GUIDELINE

THE USE OF LABORATORY RODENTS

CONTENTS

REVISIONS

Ed.	*Date*	*Description of change(s)*	*Author of change(s)*	*Approval*

0	06/06/2013	EDITION FOR PUBLICATION	Barra A.[(1)], Lacerra G.[(1)], Digilio F.A.[(1)], Lanati A.[(2)], Liguori G.L.[(1)] *(1) qPMO-WP1, IGB-CNR* *(2) VQ consultant*	**qPMO-WP1, IGB-CNR**
Ed.	*Date*	*Description*	*Drafting Group*	*Approval*

1. PURPOSE

The following are guidelines for using laboratory rodents, drawn up with the objective of providing a useful operating guide for correct animal experimentation procedures as required by the applicable regulatory regime.

2. REFERENCE SOURCES AND STANDARDS

GL: "**The Drafting of Guidelines for Scientific Research Laboratories According to Quality Principles**" Lacerra G, Digilio FA, Lanati A, Liguori GL. Edition 0, date 11.03.2013

Italian legislation:
Circular 11 March 1999
Legislative Decree n. 116 of 27 January 1992 and subsequent amendments
Legislative Decree 152/2006
Law n. 413 of 12 October 1993
DGSVA Note 10/3097-P of 28 January 2005-02-03
European legislation:
Commission Recommendation of 18 June 2007
Directive 86/609/EEC
Directive 2010/63/EU

3. ACRONYMS AND SPECIAL TERMS

Acronimo	Significato
ASL	Local health authority (Azienda Sanitaria Locale)
EEC	European Economic Community
DGSVA	Directorate-General of Veterinary Health and Food Safety
EU	European Union
H_2Oda	Distilled water
IVC	Individual ventilated cage
PPE	Personal protective equipment
WP1	Workpackage 1
qPMO	Quality Project Management Open-Lab

4. GUIDELINE AREAS

4.1. MILIEU

Organization

It is recommended that the internal space of the laboratory animal facility be divided according to the operational needs of the facility and that each area have its own specific purpose. The following are optimal arrangements:

- Entrance clean area: filter entry zone for staff and user access (for putting on single-use clothes)
- staff offices

- toilets
- housing rooms (including a post-operative holding room and a quarantine room for the intake of new animals). The management of the quarantine room, its degree of isolation and biological containment, should be proportionate to the type of pathogen to be confined, as well as its (to their, if more than one) mode of transmission. In general, it is always advisable to keep the quarantine area far away from the other housing rooms and to employ a ventilation system under negative pressure assisted by biological containment equipment such as IVC and laminar flow hoods for the handling of infected animals
- utility rooms for storing clean materials (food, bedding, cages, accessories, etc.)
- laboratory rooms for carrying out experimental procedures
- "dirty side" access (with attached storage room for materials) for staff working in the washing area
- cagewash area for the cleaning and sanitizing of cages and equipment.

In some environments in which particular activities are carried out (the transfer of potentially contaminated materials, corridors, laboratories, etc.), it is recommended that UV lamps be turned on at night.

Cleaning

The cleaning of the facilities (offices, corridors, toilets) should be carried out regularly by the Institute's contractor, according to procedures that ensure the highest possible level of hygiene. It is helpful to work with the company to define the specific cleaning procedures required if they are different from those performed in other laboratories.

The cleaning of the animal housing rooms in which animals are present is the responsibility of animal facility staff. The periodic cleaning and sanitation of the housing rooms with specific products is the responsibility of animal facility staff (Form 1).

For the cleaning of steel equipment, bleach, which corrodes it, must be avoided. The following can be used: denatured alcohol, 70% alcohol, benzyl quaternary ammonium compounds.

Monitoring

Animal facility staff are required to carry out checks even on weekends and public holidays to verify that, even on nonoperational days, all environmental parameters and climatic conditions within the facility are within the norm and to ensure the maintenance of at least minimum animal welfare conditions (availability of food and water, cleaning, etc.) (Form 2).

4.2. MANPOWER

For the optimal management of the laboratory animal facility, it is helpful to assign specific tasks to specific individuals.

Director of the Animal Facility: is the legal representative of the authorized animal house facility.

Animal Facility Manager: is selected by the Director of the Animal Facility; the Manager is responsible for the proper planning and management of the animal facility and the fulfillment of its related organizational and technical tasks.

Consulting Veterinarian: provides veterinary care, as well as advice on animal welfare by performing regular inspections, checking the conditions in which animals are housed and cared for, based on

experimental requirements. The Consulting Veterinarian also carries out the general inspection of experimental protocols, endorsing them to the extent he/she is competent.

Animal Technicians: handle the animals during experiments, assisting researchers and the veterinarian with certain procedures, such as collecting organic liquids and biological samples and administering medicines and other substances.

Animal Care Personnel: carry out the cleaning and feeding of animals and the cleaning and tidying of rooms and equipment.

Technical Support Services: This service is responsible for the care and maintenance of the equipment. Technical Support Services should be contacted in cases of equipment malfunction in the animal facility and can respond directly or make use of outside services to resolve the problem. A list of support services for the animal facility, along with contact information, can be found in section 6.

Users (researchers and experimenters): are internal staff and external personnel affiliated with the Institute (undergraduates, fellows, graduate students, post-docs, etc.) who participate in research projects requiring the use of laboratory animals. Access to the animal facility is restricted to authorized users only. To obtain the necessary authorization, it is essential to have been properly trained and to deliver the correctly filled out Security Form to the Animal Facility Manager.
To access and work in the animal facility, one must follow the directions found in the Animal Facility Rules of the facility. In particular, it is essential to respect the rules regarding the activation of research projects under the applicable law (see paragraph 4.5.1 Experiment Authorization) and the procedures for entering and working in the facility (see paragraph 4.5.2 Procedures).

4.3. MACHINES

Space Assignment

For the allocation of space (shelves in the incubators, refrigerators, and freezers), Tutors must contact the Animal Facility manager. Decisions regarding the allocation of space must be respected.

Refrigerators

Stored materials should be marked with the date and the laboratory they come from. Cardboard containers are not to be used in refrigerators. Users should periodically empty regrigerators of their unused materials, in conjunction with the cleaning of the room or as needed.
For the maintenance of refrigerators, it is advisable to periodically (every six months) check and record the internal temperature on the appropriate Form 3.

Incubators

Culture plates and flasks to be stored in the incubators should be marked with the user's or group's code and placed on the shelves assigned to each group. Users should clean the incubator shelves frequently and as needed with 70% alcohol.
Cleaning and maintenance done by the users should be recorded as per the forms attached.
The incubator shelves and chamber should be cleaned with appropriate detergents at least once every six months as reported. Removable parts should be washed under running water with detergent, then rinsed with adH_2O. After being dried and wrapped in aluminum foil, they are oven sterilized. Nonremovable parts

are washed in adH$_2$O and detergent, rinsed with adH$_2$O, and then cleaned with 70% ethanol and disinfectants which are not toxic to cell cultures, such as Incubator-Clean. For incubators that have a sanitizing cycle, it should be activated after the shelves and chamber have been cleaned with adH$_2$O and detergent, as specified above.
The water in the pan which is fitted in every incubator should be changed monthly using adH$_2$O to which a suitable disinfectant has been added (Form 4).
Below the maintenance's responsibility of Technical Support Services, which must record the details, date, and signature: temperature and CO_2 levels of each incubator should be calibrated monthly (Form 5); incubator filters should be replaced every six months or once a year (in conjunction with general cleaning) or when necessary (Form 5).

Laminar flow hoods

Hoods may be reserved using the attached Form 6. Reservations must be respected; if it is not possible, they must be cancelled as early as possible. The surfaces of all materials to be placed under the hood must be cleaned with denatured alcohol. There should be as little equipment under the hood as possible so as not to block the laminar airflow. Hoods are shared, so no materials should be left under them at the end of the activity.
At the end of their work, each user must clean the work surface and glass shield with 70% alcohol.
Users must clean the work surface, the space underneath, and the walls whenever materials are spilled and periodically, as indicated, with the date and signature recorded on the appropriate Form 1. For cleaning the hood, all parts must first be washed with adH$_2$O and detergent, followed by a rinse with adH$_2$O and cleaning with 70% ethanol. UV lights may be turned on after each use and must be turned on at the end of the day and after each cleaning of the hood.
Maintenance is provided by an external company which must record the details, date, and signature on an appropiate form (provided by the company); a semi-annual check of the filter and intake flow of the hoods is recommended; filter replacement is arranged with the Animal Facility manager, with notice posted on the hoods at least one week in advance.

Cagewashers and autoclaves

Cagewashing machines and autoclaves should be used exclusively by dedicated and properly trained staff.
General annual maintenance, with replacement of worn out parts, is performed by an external company; the details, date and signature must be recorded (Form 4).

4.4. MATERIALS

Specific materials and detergents

Specific materials for the maintenance and care of the animals (rodents) are used in the animal facility: selected feed, bedding, purified water, solutions, medications and basic medical-surgical supplies.
When non-sterile materials arrive at the facility, they must first be stored in a clean, dry place outside the housing area; later, they can be moved to the clean zone after having undergone appropriate sanitization procedures.
The animal facility provides users with single-use PPE items (coveralls, shoe covers, caps and masks) to be worn before entering the facility. These may be used solely inside the animal facility and must be placed in the appropriate containers (waste and/or recycling) at the exit of the facility (for more information, see *Animal Facility Rules*).

For cleaning and contamination prevention in the animal facility, the following materials are normally used: antibacterial soaps for hand washing and commercial detergents for surfaces and equipment (70% ethanol, bleach and specific disinfectants and detergents for washing machines). Alternatively, animal facility-specific disinfectants and detergents (e.g., Virkon s) may be used.

Users' materials

Users may bring and use their own disposable materials (e.g., syringes, plastic items, etc.) in the animal facility. All materials brought into the facility must be either sterile, sealed and single-use or correctly sterilized if not single-use. Such items must always be labeled with the date, name of the item and group it belongs to.

To reduce the risk of contamination, avoid introducing solutions which are already in use, pipettes and other items coming from other laboratories. In addition, avoid the accumulation of cardboard packaging and other items used for transport.

At the end of their activities, each user must leave the laboratory and the equipment used in order, place pipettes and other plastic materials in the cabinet and leave all surfaces free and clean.

The introduction, storage and consumption of food or drink are prohibited in the animal facility.

4.5. METHODS

4.5.1. EXPERIMENT AUTHORIZATION

4.5.1.1. ACTIVATION OF RESEARCH PROJECTS

Legislative Decree 116/92 DOES NOT PERMIT the use of animals in the laboratory—not even for pilot experiments—UNTIL the proper Ministerial Notification form has been filled out and sent.

Therefore, before starting any research project that entails the use of laboratory animals, the researcher in charge of the project must fill out the Model Annex 4 in all parts and in detail and send it in paper format to the Ministry of Health, as well as make it known to other supervising agencies (the Region, Prefecture, Municipality and local health authority).

The Model Annex 4, duly filled out, constitutes Ministerial Notification that can be presented according to the following two procedures:

1) ***Notification (self-certification)*** – for all experiments requiring the handling of and surgical procedures on animals (limited to rodents) **using anesthesia** and which do not have expected outcomes that are more painful than the operation itself; in this case, the project and the use of laboratory animals may begin as soon as the Ministerial Notification has been sent, under the tacit consent of the supervising agency (Ministry of Health) which may request clarifications and additions to the project.

2) ***Exemption*** – generally for cases in which the experimentation is prohibited, particularly when it is necessary to perform procedures **without using local or general anesthesia**, or when a treatment conducted under anesthesia results in a worse condition than that of the treatment itself or is incompatible with the purpose of the experiment itself.

Examples of such cases include: toxicology experiments on conscious animal, some drug treatments, surgical implantation of tumorigenic cells, behavioral tests, any cruel handling. To conduct such experiments, specific authorization of an exemption must be requested from the Ministry of Health (Model Application for Exemption), and the initiation of the experiments is dependent on receiving authorization from the Ministry of Health.

An exemption may also be required for the use of animals for teaching purposes or in case it is necessary to use non-rodents or species different from those provided for in Decree 116/92, in case these do not meet the purposes of the experiment.

4.5.1.2. GENERAL INSTRUCTIONS FOR DRAWING UP THE RESEARCH PROJECT

Projects must be drawn up using the appropriate form provided by the host animal facility (Model Annex 4).
The description of the experiments must detail the experimental procedures, with particular attention to describing experimental models, treatment regimen, and routes of administration used, and the duration of the observation period.
It is especially important to make a realistic estimate of the number of animals that will be utilized and to indicate the number of experimental groups and the number of repetitions required for each experiment.
If the experiment entails animal suffering, indicate the anesthetic protocol (including the dosage) and any possible analgesic therapy to be used.
If the experiment does not require the use of anesthesia, authorization must be requested using the appropriate Model Application for Exemption.
If the animals will not be acquired from a certified breeder but rather from other laboratories (e.g., genetically modified animals), provide details about model characteristcs, indicate the lab of origin and references of the scientific subjects involved, as well as the reason for the transfer (purpose of any scientific collaboration). Finally, specify the manner and time of transport.
The Animal Facility Manager and the Consulting Veterinarian should be consulted during the drafting of the protocol.
The Project Manager's CV should accompany the documentation of the experiment.

4.5.2. PROCEDURES

4.5.2.1. ENTERING AND WORKING IN THE ANIMAL FACILITY

Access to the animal facility is restricted to authorized users who have put on single-use clothes and PPE (coveralls, shoe covers, caps and masks) in the filter zone.

4.5.2.2. HANDLING OF ANIMALS

The handling of animals is permitted solely for authorized and adequately trained personnel. All of the handling procedures for the animals (cage changing, checks, numeration, provision of food and water, etc.) are to be done under a laminar flow hood wearing single-use PPE.
All experimental procedures on animals (injections, administration of drugs, anesthesia, euthanasia, etc.) are to be done under a laminar flow hood wearing single-use PPE.

4.5.3. WASTE

Animal Facility staff must ensure that special biological waste containers are hermitically sealed and properly stored in designated areas.
Glass and sharp waste are collected in the appropriate yellow containers provided by the Health and Safety Office. The glass must not overflow the container and, once it is full, it is closed and put inside the solid waste container (Form 7).
Liquid wastes are decontaminated before disposal (according to the law as specified by directives of the responsible office of the Institute).
The disposal of animal carcasses, bedding and all waste related to animal experimentation must be done in accordance with the law (Legislative Decree 152/06).

4.5.4. CONTAMINATION

Procedures for preventing the contamination of animals (e.g., staff entrance, introduction of new animals and materials, etc.) can be found in the Rules for entering and working in the Animal Facility. However, in the case of contamination of the animals by specific pathogens, appropriate measures must be taken, defined on the basis of information provided by the Consulting Veterinarian:

- isolation in quarantine of the infected animals, for the period necessary to correct the problem;
- cleaning and sanitizing the contaminated environment;
- planning a reintegration program for the decontaminated animals (embryonic rederivation) or containment procedures if the pathogen is not harmful to users and staff.

4.5.5. RISKS

To avoid physical, chemical and biological risks, animal facility staff and users must follow the instructions provided in the document "Animal Facility Risks", available in the "**Accompanying Documentation**" section.

5. FORMS

Form 1 Cleaning shifts
Form 2 Weekend/Public holidays checks
Form 3 Temperature control
Form 4 Equipment maintanance
Form 5 Incubator Calibration and Filter Changes
Form 6 Hood Reservation
Form 7 Waste removal
Security Form
Model Annext 4 (Ministerial Notification)
Model Application for Exemption

6. SUPPORT SERVICES

Health and Safety Office **Person in charge …….. Telephone ……**
For solid waste containers, sharp waste containers, and liquid waste tanks.

Technical Support Service **Person in charge …….. Telephone ……**
For problems with CO_2 or incubator temperatures, changing incubator filters, changing hood filters, maintenance, etc.

Technicians responsible for equipment and systems and maintenance staff
For furnishing technical and practical support for the operation and maintenance of equipment and systems necessary for the operation of the animal facility.

Supervision and monitoring agencies

Ministry of Health
Department of Veterinary Public Health, Nutrition and Food Safety, Directorate-General of Animal Health and Veterinary Medicine, Office VI – Animal Welfare, Via Giorgio Ribotta 5, EUR - 00144 – ROME

Local Health Authority (ASL)
Department of Prevention – Veterinary Unit

7. AUTHORS AND REVIEWERS

The following groups have contributed to the edition for publication (versione 0):

7.1. DRAFTING GROUP

qPMO-WP1, IGB-CNR Naples
Adriano Barra
Giuseppina Lacerra
F. Anna Digilio
Giovanna L. Liguori

VQ consultant
Antonella Lanati

7.2. COLLABORATORS

7.3. REVIEWERS

qPMO
Marta Di Carlo, Antonella Bongiovanni (IBIM, CNR)
Gianni Colotti (IBPM, CNR)
Annamaria Cirafici, Anna Mascia, Annamaria Kisslinger (IEOS, CNR)

External Reviewers
Marcello Raspa, DVM PhD (CNR-EMMA, Monterotondo, Roma, Italy)
Claudio I. Bernardi, DVM, M.Sc, ERT, (Director Compliance & Administration Accelera Srl, Nerviano, Milano, Italy).

8. ACCOMPANYING DOCUMENTATION

Animal Facility Risks
Animal Facility Rules