MUNI | RECETOX

Appendix 2: Laboratory Rules for Working with Biological Material

Director's Directive 3/2019

RECETOX Operating Regulations

Preliminary Provisions

1. The rules described in this Appendix to the RECETOX Operating Regulations apply to all RECETOX laboratories.
2. Any changes to this Appendix are subject to approval by the Brno Regional Hygiene Station.

General Information

Description and Focus of the Laboratories

The RECETOX laboratories analyse environmental and biological samples and study the pollutants in the environment and in biological matrices.

Location and layout of the laboratories

Location: The laboratories are located within the University Campus in Brno-Bohunice in building A29, street address Kamenice 753/5, and in building INBIT, street address Kamenice 771/34.

Layout: In building A29 (commissioned in 2013), the laboratories are located across four floors. The environmental processes and toxicology laboratories are located on the 1st basement floor and 1st (ground-level) floor. The Trace Analysis Laboratories are located on the 2nd floor. These laboratories are separated by a glass partition and are subject to restricted access rules. The ecotoxicology laboratories are located on the 3rd floor. The building provides appropriate sanitary facilities.

In building INBIT (commissioned in 2008), the laboratories are located on the 1st basement floor and on the 3rd floor. The

laboratory for processing biological material and the isolation laboratory are located on the 1st basement floor. The metabolomics laboratory is located on the 3rd floor along with the MELISA laboratory, two microbiome laboratories (pre- and post-PCR) and a room with deep freezers for storing samples.

The laboratory working hours are from 6 a.m. to 10 p.m. on working days.

Description of Biological Material Samples

Biological material is material of environmental and biological origin that comes from humans, other organisms or parts of the environment (the "biological material"). Since biological material could contain pathogens, it is a potentially infectious material and must be treated as such. The laboratories primarily analyse the following biological material:

- Human and animal samples (fat, muscle, brain, skin and liver tissue; blood and blood derivatives; hair; buccal swabs etc.);
- Human and animal secretions and excretions (breast milk, urine, stool, sperm etc.);
- Environmental samples (biological waste, wastewater, samples taken from polluted watercourses, soils and sediments etc.).

Monitored and Analysed Groups of Substances

Persistent Organic Substances

Organochlorine pesticides, cyclodiene pesticides, polychlorinated biphenyls, polychlorinated biphenyls with dioxin-like toxic effects, polychlorinated dibenzo-p-dioxins and dibenzofurans, polybrominated diphenyl ethers, new brominated flame retardants, hexabromocyclododecanes, organophosphorus flame retardants, polyaromatic hydrocarbons etc.

Polar Organic Substances

Polar pesticides, perfluorinated substances, pharmaceuticals, personal care products, sweeteners, alkylphenols, steroids etc.

Metals

Metals. The laboratories also conduct speciation analyses.

Biomarkers and Biological Macromolecules

Nucleic acids (DNA and RNA), proteins, metabolites, low molecular weight biomarkers etc.

Division of Laboratories Working with Biological Material According to the Tasks Performed

Sample Receipt and Inventory Records

Building A29

- Chemical laboratories – laboratory no. 226 and 1S09
- Toxicology laboratories – laboratory no. 325

Building INBIT

- Samples for microbiome analysis – laboratory no. 0.21
- Samples for metabolome analysis – laboratory no. 3.24
- Samples for further processing and storage in the biobank – laboratory no. 0.22

Sample Storage

Building A29

- Temperature controlled freezers – room no. 1S29 and 213
- Cryogenic storage dewars (-196°C) – room no. 1S29

Building INBIT

- Freezers (-20°C) – laboratory no. 0.21, 0.22 and 3.24
- Freezers (-80°C) – laboratory no. 3.21, 3.24

Building A1

- Freezers (-20 °C) – laboratory no. S217a
- Freezers (-80 °C) – laboratory no. S217a

Sample Preparation for Analysis

Building A29

- Drying, freeze-drying, homogenisation, fat determination etc. – laboratory no. 226
- Extraction, gel chromatography, column adsorption chromatography – laboratory no. 216, 222, 224, 225, 228 and 1S09
- Ecotoxicological testing – laboratory no. 325
- Student laboratories – laboratory no. 114, 115, 118

Building INBIT

- Isolation of biological macromolecules (DNA, RNA) – laboratory no. 0.21 and 0.22
- Separation of blood fractions and elements – laboratory no. 0.22 and 3.25
- Processing, fractionation and aliquoting of biological material (venous and cord blood, urine, buccal swabs, stool, saliva, dried blood spot) – laboratory no. 0.22
- Quantification of proteins – inflammation markers, gangliosides and metabolites (homogenisation, extraction, trypsinisation, alkylation, vacuum drying, weighing etc.) – laboratory no. 3.24
- Quantification of proteins – inflammation markers, gangliosides and metabolites (protein reduction, spectrophotometric quantification of proteins etc.) – laboratory no. 0.21

Sample Analysis

Building A29

- Instrumental methods of chemical analysis – GC-MS, GC-MS/MS, GC-HRMS, HPLC-MS, HPLC-MS/MS, ICP-MS, (GC/HPLC)-ICP-MS, AAS, UHPLC-MS – laboratory no. 1S05, 1S18, 1S21, 1S31, 214, 229, 231, 232, 233 and 325
- Other types of analysis (biomarkers) – laboratory no. 315, 316, 321, 323, 324 and 325

Building INBIT

- Analysis of biological macromolecules (DNA, RNA) – laboratory no. 0.21, 3.22, and 3.23
- Biochemical and immunochemical quantification of biomarkers in full blood, plasma, serum and urine – laboratory no. 0.21
- MELISA – detecting hypersensitivity to heavy metals and other allergens (chemicals, toxins, moulds etc.) – memory lymphocyte immunostimulation – laboratory no. 3.25

Process Experiments

Building A29

- Distribution balance – laboratory no. 1S09 and 1S11

Statutory Requirements

According to Government Regulation No. 361/2007 Coll., the minimum measures for ensuring occupational health and safety, detailed hygienic requirements and signage at the workplace, detailed requirements for work practices and health and safety information concerning tasks that could expose employees to biological agents and endanger their health must include the following:

- No eating, drinking and smoking in a workplace where there is a risk of contamination by a biological agent. Staff must not leave the designated workplace while wearing personal protective equipment.
- The sanitary facilities provided must correspond to the nature of the work.
- Personal protective equipment must be provided.
- Personal protective equipment must be stored in a designated area, checked, cleaned and disinfected, preferably before each use but always after each use. Defective personal protective equipment must be repaired or substituted before it is used again.
- An authorised employee must prepare guidelines for safe collection, handling and processing of human or animal samples, which are then approved by their superior.
- Personal protective equipment that may be contaminated with a biological agent must be removed. Prior to decontamination, cleaning or disposal, personal protective equipment must be stored separated from street clothes.
- Vaccination, when advisable, particularly for employees who are not immune to the biological agent that they are, or could be, exposed to at work.
- Employees must be informed of any incidents involving the handling of biological agents.

If the processing and/or analysis of biological material listed above in the "Description of Biological Material Samples" section of this Appendix to the Operating Regulations could involve any risk of the exposure of employees to biological agents classified as risk group 2, 3 or 4, the measures taken to protect the health and safety of employees must also include the following:

- Minimising the number of employees exposed or probably exposed.
- Adapting the work practices and technical protective measures designed to prevent or minimise the release of the biological agent into the environment at the workplace.
- Using personal protective equipment, if there is no other way of preventing the exposure of the employee to the biological agent.
- Maintaining hygiene practices designed to prevent or minimise accidental transmission or the release of the biological agent outside the workplace.
- Conducting tests to detect any presence of the biological agent used at work outside the closed system, if necessary and practicable.
- Providing resources for easy collection, storage and disposal of waste in a safe and identifiable bin, which can also be specially adapted for the purpose.
- Adjustments required for the safe handling and transport of the biological agent at the workplace.

General Measures

- Access to laboratories processing biological material is only permitted for authorised and duly trained persons.
- Performing tasks that involve biological material is only permitted for duly trained persons authorised to work with such material.
- Regular annual re-training and training of new employees are provided by an employee authorised by a RECETOX manager, who is qualified for working with potentially infectious material.
- Only persons vaccinated against hepatitis B can be authorised to work with biological material. New employees/students must submit a vaccination certificate (of at least two doses of the vaccine), a recent hepatitis B antibody screening test (HBsAg antibody titre over 10 IU/l) or a medical certificate for proven previous infection with hepatitis B.

Employees/students not vaccinated against hepatitis B will not be allowed to work with biological material until they complete the vaccination (after the second dose of the vaccine at the earliest).

When working with biological material, the persons authorised to work with biological material must follow the guidelines below to prevent contamination and the emergence and spread of infections:

- No eating, drinking and smoking in the laboratories.
- Sterile supplies and instruments must be handled aseptically.
- The instruments and the environment must be decontaminated in accordance with the Laboratory Operating Procedures.
- The recommended personal protective equipment according to the nature of the task must be worn at work: trousers, shirt, laboratory coat, shoes, single-use gloves, face masks.
- Staff must not leave the designated workplace while wearing personal protective equipment.
- Samples of biological material can only be received for analysis in standardised containers and decontaminated crates.
- Samples of biological material can only be received if accompanied by a duly completed protocol or dispatch note that includes, but is not limited to, the following information: description of the material (type of the sample), amount, origin, place and date of collection, mode of transport and storage, method of disposal and potential infection risks and other hazards.
- Samples of biological material must be moved between different laboratories so that they do not degrade due to physical influences and so that there is no threat to people.
- All waste (including gloves) produced in the laboratories while working with biological material is considered infectious and is placed in suitable labelled containers. The waste is separated according to the type: general infectious solid waste, infectious liquid waste, infectious sharp waste – glass and infectious sharp waste – metal (scalpels and needles).
- Single-use gloves must be used when handling biological material. After removing the gloves, staff must wash their hands with soap, dry them thoroughly with paper towels and disinfect them using the recommended no-rinse hand sanitiser.
- After finishing work in the laboratory, all the work surfaces (on benches and boxes) must be wiped with a disinfectant (ethanol-petrol blend, ethanol, commercial disinfection etc.).
- Only supplies that are designated for working with biological material may be used when preparing samples. Work surfaces must be designated according to the nature of the task.
- If necessary, the benches that would otherwise come in contact with biological material must be covered only with single-use supplies (aluminium foil and paper towels) and the cover changed after each series of experiments.
- Centrifugation: When centrifuge rotors or individual rotor adapters are fitted with a closing lid, the lid must always be in place during centrifugation. If there is no lid, the centrifuges must be placed in a box/fume hood or in a separate room. The tubes must always be closed properly before being placed in the centrifuge. If a tube is damaged in the

centrifuge, this can produce a large amount of aerosol. Sudden stopping of the centrifuge can also produce aerosols. In these situations, the necessary steps must be taken to prevent exposure of employees to the aerosol by using suitable personal protective equipment to protect the respiratory system. This personal protective equipment must also be used while the centrifuge and surrounding area are decontaminated.

- Only single-use plastic supplies (pipettes, tips, Pasteur pipettes, tubes etc.) may be used when working with biological material. These supplies are placed in their original packaging after use and then in strong waste bags or suitable plastic containers. After finishing the task, the bag or container is securely closed. Full bags and containers are placed in the appropriate waste bins.
- Glassware and laboratory instruments used for processing biological samples must be decontaminated immediately after use, then sterilised and stored separately from other laboratory glassware and instruments.
- Single-use instruments must never be used repeatedly, even if sterilised.
- It is recommended to remove any jewellery from your hands.
- In the case of infectious disease, the necessary steps must be taken to prevent an epidemic. If required by legislation, the presence of an infectious disease must be reported to the public health protection authority (Regional Hygiene Station of the South Moravian Region in Brno).
- In the case of an incident in the laboratory (serious injury, skin or mucous membrane contamination etc.), the employees follow the instructions outlined in the OHS fact sheet, which is available in each laboratory.

Hand Hygiene Guidelines When Working With Biological Material

Hands are washed with soap and hot water. No-rinse hand sanitisers are used for hand disinfection.

Hand Washing

When:

- Before and after any contact with biological material, including accidental contact.
- After contact with surfaces and objects (that are in direct contact with the biological material).
- After removing gloves of any kind.

Steps:

1. Wet hands with water.
2. Apply soap and rub hands to spread the soap for at least fifteen seconds.
3. Thoroughly wash the palms and backs of the hands up to the wrist including the areas between the fingers.
4. Rinse hands thoroughly with water.
5. Use your elbow to turn off the water faucet.

6. Dry hands with a paper towel.

Total time: 40–60 seconds

Hand Disinfection

While working with biological material, the hands are disinfected whenever necessary. The hands are always disinfected after completing a task that involved handling potentially infectious material and removing your gloves. Hand sanitisers are always applied to dry hands.

Steps:

1. Apply a sufficient amount of the sanitiser (according to the manufacturer's instructions) in a cupped hand.
2. Keep the hands wet for the whole duration of the sanitising process.
3. Repeat each movement five times in no fixed order:
    - Rub the hands palm to palm.
    - Place the right palm over the left dorsum with intertwined fingers and rub. Repeat for the other hand.
    - Rub the hands palm to palm with the fingers intertwined.
    - Rub the backs of the clasped fingers against the opposing palm with the fingers intertwined.
    - Rotationally rub the left thumb clasped in the right palm and vice versa.
    - Rotationally rub backwards and forwards with the clasped fingers of the right hand in the left palm and vice versa.
4. Wait until the sanitiser dries.

Do not rinse or wipe hands when the sanitiser has dried.

Total time: 20-30 seconds

Guide to Using Gloves

- Gloves are only donned once the hand sanitiser has completely dried.
- One pair of gloves may only be used for handling one series of samples.
- Single-use gloves must be removed immediately after completing the task they were used for.
- Used gloves are disposed of as infectious waste.
- Damaged gloves must not be used.
- As gloves do not completely protect users against hand contamination, the hands must be washed and disinfected after removing the gloves.

Sterilising Instruments and Tools

Instruments and tools for repeated use are sterilised to remove any microorganisms so that they can be used again with no risk of contaminating the samples. Sterilisation also protects the health and safety of employees who handle biological material.

Pre-sterilisation Steps

All instruments and tools that are used are considered to be contaminated. If they are designed for repeated use, they must be decontaminated immediately after use.

Decontamination guide:

1. Disinfect the tools with a suitable agent and remove any impurities.
2. Rinse the tools thoroughly with water.
3. Place the tools in a washer-disinfector for chemical decontamination. If required, they may be also pre-decontaminated using a decontamination agent.
4. Dry the tools and inspect them to make sure they are fully functional and in good condition.
5. Place the tools in suitable packaging for sterilisation.

Hot Air Sterilisation and Decontamination

Glassware, porcelain and earthenware are sterilised by a flow of hot air. Stainless steel tools are sterilised in a designated hot air sterilisation oven in building A29.

The hot air sterilisation requirements are listed below:

Temperature	**Time**	**Packaging material**	**Sterilised material expiration**
Glass, porcelain 400°C	8 hours	No packaging	No time limit
Stainless steel tools 200°C	120 min	Single-use bags	No time limit

After finishing the sterilisation cycle, both the high-temperature hot air ovens and the low-temperature sterilisation ovens may only be opened once they have cooled down to 150°C.

Autoclave Sterilisation

Sterilisation by wet heat and increased pressure is used to treat heat-stable solid and liquid materials. The sterilisation programme is selected according to the sterilised materials (glass, plastic, metal, liquids, objects with or without packaging). Successful sterilisation is indicated by a change in colour of the autoclave tape, which must be applied to the items before they are placed in the autoclave.

Autoclave sterilisation takes place in designated autoclaves and may only be performed by duly trained persons.

Sterilisation Packaging

Stainless steel tools sterilised in the sterilisation oven are placed in sterilisation bags with forced air circulation even after the bags are heat-sealed. Sterilisation bags, containers and aluminium foil are used for autoclave sterilisation. Liquids are sterilised in suitable closable containers.

Sterilisation and Decontamination Records

Every sterilisation must be recorded in the sterilisation log. The sterilisation or decontamination must be recorded by the employee who performed it.
A successfully performed sterilisation is documented by:

a) A record in the sterilisation log (type of material sterilised, parameters, date, name and signature of the person who performed the sterilisation);
b) Written dated results of the sterilisation test for every batch.

Performance Check of the Sterilisation and Decontamination Device

The performance of the sterilisation and decontamination device is checked by its manager.

Check schedule:

1. New devices and devices that have been repaired must be checked before commissioning.
2. Each device must be checked immediately if there is any doubt about the effectiveness of the sterilisation and decontamination process.
3. Sterilisers older than 10 years must be checked after every 100 sterilisation or decontamination cycles or once every six months, whichever comes first.
4. Sterilisers no older than 10 years from the date of production must be checked after every 200 sterilisation cycles or once a year, whichever comes first.
5. Chemical sterilisation tests are used to prove that all the requirements of the sterilisation and decontamination cycle have been met.

Disinfection

All laboratory instruments for repeated use that come into contact with potentially infectious material must be disinfected and decontaminated by the laboratory staff immediately after use.

Glassware and ceramics are first decontaminated chemically using a decontamination agent and then placed in a decontamination washer and a high-temperature decontamination oven or autoclave.
Recommended biocidal agents or disinfectants declared as medical devices or agents registered as medicinal products for use in healthcare are used for chemical disinfection.

- Disinfection solutions are prepared according to the manufacturer's instructions by carefully measuring the required amount of water and disinfectant immediately before use. Multi-day disinfectants may only be used for two-stage disinfection according to the manufacturer's instructions. Ready-made disinfectants may also be used.
- There is a regular rotation of disinfectants with different active components in the disinfection regime (once a month) to prevent natural selection and antimicrobial resistance and reduce allergic reactions in the staff. The Technical Laboratory Manager keeps records of the disinfectants used.
- **The disinfectants used must not contain the organic substances that are analysed in the RECETOX laboratories.**
- After using all the disinfectant in a dispenser, the dispenser is washed manually, refilled and labelled with the name of the disinfectant, the date of refilling and the expiration date. Alternatively, the empty package is disposed of as hazardous waste.
- Disinfecting solutions are stored in the designated containers correctly labelled with the disinfectant name, concentration, preparation date and expiration date. Open containers with disinfecting solutions must be covered.
- When handling disinfectants, staff must use suitable protective equipment and comply with the manufacturer's instructions for handling and storage.
- Areas and surfaces contaminated with a potentially infectious material are covered with cellulose wadding soaked in disinfectant, which must be left on the surface for the recommended exposure period of time (indicated on the disinfectant packaging). Afterwards, the area is cleaned as usual.

Laundry Handling Guidelines

The laundry handling guidelines are subject to Decree no. 306/2012 Coll. The staff handling used laundry must use personal protective equipment. After completing the task, the hands must be disinfected. Used laundry is placed in packaging (either washable and disinfectable or single-use) to prevent contaminating the surrounding area. Each RECETOX organisational unit with laboratories that process biological material must designate a room for used laundry storage. The room must allow ventilation and the floor and walls up to 150 cm must be washable and disinfectable. The laundry is washed at the University Hospital Brno based on a contract with the hospital. Laundry is collected according to the schedule available on the INTRANET. On the collection day, the laundry must be brought down to the garage on the 2nd basement floor by 8 a.m. The cleaned laundry is returned on the following collection day. The Technical Laboratory Managers are responsible for compliance with the laundry handling guidelines in their laboratory section.

Waste

Waste Handling Guidelines

The waste handling guidelines are subject to Act no. 185/2001 Coll. regulating waste and the alterations to certain related acts, as amended. The provisions of Decree no. 306/2012 Coll. regulating the conditions to prevent the emergence and spread of infectious diseases and the hygiene requirements for healthcare facilities and social care institutions are applied accordingly.
There is a centralised waste collection system at the laboratories.
Waste is separated at the site of production and dangerous waste is placed in labelled, separated, covered, closable, impermeable and mechanically resilient containers.
All waste produced while working with human biological materials is considered infectious.

Waste categories

- Halogenated waste;
- Non-halogenated waste;
- Waste containing heavy metals;
- Glass waste;
- Other sharp waste (scalpels, injection needles etc.);
- Infectious waste (biological material, waste contaminated with biological material);
- Other waste.

The persons authorised to work with biological material follow the principles below when working with waste:

- Single-use instruments (serological pipettes, plastic pipettes etc.) are always returned to their original packaging after use and separated according to the resulting type of waste.
- Liquid infectious waste (left after processing blood samples etc. in suction bottles) is disposed of by decontaminating with a suitable agent (Savo, Chloramine) and placed in labelled impermeable rigid containers for disposal. Alternatively, it may be collected in collection bags suitable for autoclaving and placed in suction bottles, then decontaminated in autoclaves and disposed of as general liquid waste.
- Analysed samples of biological material are decontaminated by autoclaving and then disposed of as general waste. All waste produced while working with human biological materials is considered potentially infectious.
- Separated waste is placed into labelled containers and removed from the workplace daily to designated areas (building A29). Further waste handling, including the handover to the authorised waste-management contractor, is arranged by the waste department of the Management of the University Campus Bohunice.
- Infectious waste produced in building INBIT is removed by the authorised laboratory staff at least two times a week and placed in the waste storage room (1S44 – 1st basement floor corridor; the key is available from laboratory 0.21 in building INBIT).
- All hazardous waste is visibly labelled as such on the container. The label must include the statutory waste type number, date and place of origin.
- Glass waste and other sharp waste (180101 N*), such as used needles and complete syringes without caps, glass etc., must be placed in labelled, burnable, rigid, non-pierceable and impermeable containers.

- Solid burnable waste (180103 N*) is placed in labelled, separated, impermeable and mechanically resilient containers that can be closed (such as a plastic bin with a lid and a polythene bag inside). Liquid infectious waste is collected in single-use white plastic barrels or in single-use suction bags with bacterial and hydrophobic filters (Medela).
- Prior to final removal, infectious waste may be stored in a designated enclosed space for no longer than 3 days in the winter and 2 days in the summer.
- Infectious waste may be stored in a freezer or cooler area at 8°C or less for no longer than 1 month.
- All other waste is disposed of in accordance with the Operating Regulations of the University Campus Bohunice – Appendix no. 6 (*Provozní řád odpadového hospodářství UKB*) https://is.muni.cz/do/sukb/spolecne/provozni_rad_odpadoveho_hospodarstvi_ukb/Provozni_rad_odpadoveho_hospodarstvi_UKB_2018-02-26.pdf.

Cleaning

Laboratories are cleaned centrally according to the cleaning standards (see INTRANET).

- Laboratory staff clean and disinfect laboratory benches and fume hoods as required and always after finishing work.
- Cleaning tools are always disinfected after use.
- Disinsection and pest control are provided by a specialised firm.

Water Supply and Wastewater Disposal

Drinking water is supplied by the water supply system of the city of Brno. Distilled and double distilled water is produced using the distillation and filtration apparatuses in buildings A29 and INBIT. Wastewater pipes are connected to the city sewer system. Selected laboratories have drains fitted that can be used for the disposal of weak acids and bases (but not organic solvent). These drains lead to a tank that collects chemical wastewater, which is then disposed of by a specialised firm. The laboratory bench drains leading to the chemical wastewater tank can only be used for disposing of the remaining quantities of chemical solutions miscible with water, in amounts that do not represent a hazard for the water environment, water-soluble solvents (up to 0.5 l, diluted at least ten times), and acids and hydroxides (diluted 30 times) if such waste cannot be collected in the waste bins for operational reasons.

Final Provisions

The person authorised to interpret the provisions of this Appendix and ensure that they are complied with is the OHS and FS Officer.

Brno, 9 September 2019

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