, a maximum of 2 people may work at the same time in the hood but only after receiving permission from the BSO.
2. Switch on the LF 5 minutes before starting to work in it.
3. Apply 70% ethanol on a tissue and disinfect all materials and consumables before placing them in the hood.
4. Place a yellow waste container with an autoclavable waste bag in the LF.
5. Used disposables must be discarded in the waste bag. After use, close the waste bag with autoclave tape. Autoclave the bag and discard it in the blue waste container with yellow lid.
6. If applicable, liquid cultures and possible infected fluids must be disposed of in a leak proof container. Close the container, autoclave it and discard it in the blue waste container with yellow lid.
7. Minimise disturbance of the air circulation within the LF via the following measures:
  - Place only the materials strictly necessary for the experiment inside the LF and, whenever possible, towards the rear;
  - Limit the use of a gas flame to a minimum;
  - Minimise the use of glass or metal materials that need to be annealed by using plastic disposables instead whenever possible;
  - Do not use any equipment that generates heat;
  - When using a centrifuge in the LF, do not carry out any other activities in the LF.
8. Whenever possible, carry out the work activities as far as possible towards the rear of the LF; always maintain a minimum distance of 15 cm from the front side.
9. Slide the protective glass partition of the LF down to the level indicated on the LF.
10. Keep your hands inside the LF whenever possible in order to avoid the spread of aerosols.
11. After completing the activities, close the autoclavable waste bag.
12. After completing the activities, disinfect the working surface and the materials used with 70% ethanol and empty out the LF. **Make sure that the gas has been shut off before working with ethanol.**
13. After the disinfection activities, switch on the UV lamp and leave it on until it automatically switches off.
14. The LF may be used by another employee only after decontamination has been completed (step 12).

## 5.1.5 Glass ampoules with freeze-dried infectious material

1. In principle, glass ampoules are not used. However, sometimes there is no alternative.
2. Always open the ampoule in an LF, as there may be a slight overpressure inside the ampoule as a result of which the contents may be dispersed in the atmosphere upon opening.
3. When removing ampoules stored at a temperature below 0°C from the freezer, you must always wear a "Filtermask P550" surgical mask, safety goggles and gloves. The sudden change in temperature may cause the glass to break.
4. Decontaminate the outside of the ampoule by wiping it down with a tissue in 70% ethanol.
5. Use a glass file to score the neck of the ampoule in such a spot that it will be easy to hold the top part of the neck when breaking it off.
6. Heat a glass rod in a Bunsen burner and hold the heated glass rod against the nick mark on the neck of the ampoule.
7. Take a piece of cotton wadding and use the wadding to hold the ampoule with both hands.
8. Break the neck of the ampoule and treat the broken off piece of the neck and the cotton wadding as contaminated material.
9. Gradually add the reconstitution liquid to avoid dispersion of the freeze-dried material.
10. Transfer the reconstituted material to a bottle that can be effectively sealed/closed.

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11. Treat the empty ampoule as contaminated material.


## 5.1.6 Autoclaving

1. Place all possible contaminated waste material in an autoclave bag, seal it with sterilisation tape, and write your name and the date on the tape.
2. Place the sealed bag in a metal bucket or in the bigger bucket; the buckets can be found in rooms IA3-181 and IA3-183
3. Apply autoclave tape on the autoclave bag, and write your name and the date on the tape (in case of several users, list all the names).
4. Place the bucket next to the autoclave for the next autoclave run. Carry out an autoclave run when the autoclave can be completely filled or if the waste material would otherwise remain next to the autoclave for more than 1 day. N.B.: the person wishing to autoclave material is responsible for the material until autoclaving has taken place.
5. Check whether the autoclave can be used and is not already in operation: the text 'open' should be visible on the display.
6. Check the water level in the plastic bottle. The level should be between minimum "low" and maximum "high".
7. Switch on the autoclave and move the handle towards the right, i.e. the 'unlock' position.
8. Check the water level inside the autoclave. The water level should be just above the grating. If necessary, add demineralised water.
9. Place the buckets inside the autoclave.
10. Close the autoclave by moving the handle to the left, after which 'lock' should appear on the display.
11. Select the programme using the 'mode' knob:
  - Programme 1: Large volumes of liquids
  - Programme 2: Waste
  - Programme 3: Lab coats (Duco)
12. Press start.
13. The autoclave may be opened only after the temperature has dropped to 50°C or less.
14. Remove the buckets from the autoclave, check the autoclave tape for black stripes. If that is not the case, check everything once again (water level, switching on the equipment) and autoclave again. If the second attempt at autoclaving is also not successful, notify the EO.
15. Discard the contents in the impact-resistant plastic blue waste container (with yellow lid) especially reserved for hospital waste, but do so only if black stripes are visible on the tape.
16. Rinse the buckets with tap water and allow them to dry before being used again.

## 5.1.7 Actions after the work has been done

1. Decontaminate all work surfaces as well as equipment (pipettes etc.) used with an ample quantity of 70% ethanol or disinfectant, like halamid (chloramine-T) and chlorhexidine solution.
2. Inactivate contaminated liquids and processed samples via autoclaving (see 5.1.6 Autoclaving).
3. Disinfect possible infected glasswork via autoclaving. After autoclaving, thoroughly rinse and clean the glasswork with tap water and then take it to the central washing room for thorough cleaning.
4. Bring non infected dirty glasswork in the dedicated box to the kitchen for thorough cleaning.
5. Disinfect any contaminated disposable materials by autoclaving them in sealed autoclave bags (see 5.1.6 Autoclaving).
6. Close off the blue container after it is full, wipe the outside with 70% ethanol and fill out the label on the container clearly and completely.

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7. Place the container next to the goods elevator and pick up a new one in the entrance way to the darkroom.
8. Make sure that there is always a freshly prepared stock of disinfectants in the laboratory, i.e. 70% ethanol.
9. Wash your hands and write the time of your departure (in the logbook) when you have completed your activities and leave the area.

## Cleaning the BSL-3/MLII laboratory

1. All employees working at the BSL-3/MLII lab are required to comply with the cleaning schedule and to carry out the scheduled activities. This schedule can be found above the logbook in working area IA3-179 and in the following file:  
D:\NPHL\Laboratory files\BSL-3
2. The clothing reserved especially for the BSL-3/MLII laboratory must be cleaned at least once a month. The lab coats must be autoclaved in sealed plastic bags reserved especially for that purpose before being sent to the linen department in a white plastic bag (max 10 lab coats per autoclave bag).
3. All cultures that cannot be traced to the owner will be disposed of.

## 5.2 Calamities

In case of a calamity involving a pathogenic organism, observe the following guidelines:

### Calamities can involve the following:

- Needle-, cut-. or other accidents while working with a pathogenic organism;
- fire;
- explosion;
- spillage etc. involving organisms or chemicals.

In case of fire or explosion, act in accordance with the "Fire prevention" instructions listed next to each telephone in every area.

### **In case of every calamity involving a category -3 organism:**

**IMMEDIATELY TELEPHONE 888**

Act in accordance with the instructions on the door poster: "What to do in case of a biological calamity"

1. **PREVENT** calamities by thoroughly familiarising yourself with the applicable rules and procedures, such as those described in this SOP, before starting your work activities.
2. Observe the rule never to work alone in the BSL-III laboratory. In case of an incident or calamity, someone will then always be present who can assist in taking the necessary actions.
3. In case of a calamity, act as quickly as possible in order to minimise the risks for yourself and your colleague(s).
4. Stay in the laboratory in case it is suspected that cloths are contaminated. Call for help. Replacing cloths are available in the ante-room.

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The "Central Control Room" and the BSO or LM will act together to mobilise and inform the appropriate authorities/persons, and follow-up steps will be taken.

5. Record every calamity with a pathogenic organism in the logbook.

### 5.3 Incidents

If an incident occurs involving a pathogenic organism, then the guidelines listed below should be followed:

Incidents comprise the following:

- contamination not involving penetration of the skin;
- spillage of liquid containing the pathogenic organism
- breakage of glasswork leading to spillage of the pathogenic organism.

In case of skin contamination whereby the skin is not penetrated:


1. Dab the skin for a period of 2 minutes with a 0.5% solution of chlorhexidine in 70% ethanol.
2. Cover the spilled liquid with an ample quantity of tissue paper that has first been thoroughly soaked in 2% Actisan.
3. Collect the tissue paper in plastic bags, seal the bags, autoclave them, and place them in one of the blue containers present in the laboratory.
4. Wipe the surface on which the spillage took place thoroughly with a 1% solution of hypochlorite and then with 70% ethanol and/or disinfectant. Carry this out while wearing a surgical mask and gloves.
5. Prevent any skin penetration from occurring when a glass tube or container breaks and pathogenic organisms are spilled. First remove the pieces of glass with tweezers; place the pieces of glass in one of the blue containers present in the lab after first placing a layer of tissue paper soaked in 2% Actisan in the container. After removing the glass, clean the working surface as described above under 2.
6. After first taking the necessary actions to limit the spread of the pathogenic organism, contact the biological safety officer (8158922 or 65447). Do not leave the laboratory before this officer or his replacement has arrived at the lab and has decided on a further course of action.
7. After the incident has been reported by telephone to the BSO (8158922 or 65447), the latter will initiate the following procedure as quickly as possible:
  - reporting to the "Central Control Room" of the hospital via telephone number 888;
  - reporting to the LM or his replacement.

The "Central Control Room" and the BSO or LM will act together to mobilise and inform the appropriate authorities/persons.

8. Record every incident or accident with a pathogenic organism in the logbook.



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## 6. Related Documents

- QM1 General
- P09 Waste Segregation and Disposal
- P33 Sending of (infectious) biological material
- List of persons authorised to enter the BSL-3/MLII areas: posted on the door of the entrance way and accessible at D:\NPHL\Laboratory files\Safety\BSL-3;
- Logbook ML2BSL3, D:\NPHL\Laboratory files\Safety\BSL-3
- Environmental handbook: available in every laboratory area and in IA3-150 (secretariat), IA3-224 (KF), IA3-152 (administrative dept.) and IA3-189 (technical workshop)
- Cleaning schedule for BSL3/MLII laboratory:  
N:\NPHL\Laboratory files\BSL-3
- Safety manual: areas IA3-153 (BSO), IA3-150 (secretariat), IA3-149 (LM) and IA3-151 (HAZ)
- Door posters "What to do in case of a biological calamity"

## 7. Related Forms

- P12 Form 01 "Checklist for initial training of employees at the BSL3/MLII laboratory", completed for each employee, area IA3-153 (BSO)

## 8. References

- European directive 2000/54/EG L 262/21 of the European Parliament and the Council of Europe Laboratory biosafety manual
- Information on biosafety and GMOs: <http://bggo.rivm.nl/>
- Updated information on each infectious disease: <http://www.rivm.nl/cib/>
- Packing instructions: <http://www.abc.com>
- Guidance on regulations for the Transport of Infectious Substances, WHO  
<http://www.who.int/ihr/publications/>

## 9. Attachments

N/A