UiO : Universitetet i Oslo
Institutt for medisinske basalfag, Avdeling for komparativ medisin

Standard operasjonsprosedyre: Routine health monitoring of rodents
SOP nr: 13-01
Opprinnelig dato: 17.04.2020 Forfattet av: Katarzyna Joanna Zelewska
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ROUTINE HEALTH MONITORING OF RODENTS

1.0 PURPOSE

1.1 The routine health monitoring of rodents housed in the KPM facility is central to the operation of the facility and is crucial for obtaining information about, and for controlling, the microbiological status of the animals housed inside the facility, and for reducing the risk of contamination from unwanted agents.

1.2 The aim of this document is to define the procedure for the routine health monitoring (HM) of rodents and to define criteria for the acceptance or elimination of unwanted agents. This procedure is related to the SOP “Procedure for information and actions upon receipt of health monitoring results”.

2.0 DISTRIBUTION OF RESPONSIBILITY

2.1 The KPM facility is responsible for creating, maintaining, distributing and revising this procedure.

2.2 The Head of Department (Designated Veterinarian) is responsible for approving the procedure, for gathering veterinary advice relevant to this procedure, and for generating HM reports for communication to users on the KPM facility web page. All users of the facility and facility staff are responsible for adhering to the procedure at all times.

2.3 User must share with PMSK all positive results if they take samples themselves and send to commercial diagnostic suppliers.

3.0 PROCEDURE

3.1 Special guidance on testing Clostridium piliforme is included in appendix I and II.

3.2 KPM, The University of Oslo, has an agreement with Idexx Bioanalytics to provide an analysis of HM of rodents. Routine HM is performed by the KPM staff every third month by the sampling of sentinels and random animals. Once a year (in December), an extensive panel of agents is screened, followed by three intermediate screenings (in March, June and September), also for a defined panel of agents. For all samples, routine HM is based on serological analyses (dry blood spots and virus) and PCR analyses of fecal pellets and fur swabs (virus, bacteria and parasites). The panel of agents included in the annual and intermediate screens can be found in Tables 1-2.

3.3 **Definition of microbiological units:** One room with one or more IVC racks comprises the regional microbiological unit. Routine HM results are presented both per room (in the microbiological unit) and per species (in all rooms).

3.4 The movement of animals between units must be approved by the Designated Veterinarian with the cooperation of PMSK (personnel with special screening responsibility). Animals can only be moved from a unit with a higher microbiological status to one of a lower status. In some cases, the Designated Veterinarian can make an exception to this procedural rule.

Use of sentinels

3.5 Sentinels from outbred stock of minimum SPF-status are ordered from an approved supplier. Current sentinels are Sprague-Dawley (RjHan:SD) and SWISS (RjOrl:SWISS). Sentinel mice must be females of 4-5 weeks of age upon delivery to the facility. Sentinel rats can be of both sexes of 4 weeks of age upon delivery. Female sentinel rats are preferred in the KPM facility as they are less potent allergen shedders than males. Sentinels are housed in groups of two animals per cage per IVC rack. The sentinels serve up to 70 mice cages, 35 standard rat cages (GR900) or 16 double decker rat cages (GR1800). The sentinel cages must be placed in the bottom right space on each rack.

3.6 Sentinels are exposed to unwanted agents present in the rack by transferring a standardized amount of dirty bedding from all cages in the local microbiological unit (IVC-rack) to a clean cage during cage changing. Dirty bedding from each cage is transferred to the empty cage and the amount will vary according to the number of cages currently in use in the local microbiological unit, but at least 50% of the sentinels' bedding should be dirty. When all cages in the local microbiological unit are changed, the collected dirty bedding is transferred in a suitable standardized amount to a clean cage, and the sentinels are transferred to this cage. The sentinel cage is always changed last in the local microbiological unit. Sentinel mice cages should have running wheels. The water bottle in the sentinel cage is replaced with a dirty water bottle from a random cage from the local microbiological unit, refill the bottle with any other bottles in the same unit.

Use of random animals

3.7 Random animals are animals housed in the local microbiological unit, but not sentinels. The sampling of random animals increases the likelihood of detecting any agents present in the local microbiological unit that are not easily transmitted horizontally. Eight (8) random animals should be selected from each local microbiological unit. If possible, random animals should be older than 7 weeks of age and preferably selected from various strains and dates of arrival. The sampling of random animals is non-invasive and non-terminal.

Sampling

3.8 Hygiene procedure between sampling from different racks (units): Remove outer gloves. Forceps and the restraining tube, LAF-bench/laboratory bench etc. must be rinsed with soapy water and disinfected with 70% ethanol. Pens, notes etc. have to be disinfected with 70% ethanol. Put on new gloves.

3.9 Materials for sampling and shipping can be ordered at http://www.idexxbioresearch.eu/resources-health-monitoring. For assistance send e-mail too: IDEXXBioAnalytics-Europe@idexx.com. Given correct packaging and storage, samples can be stored for a few days before pick-up without affecting the analyses. The Head of Department (Designated Veterinarian) delegates tasks related to routine HM among the facility staff. Sampling is carried out according to instructions from Idexx

Bioanalytics. The way PCR samples are pooled for a microbiological unit is decided by Head of the department.

3.10 It is sometimes necessary with two facility staff members for sampling (one person does the sampling, while the other is responsible for assisting with the system of sampling tubes and bringing cages for sampling). Before sampling, the staff agree on which animals to select as random animals. All sampling tubes and Opti-spot cards are marked with the sample ID prior to sampling and bags for samples are also labelled. Cage cards are updated with information about routine HM sampling and information is added to the animal journal in SL.

3.11 **Materials needed:** 70% ethanol in a suitable beaker; two forceps (if desired), test tube rack, cage lid, bench paper, 25 G syringes (blue). Two forceps are used – one is placed in 70% ethanol while the other is in use within a local microbiological unit. When proceeding from one local microbiological unit to another, the second forceps are used and the first is placed in 70% ethanol (to eliminate DNA/RNA contamination between local microbiological units). The forceps are wiped clean and placed in 70% ethanol for a minimum of 5 minutes. The cage lid is placed on the bench paper (for collection of feces), and the animals are handled on the cage lid. The collection of samples from rats can be done using general anaesthesia (Isoflurane) as alternative to handling. Samples are collected in the following order (feces whenever available):

- **Fur swab:** The animal is placed on the cage grill and is restrained by the tail. A sterile, dry swab is rolled thoroughly over the fur over the back, behind the ears, in the groin and under the abdomen. The swab is then placed in the collection tube and cut at the weak point. Up to 10 fur swabs can be pooled per tube and samples are taken from sentinels and random animals for each routine HM.

- **Blood samples (Opti-spot cards):** Place a mouse in a custom fitted 50 mL NUNC tube or cage grill, hold at the base of the tail, poke vein with needle/lancet, blot 2-3 blood drops onto the circled area of the Opti-spot strip. The cardboard within the circle must be saturated with blood and blood should preferably fill the whole marked circle. Blood samples for sentinels in the same cage are pooled on the same card. The Opti-spot cards must be left open to dry for at least 30 minutes on the laf bench before they are closed and packed in a sealed bag with silica gel, one bag per local microbiological unit. Place the bag on the bench and extract the air before sealing it. For routine HM, blood samples are taken from sentinels only. Blood from rats is taken by puncturing the tip of the tail.

- **Feces:** Fresh fecal pellets are collected directly from the animal or by using sterile forceps from the bench paper/cage lid. One pellet is collected per animal, and up to 10 fecal pellets can be pooled in the same tube. Fecal pellets are sampled from both sentinels and random animals.

  Clean all bench surfaces with soapy water and disinfect with 70% ethanol after sampling is completed. Clean and/or disinfect all equipment. Sweep the floor.

Special precautions for DU-008A and DU-035

3.12 Taking of samples from animals under toxicological studies or animals treated with isotopes is not allowed. Take samples from control animals or other non-treated animals.

Packaging and shipping of samples

3.13 All swab samples are to be stored at room temperature until shipping. Fecal samples can be stored at room temperature if shipped on the same day as they are sampled, or in a fridge if shipped the next day or later. Opti-spot cards must be placed in sealed bags with silica gel the same day as they are sampled, and be stored in room temperature until shipping.

3.14 All plastic bags containing samples must be marked with the correct ID and packed in the silver transport box provided by IDEXX Bioanalytics. Before closing the box, the responsible person handling the shipping ensures that the number of samples and the packaging and marking of the samples are in accordance with the submission form, that the transport box is correctly marked and that the number of boxes are in accordance with the transport documents to be delivered to DHL. Samples are submitted online at: https://secure.idexxradil.com/customer/CSLogin.aspx. A proforma invoice must be filled out and placed on the box together with the other transport documents. A printed copy of the submission forms must be placed inside the box.

3.15 Remember to be consistent in the use of names for the relevant building, area, room and rack when registering samples for submission, so that historical data can be generated.

3.16 To book DHL for pick-up, follow the separate booking instruction provided by IDEXX Bioanalytics. Note the tracking number for the shipping of the samples. Deliver the transport box(es) to the Reception room at KPM, where it/they will be picked up by DHL.

Communication of results

3.17 The results of routine HM must be communicated in FELASA-style format, including results for the past 18 months. Reports must be generated for each room level and also in the form of complete reports for each species. Reports are made available to users on the KPM web page. See: SOP: “Procedure for information and actions upon receipt of health monitoring results”.

4.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)

4.1 Everyone who handles animals must have adequate training and practice.

4.2 Everyone must have adequate training to ensure that they use the proper PPE.

4.3 The work must be carried out under ventilation to avoid exposure to allergens and the spread of potential contamination to the surrounding areas.

4.4 Everyone who handles chemicals must have adequate training and access to proper protective gear to ensure the safe use of chemicals.

4.5 Everyone should be familiar with the Eco Archive and Safety Data Sheets for the chemicals they may be exposed to.

4.6 Users must familiarize themselves with the SOP “Clothing in the Department of Comparative Medicine”.

4.7 **Chemicals/biological agents**

Kemetyl technical ethanol 96%	CAS no	Pictogram	Hazard statements	Precautionary statements
Ethanol	64-17-5		H225 Highly flammable liquid and vapour H319 Causes serious eye irritation	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P370 + P378 In case of fire: Extinguish with carbon dioxide (CO2), foam, powder or water.

Chemical	CAS no	Pictogram	Hazard statements	Precautionary statements
Isoflurane*	26675-46-7		H319: Causes serious eye irritation H361: Suspected of damaging fertility or the unborn child H373: repeated or prolonged exposure may cause damage to organs	P280: Wear protective gloves/protective clothing/eye protection/face protection. P260: Do not inhale dust/fumes/gas/mist/vapours/spray.

* Use substance under proper ventilation. Wear uniform and chemical-resistant gloves, covering sleeves. No extra PPE is mandatory under normal use and when executing regular technical tests. Safety glasses are recommended. A proper facemask to protect the user from vapours is mandatory if levels of exposure exceed approved limits. **It is recommended not to handle Isoflurane if you are pregnant or planning to be pregnant. Pregnant or breastfeeding women must not under any circumstances handle Isoflurane without a prior risk assessment and proper PPE.**

SPECIAL PRECAUTIONS NECESSARY DUE TO REPRODUCTIVE TOXICITY

4.8 Generally, it is not recommended to work with a chemical that is cytostatic, carcinogenic or has potentially negative effects on the reproductive system if you are planning to be, or are, pregnant. If a chemical is proven to pass into breast milk, it is not recommended to perform a procedure involving the use of this chemical if you are breastfeeding. If you are working with Class 2 biological agents that may cause infections, you should consider the risks, using the relevant PSDS and other relevant documentation.

4.9 Planning pregnancy (men and women): Evaluate the individual procedure for the specific SOP or MSDS (Material safety data sheet) for each activity. If you are pregnant: Based on the activity in the facility, pregnant women are not recommended to stay in the area. If you are breastfeeding: Due to the activities in the facility, nursing women are not recommended to stay in the area.

4.10 Special precautions are related to the SOP: "Eksperimentoppsett med helsefarlige stoffer" (Conducting experiments with hazardous substances).

- **Biological agents**

Rodents are classified as class 2 biological agents. Because rodents can be wild species, gene modified or exposed to gene-modified microorganisms, all exposure/handling of rodents should be treated accordingly.

Rodents and their waste products	Containment Level 2 facilities are required
Risk class 2	Risk factors can be related to: -Allergy -Asthma -Tetanus All procedures involving such agents require an individual SOP and must be risk evaluated.

GMM2 agents	Containment Level 2 facilities is required
Risk class 2	Risk factors can be related to: Infection from work with viral vectors All procedures involving such agents require an individual SOP and must be risk evaluated.

RISK ASSESSMENT

Risk assessment with focus on human HSE:

Part of procedure	Unwanted scenarios	Necessary precautions	S*K (Probability*Consequence Scale from 1 to 4)
Animal handling	Exposure to allergens	Wear personal protective equipment.	1*4
Animal handling	Animal bite	Use proper restraining technique. Tetanus shot in accordance	2*2

		with targeted health check programme.	
Blood sampling	Needle prick	Use proper restraining technique. Do not re-cap syringes – place used syringes directly into risk container. Tetanus shot in accordance with targeted health check programme.	1*4
Waste management	Risk waste not disposed of according to established policies	All contaminated material must be disposed of as hazardous waste. Syringes and other sharps to be disposed as hazardous waste.	1*2

5.0 EQUIPMENT AND MAINTENANCE

5.1 Fur swabs
5.2 Opti-spots
5.3 70% ethanol
5.4 Suitable beaker
5.5 Two forceps
5.6 Test tube rack
5.7 Custom-fitted 50 mL NUNC tube for restraining mice
5.8 Anaesthesia unit
5.9 Isoflurane
5.10 Paper towels
5.11 25 G syringes (blue)
5.12 Permanent marker
5.13 Eppendorf tubes
5.14 Scissors
5.15 Soapy water
5.16 Opti-spot bag
5.17 Biohazard bag
5.18 Transport box

5.19 Waybill and waybill bag

6.0 HISTORY OF EDITING

6.1 This procedure is based on the current procedure in place at University of Oslo – InVivo facility IBV, and adapted to local routines following an agreement between the Facility Management, the Head of Department and Group Leaders and based on advice from the Designated Veterinarian. The procedure is revised annually, or when new information of relevance becomes available.

6.2 08.05.2020; Updates on hygiene between sampling from different racks (units). Items added to "Equipment and maintenance". Points added to "HSE".

6.3 19.06.2020; appendix 1 and 2 added.

6.4 19.04.2022 added sentence: User must share with PMSK all positive results if they take samples themselves and send to commercial diagnostic suppliers. (K. Zelewska and H. Tandberg)

7.0 REFERENCES

7.1 SOP University of Oslo – InVivo Facility IBV

7.2 Mähler M. at all. FELASA recommendations for health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab. Anim. Vol. 48, 2014.

Tab.1 Health monitoring profiles MOUSE

Agent	**Analytical technique**	**Intermediate**	**Annual**
VIRUSES			
Mouse hepatitis virus	MFI/IFA	X	
Mouse rotavirus (EDIM)	MFI/IFA	X	
MNV, murine norovirus	MFI/IFA	X	
Parvoviruses: - Minute virus of mice - Mouse parvovirus	MFI/IFA	X	
Theiler's murine encephalomyelitis virus	MFI/IFA	X	
Lymphocytic choriomeningitis virus	MFI/IFA		X
Mouse adenovirus type 1 (FL)	MFI/IFA		X
Mouse adenovirus type 2 (K87)	MFI/IFA		X
Mousepox (ectromelia) virus	MFI/IFA		X
Pneumonia virus of mice	MFI/IFA		X

Reovirus type 3	MFI/IFA		X
Hantaviruses	MFI/IFA		X
Sendai virus	MFI/IFA		X
Lactate-deydrogenase elevating virus	MFI/IFA		X
Polyomaviruses (mouse polyomavirus, K virus)	MFI/IFA		X
Herpesviruses (mouse cytomegalovirus, mouse thymic virus)	MFI/IFA		X
BACTERIA AND FUNGI			
Helicobacter spp (with differentiation to species level if positive: H. hepaticus, billis, typhlonius, ganmani, mastomyrinus, rodentium)	PCR	X	
Pasteurella pneumotropica Heyl	PCR	X	
Pasteurella pneumotropica Jawetz	PCR	X	
Streptococci b-haemolytic (not group D)	PCR	X	
Streptococcus pneumonia	PCR	X	
Citrobacter rodentium	PCR		X
Clostridium piliforme	MFI/IFA		X
Corynebacterium kutscheri	PCR		X
Mycoplasma pulmonis	MFI/IFA		X
Salmonella spp.	PCR		X
Streptobacillus moniliformis	PCR		X
Filobacterium rodentium (Cilia-associated repiratory bacillus)	MFI/IFA		X
Klebsiella oxytoca	PCR		X
Klebsiella pneumonia	PCR		X
Pseudomonas aeruginosa	PCR		X
Pneumocystis murina	MFI/IFA		X

Staphylococcus aureus	PCR		X
PARASITES			
Fur mites (Myobia, Mycoptes, Radfordia)	PCR	X	
Pinworms (Aspicularis, Syphacia) Syphacia muris, Syphiacia obvelata, Aspiculuris tetraptera	PCR	X	
Giardia muris	PCR	X	
Spironucleus muris	PCR	X	
Cryptosporidium spp	PCR	X	
Entamoeba muris	PCR	X	
Tritrichomonas muris	PCR	X	
Encephalitozoon cuniculi	MFI/IFA	x	x

Tab. 2. Health monitoring profiles RATS

Agent	**Analytical technique**	**Intermediate**	**Annual**
VIRUSES			
Parvoviruses: Kilham rat virus, Rat minute virus, Rat parvovirus, Toolan's H-1 virus	MFI/IFA	X	
Pneumonia virus of mice	MFI/IFA	X	
Rat coronavirus/Sialodacryoadenitis virus	MFI/IFA	X	
Rat theilovirus	MFI/IFA	X	
Hantaviruses	MFI/IFA		X
Mouse adenovirus type 1 (FL)	MFI/IFA		X
Mouse adenovirus type 2 (K87)	MFI/IFA		X
Reovirus type 3	MFI/IFA		X
Sendai virus	MFI/IFA		X
BACTERIA AND FUNGI			
Clostridium piliforme	PCR	X	

Helicobacter spp (with differentiation to species level if positive: H. hepaticus, billis, typhlonius, ganmani, mastomyrinus, rodentium)	PCR	X	
Mycoplasma pulmonis	PCR	X	
Pasteurella pneumotropica Heyl	PCR	X	
Pasteurella pneumotropica Jawetz	PCR	X	
Streptococci b-haemolytic (not group D)	PCR	X	
Streptococcus pneumonia	MFI/IFA	X	
Filobacterium rodentium (CAR bacillus)	PCR		X
Pneumocystis carinii	MFI/IFA		X
Salmonella spp.	PCR		X
Streptobacillus moniliformis	PCR		X
Bordatella bronchiseptica	PCR		X
Corynebacterium kutscheri	PCR		X
Klebsiella oxytoca	PCR		X
Klebsiella pneumonia	PCR		X
Pseudomonas aeruginosa	PCR		X
Staphylococcus aureus	PCR		X
PARASITES – ENDO- AND ECTOPARASITES (REPORTED TO THE GENUS LEVEL)			
Fur mites (Myobia, Mycoptes, Radfordia)	PCR	X	
Pinworms (Aspicularis, Syphacia) Syphacia muris, Syphiacia obvelata, Aspiculuris tetraptera	PCR	X	
Giardia muris	PCR	X	
Spironucleus muris	PCR	X	
Cryptosporidium spp	PCR	X	
Entamoeba muris	PCR	X	
Tritrichomonas muris	PCR	X	

Encephalitozoon cuniculi	MFI/IFA		x

Sampling plan

Month	**Mars**	**June**	**September**	**December**
Testing plan	Intermediate	Intermediate	Intermediate	Annual
Changing sentinels	After sampling and receiving results			After sampling and receiving results

Appendix I

Clostridium piliforme – routine health monitoring program for mice

1. The first sampling:
    a. Serological examination as standard practice
    b. Clinical examination

2. Results:
    a. Negative: serological/clinical examination

        Non-action

    b. Positive serological examination

        Action:

        i. Room(s) with positive serological results are closed for users
        ii. Animal testing is discontinued
        iii. Biosecurity is increased in the suspected area: use of chlorine-based disinfectants, shoes and equipment are changed, cages leaving the room are treated as contaminated material and undergo autoclaving before washing
        iv. Second sampling:
            - PCR - feces samples from sentinel cages and random cage
            - PCR environmental tests – samples from racks, laminar flow cabinet, floor

3. Results:
    a. Negative: clinical/serological/PCR testing

Non-action

b. Positive: serological testing/negative: PCR

Action:

i. Histopathology of seropositive animals
ii. Positive histopathology: radical elimination

c. Positive: PCR/negative serology

Action:

i. Radical elimination

d. Positive: serological/PCR testing

Action:

i. Radical elimination

Appendix II

Clostridium piliforme – routine health monitoring program for rats

1. The first sampling
   a. Clinical examination
   b. Serological examination
   c. PCR testing - feces samples from sentinel cages and random cage
   d. PCR environmental tests – samples from racks, laminar flow cabinet, floor

2. Results
   a. Negative: clinical/serological/PCR testing

      Non-action

   b. Positive: serological testing/negative: PCR

      Action:

      i. Histopathology of seropositive animals

- Positive histopathology: radical elimination

c. Positive: PCR/negative serology

   Action:

   i. Radical elimination

d. Positive: serological/PCR testing

   Action:

   i. Radical elimination