Animal Testing Regulations

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I.

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Decree No. 52/2020. of the

Semmelweis University

Senate on 14. April

on the acceptance of the Animal Testing Regulations

The Senate of Semmelweis University authorized by Part I.1. Paragraph 18. Section (9) d) of the Organizational an Operational Rules (hereinafter: SZMSZ) made the following Decision:

Section 1 The Senate of Semmelweis University approved the proposal for the adoption of the Animal Testing Regulations.

Section 2 This Decision, and with it the Animal Testing Regulations, **shall enter into force on the day following its publication** on the sub-website of the Directorate-General for Legal and Administrative Affairs (JIF).

Section 3 Rectoral Decision Nr. R/5/2017. (IX.28.) **shall be repealed** with effect from the date of entry into force of this Decree.

17. April 2020.

Dr. Béla Merkely

Rector

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1. GENERAL PROVISIONS

1.1. Scope of the Regulations

The material scope of the Regulations covers the breeding, keeping and experiments of animals used in scientific and educational experiments, as well as the breeding and keeping of other animals - their organs or tissues for scientific or educational purposes.
For the purposes of this Code, animals include live cephalopods and living vertebrates other than humans, as well as their independently feeding larval forms and mammalian fetuses in the last third of their normal development. For the purposes of this Code, animals include live cephalopods and living vertebrates other than humans, as well as their independently feeding larval forms and mammalian fetuses in the last third of their normal development.
This Regulation shall apply to the rearing of animals with organs or tissues specifically for scientific or educational purposes.

1.2. Definitions

animal house:	A building or part of a building consisting of rooms with different functions for housing and / or breeding experimental animals under specified hygienic conditions.
animal experiment[1]:	The use of an animal for experimental, educational or other scientific purposes which may cause it pain, suffering, distress or lasting harm equivalent to or greater than that caused by a needle stick in accordance with good veterinary practice, including any activity involving the birth of an animal, including hatching. The use of an animal for experimental, educational or other scientific purposes which may cause it pain, suffering, distress or lasting harm equivalent to or greater than that caused by a needle stick in accordance with good veterinary practice, including any activity involving the birth of an animal, including hatching. Such use of an animal shall be considered an experiment even if anesthesia, analgesia or other methods have been successfully used to eliminate pain, suffering, distress or lasting harm. The use of non-experimental agricultural or veterinary activities or the use of modern methods of killing or marking animals which are considered by the natural sciences and which are considered to be less painful, and the killing of an animal solely for the purpose of using its organs or tissues, shall not be considered as animal testing
the use of animals for educational purposes:	the use of animals bred for educational purposes in practical classes, during which instructors give demonstrations,

[1]Ávtv: Act XXVIII of 1998 on the protection and welfare of animals Section 3

	experiments to expand the students' theoretical knowledge in practice, and the students themselves carry out interventions to deepen the practical knowledge.
animal room:	a room for carrying out the experiment or keeping the animals, which may be properly applied in accordance with Act XXVIII of 1998 on the protection and welfare of animals. (hereinafter: Ávtv.) and Gov. Decree Nr. 40/2013 (II.14.) on animal testing (hereinafter: Gov.Decree) and the requirements thereto.
narcosis-overanaesthesis experiment:[2]	an experiment under full anesthesia, after which the animal does not regain consciousness and dies in the event of over-anesthesia. The fact of the occurrence of death must be established beyond a reasonable doubt.
mild experiment[3]:	an experiment in which the animal is likely to be in mild pain, suffering or distress for a short period of time, and an experiment in which the welfare or general condition of the animal is not significantly impaired.
placement:[4]	the re-introduction of an animal used in the experiment or intended for such use to a habitat appropriate to that species or its return to the animal husbandry system (hereinafter together referred to as "placement")
moderate experiment:[5]	an experiment in which the animal is likely to be in moderate pain, suffering or distress for a short period of time, or in mild pain, suffering or distress for a long period of time, and an experiment in which the welfare or general condition of the animal is likely to be moderately impaired.
project:[6]	a program of work designed and then carried out for a specific scientific or educational purpose and involving one or more experiments.
grave experiment[7]:	an experiment in which the animal is likely to be in severe pain, suffering or distress or of prolonged moderate pain, suffering or distress and an experiment in which the welfare

[2]40/2013. (II.14.) on animal testing Gov. Decree Section 2.
[3]40/2013. (II.14.) on animal testing Gov. Decree Section 2.
[4]40/2013. (II.14.) on animal testing Gov. Decree Section 15.
[5]40/2013. (II.14.) on animal testing Gov. Decree Section 2.
[6]40/2013. (II.14.) on animal testing Gov. Decree Section 2.
[7]40/2013. (II.14.) on animal testing Gov. Decree Section 2.

or general condition of the animal is likely to be significantly impaired.

2. GENERAL PROVISIONS

2.1. Operating permit

(1) All breeding and user sites for experimental animals must have an operating license issued by the Food Chain Safety and Animal Health Authority. The permit is valid for a maximum of 10 years.
(2) The application for the permit is prepared by the head of the institute and the Gov. Decree to the Food Chain and Animal Health Authority in accordance with Annex 1 thereto. A copy of the permit is to be kept by the institute. A copy of the permit shall be sent by the institute to the Committee for Animal Welfare at Work (hereinafter referred to as: MÁB, the number of which is registered by MÁB.
(3) An amendment to the permit should be requested from the Food Chain and Animal Health Authority if there has been a significant change in the structure or function of the establishment which could adversely affect the welfare of the animals.
(4) The food chain safety and veterinary authority shall be notified of any change in the persons identified in the operating license within 30 days of the change.
(5) It is the responsibility of the head of the institution to amend the operating license and to make the change in the person (s) indicated in it, and to send a notification, about which he / she informs the MÁB at the same time.

2.2. Rules for animal testing

During the animal experiments, the Ávtv. and the provisions of the Government Decree, as well as the contents of the project permit, the exemption decision of the animal protection authority must be obtained in case of the need to deviate from them.

2.2.1. Project permit

(1) All animal testing activities can be started on the basis of a project permit for a maximum of 5 years (and renewable for a maximum of 10 years). A project cannot be carried out without the prior permission of the Food Chain Safety and Veterinary Authority.
(2) The application for a project permit is drawn up by the person responsible for the project. The person responsible for the project is responsible for ensuring that the staff working on the project have the qualifications required by law.
(3) If an extension of the authorization is necessary and there is no change in the conditions set out therein, an extension of the authorization shall be requested no later than 60 days before the expiry date.
(4) The person responsible for the project may apply to the Food Chain and Animal Health Authority for an amendment to the project authorization in order to change one

or more of the conditions of the original authorization. Modification of the permit does not change the expiry of the permit.

(5) If the extent of the change in the conditions set out in the permit justifies the granting of a new permit, the person responsible for the project may initiate the renewal of the project permit with the Food Chain and Animal Health Authority. An amendment or renewal of the project authorization shall be requested in the event of any change in the project which may adversely affect the welfare of the animals.

(6) The plan of experiments included in the project must be sent to the MÁB for approval, which will decide on the approval of the experiments within 30 days.

(7) The approval shall cover the suitability of the materials and methods used in the experiments for the purpose of the experiment and shall include a proposal for the preliminary severity classification of the experiments.

(8) For approval, the following must be presented to the MÁB:

a) the purpose of the experiment, data on its scientific validity;
b) the species, genetic and microbiological status of the animals, the planned number of animals;
c) the species, genetic and microbiological status of the animals, the planned number of animals;
d) the applied test procedures and measurement methods;
e) the experimental setup;
f) the expected duration of the experiment
g) measures aimed at the pain, suffering, distress, damage that may be caused to the animal, and the measures to prevent and eliminate them.

(9) After the approval of the MÁB, the application for the project permit is submitted by the MÁB in accordance with Government Decree No. 2. to the food chain safety and animal health authority (competent department of the Pest County Government Office) by filling in the electronic form on the website of the National Food Chain Safety Authority (hereinafter referred to as: NÉBIH.

(10) The person responsible for the project must keep all relevant documentation, including project authorizations and the results of the project evaluation, for at least 5 years from the expiry date of the project authorization or from the receipt of the rejection decision.

2.2.2. Personal conditions for animal testing

(1) The breeding and user institute must ensure that an adequate number of staff is available at the establishment.

(2) Persons participating in animal experiments must participate in the annual MÁB training in order to maintain the expertise acquired in accordance with the law and the right to perform related activities.

(3) Persons involved in animal experiments are those by whom

a) the animal is cared for;
b) the animal experiment is performed;
c) the animal experiment is planned and conducted;
d) the animal is killed.

(4) Person responsible for supervising the welfare and care of the animals: the head of the animal house, who is responsible for supervising the welfare and care of the animals in the establishment.
(5) Designated veterinarian: a veterinarian experienced in laboratory veterinary medicine or a suitably qualified, highly qualified expert in the field of animal welfare and treatment.
(6) Person responsible for the project: the person responsible for the overall implementation of the project and for compliance with the conditions set out in the project authorization.
(7) Other responsible persons: in the case of stock and breeding breeds, the head of the breeding is responsible for the observance of the provisions of the Regulations, and the supervising researcher is responsible for the animals caught in the experiment.

2.2.3. Provisions for animals in experiments

2.2.3.1. Acquisition of experimental animals

(1) An animal authorized by the animal welfare authority in the project permit may be used for experimental purposes.
(2) The animals purchased must comply with the requirements of veterinary and public health legislation. This fact must be attested by a quality certificate attesting to a level of hygiene and / or a valid vaccination certificate. Certificates of quality and vaccination must be kept in the records of the experiment using the animal.
(3) The procurement of experimental animals is carried out by the person responsible for the project, or the head of the breeding, or the head of the animal house.
(4) The provisions of the University Commitment Regulations in force at any time shall apply to the commitment to procure experimental animals.
(5) The procurement of genetically modified animals is subject to special rules, which are regulated by Act XXVII of 1998 on genetic engineering activities as well as the relevant provisions of the 82/2003. (VII. 16.) FVM (Ministry of Agriculture and Rural Development) Decree on the procedure for registration and data provision for genetic engineering activities and on the documentation to be attached to the application for a permit required for genetic engineering activities) shall apply.

2.2.3.2. Action on experimental animals after completion of the experiment

(1) At the end of the experiment, a veterinarian or other suitably qualified person will decide whether to keep the animal alive. At the end of the experiment, the experimental animal is either killed or reused or placed.
(2) An animal should be killed if it is expected to continue to experience moderate or severe pain, suffering, distress or lasting harm after the experiment. The killing of the animal must be carried out in accordance with the instructions in Annex 4 to the Government Decree.
(3) The person responsible for the project is obliged to ensure the painless extinction of the life of any non-viable, sick or aged animal whose continued survival would result in irreparable pain or suffering.

(4) An animal used in the experiment or intended for such use may be re-housed or returned to a habitat or animal husbandry system corresponding to the given species (hereinafter together: landing) if the conditions prescribed in the Gov. Decree are met.
(5) An animal may be used in a new experiment only if the actual severity of the previous experiments was ‘mild’ or ‘moderate’, the general health and welfare of the animal has been restored, and the new experiment is classified as ‘mild’, ‘moderate’ or ‘anesthetic’. over-anesthetic ’and re-use is in line with the veterinary proposal, which takes into account the effects on the animal throughout its life cycle.

2.2.3.3. The action plan

(1) No later than 60 days before the planned placement, the deployment action plan prepared by the head of the animal house and reviewed by the MÁB must be submitted to the Food Chain Safety and Animal Health Authority, which decides on its approval. Following the opinion of the MÁB, the action plan is submitted by the head of the animal house to the food chain safety and animal health authority through the head of the institute.
(2) The action plan must ensure the integration of the animal at the place of deployment. In the case of wild animals, a rehabilitation program should be carried out, if necessary, before returning to the habitat, taking into account the animal's state of health, behavior and experimental history. An animal may only be moved within the framework of an action plan approved by the Food Chain Safety and Animal Health Authority.

2.2.4. Inspection by the veterinary authority

(1) The food chain safety and animal health authority regularly inspects both the experimental breeders and the user establishment and the animal house.
(2) The record of the inspections must be kept for at least 5 years by the head of the animal house and the person responsible for the project.
(3) The implementation of the measures provided for in the protocol is monitored by the MÁB.

2.3. Operational requirements for animal houses

2.3.1. Personal conditions for operating animal houses

Only persons with the qualifications specified in the Gov. Decree may be employed in an animal house.

2.3.2. Monitoring the operation of animal houses

(1) Animal houses must prepare rules of procedure, which are prepared by the head of the animal house and adopted on the basis of Section 3 (18) of the SZMSZ.
(2) The annexes to the rules of procedure are as follows:
a) the house rules,

b) floor plan of the animal house (with markings showing the functional structure).

(3) The policy and, as part of it, the health preservation and environmental enrichment strategy must be reviewed every 5 years from the entry into force of the rules of procedure - or, if necessary, out of turn - by the head of the animal house, including the opinion of the MÁB. In case an amendment is necessary, paragraph 1 shall apply.

(4) The house rules must be placed in a clearly visible place in printed form in the animal house, and the rules of procedure of the animal houses must be published electronically on the website of the MÁB.

2.3.3. General requirements for animal houses

(1) The head of the animal house is responsible for the implementation of and compliance with the requirements for animal houses, taking into account the obligation to comply with the framework provided.

(2) A maintenance program should be established to protect the equipment of the animal houses and to organize regular troubleshooting and rectifying defects. The heating and ventilation system of the animal house must be equipped with monitoring and alarm devices. Temperature and humidity should be measured and logged daily with manual tools while retaining data from the monitoring system.

(3) The air system of animal houses and animal rooms must not be common with the air system of offices and research laboratories (the air in the animal room must not be common with the other ventilation systems of the building, or closed, lower pressure animal housing must be used, the outlet of which is not the same as).

(4) Animal experiments with infectious pathogens should only be performed in an isolator / room with a HEPA filter-protected extractor.

(5) If a breeding or experimental animal escapes, breaks loose or is released (hereinafter: escaped animal) despite complying with the conditions prescribed in the Government Decree, the operator of the Building must be notified immediately and the animal must be captured as soon as possible, if possible. The escaped animal must not be reintroduced among the other bred or participating animals, its termination must be carried out in accordance with the provisions of the Gov. Decree.

(6) If the escaped animal bites anyone, the animal must be treated in accordance with the veterinary legislation in force, the animal must be quarantined for a definite period of time and then terminated after observation. In such cases the University Occupational Safety Regulations apply

(7) If an animal bite occurs during working, the provisions of the University Occupational Safety Regulations must be followed.

(8) The procedures to be followed in the event of an emergency in the animal house are set out in the animal house's policy.

(9) The hygiene requirements of Semmelweis University Animal Houses are governed by the Annexes to these Animal Hygiene Regulations.

(10) In the case of a breeding (propagating) animal house, compliance with the hygiene requirements must be continuously monitored using sentinel animals, following the appropriate sampling protocol.

(11) Separation of disinfected and cleaned as well as contaminated tools and equipment must be ensured.

(12) The executive head of the animal house shall take care of pest and insect control as often as deemed necessary.
(13) Hygienic storage and safe disposal of animal carcasses and wastes must be ensured. Animal carcasses must be stored in a refrigeration plant set up for this very purpose and transported to a specialist company for eco-friendly disposal.
(14) Carcasses of experimental animals and litter waste are considered hazardous waste, and the University's hazardous waste management regulations apply to their disposal.
(15) As part of the house policy of the animal house, a health maintenance strategy must be developed to ensure animal welfare and compliance with scientific requirements, including a microbiological surveillance program, a disease management plan, health parameters and procedures for the admission of new arrivals (Annex 1). Isolation of the newly-acquired animals is to be ensured from the already established animals and the sick or injured animals from the healthy animals.
(16) In order to create and maintain a stimulus-rich environment, an enrichment strategy should be developed and esetablished in the house policy. In this context, an adequate stimulus-rich living space should be provided by providing at least one of the following conditions:
a) According to the possibilites, isolation should be avoided. Group housing, which takes into account the minimum housing area required for each species, allows the animals to develop their natural hierarchy, which affects them as an enrichment of the environment. A toy suitable for the species, e.g. in the case of rodents, an autoclavable paper roll, a red hiding place, an autoclavable wooden cube or a cylinder shall be used.
b) Where possible, nesting material, litter and sleeping accommodation should be used for animals. This is especially important for mothers who are about to have a litter, because here the mothers can form a nest and thus later the newborns will also find a stimulus-rich environment.
(17) Animals can only be provided with quality guaranteed feed and litter. These materials should be stored in a dry and dust-free place. It is forbidden to feed with feedstuffs that have passed their date of expiration.
(18) Continuous care of the animals must be provided including on workfree days and public holidays. The caregiver keeps log records of the care measures taken.
(19) Animals must be kept in accordance with the housing and care regulations set out in Annex 3 to the Gov.Decree.

2.4. Workplace Animal Welfare Committee

(1) The University operates a Workplace Animal Welfare Committee (hereinafter: MÁB).
(2) The President and members of the MÁB are elected by the Senate for a fixed term that is not exceeding 3 years, based on the proposal of the rector and taking into account the proposal of the relevant faculties. The rules of procedure are determined by the MÁB itself.
(3) Tasks of the MÁB
a) on the acceptance of the Animal Testing Regulations
b) preparation and review of animal experimental regulations;

c) monitoring of experiments woth regard to animal welfare, including the identification of elements that can further enhance compliance with the requirements for replacement, reduction and refinement;
d) organizing the education and training of persons authorized to conduct and conduct the experiment, to care for animals and kill the animals, to keep records of the subjects and topics of education and the list of participants;
e) conducting and recording examinations following the training of local animal caregivers;
f) animal welfare advice on the following topics:
   fa) animal welfare issues concerning the acquisition, accommodation, care and use of animals,
   fb) technical and scientific developments concerning the application of the requirements of replacement, reduction and refinement,
   fc) in relation to systems to facilitate the repeated placement of animals, including the proper reintegration of the animal to be moved into the community;
g) monitoring the implementation of counseling, recording the decisions made following and upon counseling;
h) prior approval and registration of project applications;
i) keeping the records of project authorizations;
j) forwarding the project authorization to the head of the animal house indicated in the project authorization;
k) regular inspection of the conditions of animal housing and care in breeding places and collection and processing related statistical data threof;
l) regular inspection of research sites performing chronic animal experiments and collection and processing related statistical data thereof;
m) to discover and investigate the cause of possibly occuring mass animal mortality;
n) collection of statistic data on the introduction of animals into experiments.

(4) In case of violation of the provisions of the Act, the Gov. Decree or these Animal Experiment Regulations, the MÁB is entitled to immediately stop the experiment, along with simultaneously notificating of the food chain safety and animal health authority.

(5) At Semmelweis University, each institute is obliged to designate a contact person responsible for everyone abiding by the rules of supervision, reporting and monitoring of animal welfare and use within the institute, who also maintains contact with the designated MÁB member and supplies MÁB with the required data.

2.5. Records of animals and experiments

(1) The registration of animals and experiments is governed by Sections 30, 31 and 51 (2) of the Gov. Decree, with the exception that all data are to be kept for at least 5 (five) years after the experiment or project has been finished or terminated.

(2) A person responsible for a project is obliged to provide the MÁB by 1 February each year with all statistical data on the animals used and the experiments performed.

(3) The MÁB is to send by 28 February each year all statistical data on the use of animals in experiments, including information on the actual severity of the experiments used and the origin and species of non-human primates used as well as data on the number

of animals killed solely for the use of their organs or tissues to the Directorate of Food Chain Safety and Animal Health, according to the tables published on the NÉBIH website.

(4) The facility must keep the data in the register for at least five years.

(5) The head of the animal house keeps the following records of the animal house:

a) the number and species of animals bred, procured, transported, used in experiments, released or re-housed;

b) information on the origin of the animals (names and addresses of suppliers) and whether they have been bred for experimental use;

c) the date of acquisition, dispatch, release or re-dispatch of the animals;

d) the number and species of animals killed or killed in each establishment and the cause of death, if known; and

e) in case of users the projects using animals;

f) the actual severity of the experiments used.

(6) Records must be kept of all dogs, cats and non-human primates on the following:

a) the identification data of the animals;

b) the place and date of birth of the animals, if known;

c) whether the animals have been bred for use in an experiment;

d) in the case of a non-human primate, whether it is the offspring of a captive-bred non-human primate;

e) information on the origin and species of non-human primates.

(7) An individual event log shall be kept for each dog, cat and non - human primate for as long as the animal is kept for the purposes of this Regulation. The event log shall start at the earliest possible date of birth and shall include relevant data on the animal's reproduction, health status, vaccination status and community status, as well as data on the projects in which the animal has been used.

(8) Anyone who breeds, reproduces, supplies or uses an experimental animal must keep records of the names and addresses of the persons from whom the animals were obtained and to whom the animals were passed on.

Annex 1
Hygiene Regulations for Animal Houses

Preservation and control of high hygienic status (Specific Pathogen Free / SPF)

There is no specific binding rule for determining the hygienic situation of a given animal house. Tests for the presence of pathogens are determined by the responsible veterinarian and the results are used to determine the hygiene classification together with the management. Due to a different interpretation of the pathogen-free recommendation, the hygienic status of two animal houses both considered to be SPF may not be the same. The significance of SPF hygiene status is explained in Amendment "b".
The SPF hygiene rating places a great responsibility on the operator of the animal house. Categorization is voluntary, as there is no official body or institution that would determine the classification of an animal house on the basis of laboratory results and then decide on the maintenance of the status after regular official sampling. The management of several domestic (and foreign) animal houses classifies the holding as SPF, citing the strict meaning of the word, however, a thorough investigation may point out its falsity.
In the present situation, therefore, an animal coming from an SPF animal house does not in itself constitute a hygienically clean animal. Before registration of arrival, a decision on admission can be made after a thorough analysis of the latest status check result. In order of the protection of own stock, hygiene certificates should be accepted with reservation only and quarantine or other measures should be put into effect.
The denomination SPF (SPF: Specific Pathogen exemption from specified pathogens) carries the multitude of possible pathogens in itself. In order to find a way around the confusing denomination, FELASA (FEDERATION OF EUROPEAN LABORATORY ANIMAL SCIENCE ASSOCIATIONS) has developed a recommendation to standardize the hygiene status of laboratory animals. Semmelweis University (hereinafter: SE) follows this recommendation to establish the hygiene status and has set up a monitoring system following the recommended methods.
The SPF status of an animal house that meets high professional requirements strictly follows the FELASA list, and all animals leaving it are free of all pathogens on the list. Animals entering SE SPF animal housing must also meet this criterion. Common infections and some pathogens in animal houses can be found in Appendix "a".
The main criteria for selecting suppliers are compliance with the hygiene criteria of the purchasing animal house and previous favorable experience. Only animals from breeders may cross the barrier and be taken directly to the animal rooms, which will adapt the series of hygiene tests to the status of the receiving animal house and the results will be considered authentic by the supplying veterinarian.
The hygiene certificate for the status of animals must be complete, valid and valid for the pathogens tested. The veterinarian must assess all the criteria in accordance with the strict rules, in which case he may not deviate from this. Incoming wild-type animals may only come from suppliers described later due to loss of confidence due to adverse experiences. (Amendment "b").

The sentinel program

For FELASA investigations so-called sentinel (sentry) animals are to be prepared. The possibility of this is given by the fact that all viruses, bacteria and parasites are spread by

direct contact, i.e. either by the agent itself or by parts carrying the infection, e.g. bacteria, cysts or eggs are found in the contaminated litter.

Criteria for sentinel animals

In order for sentinel to be as likely to occur and to faithfully represent the status of the holding, the following conditions must be met:

Its hygiene status is proven to be SPF, a freshly purchased animal from a reliable supplier.

Strain: Balb / c in mice, Wistar in rats.

Age: 6 weeks (in "healthy" animals 4 months of age or older, vacuolatic degeneration or rarely even cancerous lesions may be revealed by a post-mortem examination).

Sex: female or littermate males.

Placement in a test cage: at least 6 weeks before the test.

Immediately upon arrival of the animals, we performed our own serological status test, which was supplemented with a Helicobacter test from the faeces.

Sampling protocol

According to the international recommendation, the hygiene status should be documented by repeated inspections once a year and then quarterly. The complete test series, test laboratory and diagnostic method used are included in *Amendment "a"*.

The accompanying document shall be written by the attending veterinarian. In addition to the sample number, this should include the unique identifier of the animal or, if this is not possible, information such as in the case of a wild type, the name of the strain and the cage number must be given - by which the individual can be identified with certainty. The cover letter is based on the sampling report. Test request letters and test results received must be archived.

For each animal rack (usually 80 cages / rack), one box of sentinel is counted in which 2 animals are housed. The sentinel cage is placed in the bottom row of the rack, in the right-hand position, and during litter changes, contaminated litter is loaded from the other cages on that rack, so these animals do not receive fresh litter.

After the end of the incubation period, the animals are sent alive to the Department of Mammalian Pathology and Wildlife Diseases (Institute of Veterinary Medicine) of the Veterinary Diagnostic Directorate of the Agricultural Administration. (*Amendment "c"*) The identification must be clear, which can be grouped by color or sex or painted on the tail. The animals are preferably sent in a paper box. The number of the animal cage and the clear marking of the animals in it must be indicated in the accompanying document Due to the administration of the diagnostic institute, the sending box is also numbered.

Due to the extensive line of tests, the date must be agreed with the Hungarian Institute before the submission of the test material, where the samples will be received from Monday to Wednesday due to the time-consuming nature of the bacteriological tests.

Serological and PCR testing was performed by another laboratory, so blood and faeces that were not inhibited from coagulation were returned. The day after the test, the recovered blood was pelleted in a centrifuge at 8000 rpm for 5 minutes, and then 100 µl of serum was pipetted into an Eppendorf tube. The serum and faecal sample are marked in the same way, with the serial number on the tubes in the cover letter, thus ensuring that all tests are performed on the same individual.

Serum was sent to BioDoc (Hannover) for complete serological testing and faecal Helicobacter PCR detection. (Appendix "c") The sample should be delivered by express mail.

What to do in case of a positive result

In case of laboratory results indicating infection, the animal house loses the SPF status, its recovery is possible only by replacing the herd.
Individual exemption can be achieved through drug intervention, however, treatment does not eliminate herd-level infection. For example, a nematode infection with a theoretically strong anthelmintic (Mebendazole, Allbendazole, etc.) can be eradicated from the individual, but at the herd level it is only suitable for symptomatic treatment. As eggs are extremely resistant, making it only a matter of time before they reappear and infect the entire animal house. Some bacterial infections can however be overcome with broad spectrum antibiotics, taking the former into account.
Maintaining SPF status is paramount, so in the event of a result indicating the presence of a pathogen, the entire herd should be replaced. Depending on the nature of the infection detected by the laboratory, the animals may be used for the experiment or replaced immediately. No result can be expected from the self-evacuation of some rooms or "sections", so the SPF space of the whole animal house must be evacuated uniformly.

What to do in case of any illness detected

If a dead animal is found, it is taken out of the cage and placed in the freezer until the arrival of the veterinarian, who decides his further fate. Mice autolyze within a few hours after death (due to their very rapid metabolism), an evening corpse can no longer be examined by morning, so there is little hope for any diagnostic results. Rats can be examined for a longer period of time after death.
It must be ascertained whether this is an individual case or whether several animals are involved. In the event of the simultaneous illness of several animals, the veterinarian must be notified immediately.
Observation of live animals is of diagnostic value. Sluggishness, lethargy, malaise, diarrhea, musculoskeletal disorders are anomalies, which can be a sign of infection or even a malnutrition (e.g., rancid or toxinous feed).
The veterinarian must not dissect the carcass for diagnostic purposes. Pathological examination in the animal house can only be used for a partial diagnosis, e.g. pneumonia or spleen enlargement, etc., which is unsuitable for detecting diseases. In sporadic cases resulting from the individual existence of the animal, e.g. in the case of bloating due to intestinal obstruction, a home autopsy is indeed of diagnostic value, however, such a case is very rare in the animal house. The microclimate of the animal rooms is artificially regulated, so signs of a common cold can only occur if the climate fails. Some infectious diseases are called they can cause smudged symptoms in the form of atypical pneumonia or mild bleeding throughout the body under the serosa of the internal organs, so that relying on such symptoms alone can be a victim of serious diagnostic error. The laboratory sends the result based on a complex test sequence.
The veterinarian must immediately send the animal to a diagnostic institute. The Hungarian institute - although its autopsy department is professionally well prepared - does not make a diagnosis of a disease, but only gives an opinion on a specific organ or tissue, which makes the situation of the referring colleague more difficult. In Amendment, the results will be returned to the hands of the veterinarian with a delay of 2-3 weeks, during which time measures might be taken that could significantly affect the further fate of the stock. In possession of the finding, it can be decided whether the herd is suffering from an infectious disease or whether only an individual disease has occurred.

Quarantine rules

When purchasing sentinel animals or to verify the hygienic status of the incoming animal, place them in quarantine.
Upon arrival, one animal per consignment and per strain was bled, the whey was separated as previously described and sent to a serological laboratory (BioDoc lab).
Until the result arrives, they remain in the quarantine room, in a closed cage with a filter lid, in quarantine. During this time, no animal caretaker can enter the room, so they must be provided with food and drinking water properly. No more than 5 animals can be kept in a box.
In the case of a positive test, the animals must be dispensed and destroyed in the filter-covered cage. The caregiver's gloves should be discarded and your clothing should be disinfected. The quarantine room must be thoroughly sterilized.
In case of a negative result, no other intervention is required, the animals can be moved to the animal rooms.
Animals arriving continuously from a reliable vendor from the same lock can be placed directly in the animal rooms, no control tests are required.
Room sterility
At the start of the clear space, sterility is achieved by washing the walls and floor with a broad-spectrum (bactericidal, virucidal and fungicidal) disinfectant and then by disinfection gaseous form of formalin. Maintaining it is one factor in maintaining the hygienic level of the SPF.
Animal rooms and Amendmental rooms (corridor, litter, etc.) should be emptied quarterly, racks (both open and IVC) walls and floors should be thoroughly washed and disinfected.
At the time of testing the status of the animals, a swab sample (walls, floor, scaffolding, footwear, etc.) must be taken to check sterility and taken to the NÉBIH Veterinary Diagnostic Directorate. They also provide a sterile swab. The number of samples is determined by the number of rooms and objects to be examined. A record must be kept of the sampling. The place of sampling shall be indicated next to the sample number in the accompanying document.

Significance of hygiene status

In a clear space, it is a particular requirement that mice be kept and bred under high hygienic conditions.
1) The hygiene requirements for an animal house set up for research on genetically modified (GM) animals are significantly higher than for animal houses set up for other purposes.
2) A significant proportion of special strains bred in a clean space cannot or are very difficult to replace in the event of destruction due to disease, infection or other reasons (unlike, for example, commercially available strains).
3) Acquiring special GM mouse strains housed in a clean space and establishing individual colonies usually costs in the order of millions, so losing them can cause severe damage. It is likely that there are / will be lines that are not found elsewhere (worldwide). The loss of individual strains also results in a significant time lag in international competition. Therefore, the expected total market value of mice to be kept in a clean space is in the scale of hundreds of millions of HUF and their ideal value is even higher. Such valuable colonies deserve outstanding hygienic protection.
4) The operation of a clean space is also most appropriate in terms of cost efficiency and work organization when the entire clean space operates as a unit. In such cases, however, routine work processes (eg.: washing cages together) do not allow the protection of mice of certain strains and research groups against infections in other mice. It is therefore in the best interests

of researchers to keep all animals under high hygienic conditions. In the event of a possible infection, it is of course possible to isolate the infection (quarantine, mandatory autoclaving, etc.), but this goes beyond routine workflows.
5) A number of mouse strains with weakened immune systems can be housed in the clean space. They are more susceptible to infections.
6) The possible infection of the animals may affect the outcome of the particular test / experiment. A classic example of this is the examination of the immune system, but in other tests the infection of the animal can be a significant influencing factor.
7) Serious scientific research now expects the transgenic mice used to be kept under special conditions (e.g., SPF). This demand is likely to become more widespread in the future.
8) The European Union is laying down increasingly strict rules for the keeping of experimental animals. Only a high-purity animal house can meet these conditions.

Significance of SPF animals in research

In pharmaceutical trials, the effects of the active substance on animals can be assessed if the body is not adversely affected by any infection.
An important measure of scientific results is the repeatability of experiments. The results of a work can be reproduced if animal-related factors such as genetic background and housing conditions are similar under housing conditions.
Animal houses loaded with pathogens differ in terms of hygiene status. They are burdened with different diseases in each animal house, in many cases the presence of several pathogens can be detected. SPF animal houses, on the other hand, can be considered hygienically identical, whether in Japan or in a European country, if they are disease-free according to the international recommendation. The repeatability criterion of the experiment is thus hygienically ensured in two SPF animal houses if the hygiene status is determined according to the same criteria.
The results of research are greatly influenced by infections. Some pathogens can cause a coarse infection, e.g. the mouse hepatitis virus (MHV) affects the results of immunological and tumor research, or the Interleukin 10 knockout mouse is prone to severe intestinal inflammation (colitis), rectal prolapse, and poor reproduction in the conventional animal house due to genetic modification. When kept under SPF conditions, these unfavorable properties disappear.

Hygiene of conventional animal houses

Getting to know the infections

Animal houses where the surveys outlined above have not been carried out or the presence of pathogens on the FELASA list has been established are classified as conventional, i.e., infected.

In many cases, the infections are not uncommon and need to be assessed and repeated annually.

The hygiene status of the animals entering the animal house shall be certified by the veterinarian of dispatch.

In the case of periodically operating livestock houses (where there are only occasional animals, there is again an animal-free period after an experiment), it is not necessary to perform these tests. The infection of the animals can be considered as identical to the certificate issued by the sending veterinarian.

Control of infection access

According to the existing infestation of the holding, the responsible manager shall decide, on the basis of a previously received status certificate of the animals to be received, on the recommendation of the attending veterinarian, whether or not to accept the animal from outside. There are infections that cannot occur in livestock under any circumstances, such as mouse hepatitis virus (MHV), external parasites (lice and ryegrass), nematodes (Syphacia and Aspiculuris species).

If an infection enters the animal house, it cannot be removed because continuous animal husbandry does not break the chain of infection. The only possible way to get rid of infectious agents is by complete evacuation and thorough liquid and then gas disinfection with a broad-spectrum disinfectant. However, all external / internal parasites and some viruses (eg parvovirus) are highly resistant to external influences, so that disinfection may reoccur over time despite disinfection. Thus, the person authorizing admission bears a great responsibility.

Amendment "a"

FELASA list

http://www.felasa.eu/recommendations/recommendation/recommendations-for-health-monitoring-of-rodent-and-rabbit-colonies/

Amendment "b"

Reliable suppliers as of 2020

Supplier of imported animals:

Akronom Kft
CEO: Novotta Csaba,
1149 Bp, Angol utca 34.
Landline phone: +3617999212
info@akronom.hu

Innovo Kft.
http://innovokft.hu/

Company head office and site:
2117 Isaszeg, Ady Endre út 47.
Landline: +36 28 582035
Landline: +36 28 582036
Cellphone: +36-30/940-4173

Animalab Hungary
CEO: Kovács Róbert
H-2600 Vác, Naszály út 18.
Cellphone:06 70 431-4441
info@animalab.hu

Domestic supplier:
Toxi-coop Kft
Contact person: Balogh Zoltán
1103 Budapest, Cserkesz utca 90.
Cellphone: 20/926-4459, 261-8266
Fax: 261-9162

All documents received with the animals (consignment note and status certificate) must be filed.
Genetically modified (GM) animals may enter the SPF space only in the case of animals purchased from trusted vendors, and animals from research laboratories, without exception, may only enter the SPF space by embryo transfer (ET) rederivation.

GM service can be ordered at the following address:
MTA KOKI OGR
Contact person: Dr. Erdélyi Ferenc
1083 Budapest, Szigony utca 43.
Cellphone: 20/927-1520, 299-1002

Amendment "c"

Testing institutes

Tests confirming the hygiene status of the animal house may only be performed in an accredited laboratory.

Domestic research institute

National Food Chain Safety Office (NÉBIH)

Veterinary Diagnostic Directorate

1149 Budapest, Tábornok utca 2.

Dr. Erdélyi Károly

Cellphone: 20-957-2776, 460-6356

Fax: 222-6071

Institute for Serological and Helicobacter Testing

BioDoc
Biomedical Diagnostics
Feodor-Lynen-Strasse 23
30625 Hannover
Germany

Phone: +49 (0) 511 548885
Fax: +49 (0) 511 548886

Annex 2
Audit trails

process steps	**preparation steps**	**task manager**	**controlled by**	**control method**	**approved by**	**mode of approval**	**document generated as a result of a process**
1) Project permit - approval of the permit application by MÁB - submission of a permit application to the Pest County Government Office	the project manager prepares the project authorization application document	project manager	n.a.	n.a.	MÁB	signature	approval document
2) accredited course organization, courses conducted	- renewal of accreditation - recruiting instructors - announcement of the courses	MÁB (chairperson)*	ÁTET	- request of course documentation - personal appearance	n.a.	n.a.	Certificate
3) annual MÁB education organization, holding education in 2-3 times	announcement of the courses	MÁB (chairperson)*	Pest County Government Office	document verification	n.a.	n.a.	Attestation
4) submission of an operating permit application to the Pest County Government Office	preparation of the permit application document	Head of Institute	Pest County Government Office	personal inspection	n.a.	n.a.	operating permit

5) providing of annual data: - aggregation of data - submission of data to NÉBIH	collecting data from users	MÁB (chairperson)*	n.a.	n.a.	n.a.	n.a.	chart on university annual animal use
6) register of animals: - documentation of incoming animals - documentation of issued animals - documentation of dead and killed animals - individual identification where necessary - documentation of sick animals	development of a registration system	leader of the animal house	MÁB	personal inspection	n.a.	n.a.	registration document
7) action plan - creating an action plan - submission of the action plan to the Pest County Government Office	assessment of the health of the animals	leader of the animal house	MÁB	check of documents	experiment leader veterinarian	approval assessment of the health of the animals	document of the elaborated action plan
8) house rules: - creating house rules - publication at the university (in a visible place and on the MÁB website) - review every 5 years or less	part of the house rules: - developing a health strategy - developing an environmental enrichment strategy - review of the stretegies every 5	leader of the animal house	MÁB	check of documents	n.a.	n.a.	document of the house rules

	years or less						

9) acquisition of experimental animals - demand assessment - consultation with breeder - receiving animals	order	the person responsible for the project or the person in charge of breeding or the head of the animal house			leader of the animal house	approval	order sheet

*Basically, it is the task of the MÁB, if a person has to be nominated, this is to be the chairperson of MÁB.