UNIVERSITÉ LAVAL

Direction des services vétérinaires **Standard operative procedure**

Title: Health monitoring program – rodents	Number: CQ-3
Scope: This is a directive from the Direction des services vétérinaires (DSV) for the animal facilities managers of the Université Laval (campus and affiliated research centres).	
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Purpose: Describe the health monitoring program to be followed using sentinels in rodent animal facilities.	Version 4 – EN

General information

- Many pathogens have known effects on the overall health status of rodents and cause significant clinical signs.
- Eradication of pathogens known to have an influence on the validity of experimental results and therefore on the rational use of animals is paramount.
- A sentinel program must be in place to rapidly **detect** the onset of infection and to **prevent** transmission of the pathogen into colonies or animals involved in an experimental protocol.
- The assessment of health status of animals used in research is an integral part of any quality control program and is under the responsibility of the DSV veterinarians.
- A surveillance program with sentinel animals is mandatory for any colony of specific pathogen free (SPF) and for any long-term experiment with rodents.
- The use of EZ-Spot®, HemaTIP™ serology technologies are recommended because they require less blood, thus fewer sentinels.
- It is desirable to test several animals in order to increase pathogen detection. The sentinels may be put directly in contact with colony or experimental animals or indirectly by exposing them to contaminated bedding.
- Rodent colonies should be checked at least four times a year. The frequency of testing in experimental rooms will vary according to the analysis performed by the DSV veterinarians.

- It may not be necessary to have a sentinel program for animals housed for a short period. It must, however, be evaluated by the DSV veterinarians. Here are some criteria to consider:
    - Animals housed for less than 8 weeks.
    - All in-all out management of the room.
    - No equipment shared with colonies having a sentinel program.
    - Animals experimentally infected by a pathogen that can infect the sentinels.

Procedures

Origine of sentinel animals

- Purchase the rodents from recognized specific pathogens free vendors such as Charles River Laboratories, Harlan Laboratories or Taconic.
- Ensure that the animals purchased are free of pathogens that will be tested (eg. immunodeficient mice free of *Pneumocystis carinii*). Respect the PNF CQ-2, Health Status of rodent animal facilities.

Characteristics of sentinel animals

- Use animals of the same species as those housed in the room.
- Choose a strain of animal with an important immune response (outbred strain, young animals).
- Purchase sentinel animals with the following characteristics:
    - Mice: females ≈ 4-6 weeks, CD-1® or Swiss Webster®.
    - Rats: females ≈ 4-6 weeks, Sprague Dawley® or Long Evans®.

Housing and manipulations

- House 2 to 3 sentinels in the same cage while considering the following ratio: 1 sentinel cage for a maximum of 80 cages of mice and a maximum of 50 cages of rats (eg. 1 cage of sentinels by side of ventilated rack).
- Implement the same housing conditions for the sentinels than animals to be monitored (ventilated cages, bedding, diet, etc.).
- Identify sentinel cages with a “SENTINEL” cage card. A label containing relevant information must also be place on the cage card.
- Clearly write the date of the initial exposure on the cage card as well as the exposure method (“direct contact” or “indirect contact: bedding”).
- In order to facilitate the identification of sentinel animals, place their cage at the bottom of the rack.

- Always handle the sentinels **after** other animals. Thus, the sentinel cage must be changed last. If sentinel animals need treatments, take care to change gloves and sleeves before handling other animals.
- Never move the cages of sentinel animals; they must always be in contact with the same group of animals.

Exposition

Indirect contact with dirty bedding

- During cage change, collect one teaspoon (~5 ml) of dirty bedding containing feces and urine from every cage monitored. Total amount of dirty bedding should not exceed 500 ml.
- Make sure that all material used to transfer the dirty bedding is sterile (clean cage, spoon) in order to affect the results. Disposable material can be used.

Direct contact or tests carried out on experimentation/colonies animals

- Use this program after veterinary recommendation to test viruses and bacteria that are poorly transmitted by indirect contact (*Helicobacter*, MNV, external parasites).

Tests and analysis

- Send samples to a recognized laboratory for laboratory animal pathogen testing: Charles River Laboratories (IDEXX RADIL can also be used, upon request).
- Perform tests at least 4 times a year for colonies and as recommended by the DSV veterinarians for experimental rooms.
- Keep the spleen of the animal at every terminal sample collection until the negative results are received. Freeze the spleen at -80 °C and properly identify the tube.
- Never pool samples for serology given the risk of dilution of antigens.
- If the room holds more than one rack, perform the tests in alternation in order to have a continuous assessment of the room. Four tests per year should be performed by support.
- If required, take organs for histopathology analysis.
- Perform the tests as directed.

Colonies: mice and rats

Mice colonies: number of sentinels to test by side of rack

	Bacteria testing	Parasitology	Serology
3 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[2]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[2]
6 months	-----	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[2] **Replace the sentinels**
9 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[3]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[3]
12 months	PCR Helicobacter Microbiology culture[1] 1 sentinel/2	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] CARB, CPIL, ECTRO, HANT, ECUN, EDIM, LCMV, LDV, MAD1 et MAD2, MCMV, MHV, MNV, MTLV, MPUL, Parvovirus (MPV1, MPV2, MVM, et NS-1), POLY, PVM, REO, SEN, TMEV[4] **Replace the sentinels**

[1] Bacteria testing: Upper respiratory culture and Gastrointestinal tract culture.

[2] Serology profiles: QC MFIA Mouse Prevalent Profile.

[3] Keep the spleen of the animal at –80 °C and the other sentinel alive.

[4] Serology profiles: QC MFIA Mouse Assessment Plus.

Rat colonies: number of sentinels to test by side of rack

	Bacteria testing	Parasitology	Serology
3 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[2]
6 months	-----	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2] **Replace the sentinels**
9 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[2]
12 months	Microbiology culture [1] 1 sentinel/2	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] CARB, CPIL, ECUN, H-1, HANT, IDIR, KRV, LCMV, MPUL, MAV, NS-1, PVM, PCAR, REO, RMV, RPV, RTV, Sendai, SDAV[4] **Replace the sentinels**

[1] Optional bacteria testing: Upper respiratory culture and Gastrointestinal tract culture.

[2] Serology profiles: QC MFIA Rat Prevalent Profile.

[3] Keep the spleen of the animal at –80 °C and the other sentinel alive.

[4] Serology profiles: QC MFIA Rat Assessment Plus.

Experimentation: mice and rats

Sentinels are only needed in housing rooms where the protocol lasts more than 8 weeks and in housing rooms constantly occupied by several protocols (1 sentinel cage/80 cages of mice in experimentation and 1 cage/50 cages of rats in experimentation).

Conventional cages: number of sentinels to test by side of rack

	Bacteria testing	Parasitology	Sérology
End of protocol or 6 months	-----	Internal parasitology Terminal: 1 sentinel/2 External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[2] Mice: EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[1] Rats: H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[1] **Replace the sentinels**
End of protocol or 12 months	-----	Internal parasitology Terminal: 1 sentinel/2 External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[2] Mice: EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[1] Rats: H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[1] **Replace the sentinels**

[1] Serology profiles: QC MFIA Mouse Prevalent Profile or QC MFIA Rat Prevalent Profile.

[2] Keep the spleen of the animal at –80 °C and the other sentinel alive.

Ventilated cages: number of sentinels to test by side of rack

	Bacteria testing	Parasitology	Serology
3 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] Mice: EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[2] Rats: H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[2]
6 months	-----	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] Mice: EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[2] Rats: H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2] **Replace the sentinels**
9 months	-----	Internal parasitology Non terminal: 2 sentinels/3	Terminal: 1 sentinel/3[3] Mice: EDIM, MHV, MNV, Parvovirus (MPV1, MPV2, MVM et NS-1), TMEV[2] Rats: H-1, KRV, NS-1, PCAR, RMV, RPV, RTV, SDAV[2]
EZ-Spot or HemaTIP		Non terminal: 2 sentinels/2	Non terminal: 1 sentinel/2[2]
12 months	PCR Helicobacter Microbiology culture [1] 1 sentinel/2	Internal parasitology Terminal: 1 sentinel/2 Non terminal: 5 cages/side of rack External parasitology Terminal: 1 sentinel/2	Terminal: 1 sentinel/2[3] Souris : CARB, CPIL, ECTRO, HANT, ECUN, EDIM, LCMV, LDV, MAV1 et MAV2, MCMV, MHV, MNV, MTLV, MPUL, Parvovirus (MPV1, MPV2, MVM, et NS-1), POLY, PVM, REO, SEND, TMEV[4] Rats : CARB, CPIL, ECUN, H-1, HANT, IDIR, KRV, LCMV, MPUL, MAV, NS-1, PVM, PCAR, REO, RMV, RPV, RTV, SDAV, SEND[4] **Replace the sentinels**

[1] Bacteria testing: Upper respiratory culture and Gastrointestinal tract culture.

[2] Serology profiles: QC MFIA Mouse Prevalent Profile or QC MFIA Rat Prevalent Profile.

[3] Keep the spleen of the animal at –80 °C and the other sentinel alive.

[4] Serology profiles: QC MFIA Mouse Assessment Plus or QC MFIA Rat Assessment Plus.

Experimentation: guinea pigs and hamsters

Sentinels are only needed in housing rooms where the protocol lasts more than 8 weeks and in housing rooms constantly occupied by several protocols (1 cage with 2 sentinels/50 cages in experimentation).

Conventional cages: number of sentinels to test by room

	Bacteria testing	Parasitology	Serology
End of protocol or 6 months	-----	Internal parasitology Terminal: 1 sentinels/2 External parasitology Terminal: 1 sentinels/2	Terminal: 1 sentinels/2[2] Guinae pigs: CPIL, ECUN, LCMV, PI3, PVM, Sendai, SV5[1] Hamsters: CPIL, ECUN, LCMV, PVM, Reovirus 3, Sendai, SV5[1] **Replace the sentinels**
End of protocol or 12 months	-----	Internal parasitology Terminal: 1 sentinels/2 External parasitology Terminal: 1 sentinels/2	Terminal: 1 sentinels/2[2] Cobayes: CPIL, ECUN, LCMV, PI3, PVM, Sendai, SV5[1] Hamsters: CPIL, ECUN, LCMV, PVM, Reovirus 3, Sendai, SV5[1] **Replace the sentinels**

[1] Serology profiles: QC MFIA Guinae Pig Assessment or QC MFIA Hamster Assessment.

[2] Keep the spleen of the animal at –80 °C and the other sentinel alive.

Sampling

Helicobacter

- Collect fresh feces with sterile forceps and put them in a sterile tube.
- Pool 10 feces in a tube, one for each rack (5 feces by side of rack). Take feces from different cages including the sentinels.
- Send sample to the diagnostic laboratory for PCR testing PCR.

Microbiology culture

- After euthanasia, swab upper respiratory tract and gastrointestinal tract for each animal.
- If sending at Charles River Laboratories, use *Cary Blair* swab (red cap) for gastrointestinal tract and use *Aimes* swab (blue cap) for upper respiratory tract.

  Note: Swabs of the same type from both sentinels can be combined for shipment if both sentinels are euthananized.

- Keep the samples at 4°C and send them to the diagnostic laboratory as soon as possible. The analysis must be done in the following 48h after collection.

Internal parasitology: non terminal or terminal

- 2 possible techniques:

  PCR testing:

  - Collect fresh feces (non terminal) or caecal contents (terminal) with sterile forceps and put them in a sterile tube. Feces from different animals can be pooled.
  - Send sample to the diagnostic laboratory for pinworm testing.

  Fecal centrifugation:

  - Collect fresh feces (non terminal) or caecal contents (terminal) with sterile forceps and put them in a sterile tube. Feces from different animals can be pooled.
  - Perform a fecal centrifugation test using a flotation solution.
  - Examine the slide under a microscope at 10X to detect the presence of pinworms.
  - In addition, perform an adhesive tape test (anus) on the sentinel animals.

External parasitology

- 2 possible techniques:

  PCR testing:

  - Swab the skin of the belly, back and neck.
  - Combine swabs of the 4 animals in the same tube.

    Note: In order to pool the samples, use the appropriate material provided by the lab.
  - Send sample to the diagnostic laboratory for fur mites testing.

  Skin scraping:

  - Use a sterile #10 scalpel blade.
  - Scrape 3 sites of approximately 1 cm^2: neck, back and belly.
  - Transfer loose hairs and debris to a slide and cover with a coverslip.
  - Examine the slide under a microscope at 100X to detect the presence of fur mites.

  Note: This technique must be done on a **previously euthanized** animal.

Non terminal serology

- Collect a drop of blood at the saphenous vein following the blood collection SOP of the correct species and lay it on the EZ-Spot® or absorb it onto the HemaTIP™.

- Follow manufacturer's instructions for blood quantities and shipping conditions.

Terminal serology

- Collect maximum blood volume (intracardiac; terminal method) following the blood collection SOP of the correct species.
- Centrifuge the blood and collect the serum in an aseptic method in order to prevent contamination of the sample.
- Alternatively, lay a drop of blood on the EZ-Spot® paper or the HemaTIP™ and follow manufacturer's instructions for blood quantities and shipping conditions.

Sample packaging

Samples of animal origin which are believed not to contain infectious substances are partly exempt from the regulations on shipping infectious substances. It is therefore not necessary to use packaging meeting the packing instructions 620 or 650 for samples of sentinel animals.

- Use a triple packaging system:
  - Primary receptacle: sample tubes (plastic tubes, culturettes, etc.) closed to prevent leakage.
  - Watertight secondary packaging: watertight plastic bag (eg. Ziploc®) or cooler, depending on the number of samples. This package must include the primary receptacles as well as absorbent material in sufficient quantity to absorb the totality of the samples in the event of a leakage.
  - Outer packaging: cardboard box or bubble mailers.
- Write "Exempt Animal Specimen" on outer packaging.
- Send samples according to laboratory guidelines where they will be transported (dry ice, etc.).

Replacement of sentinels

- Buy new animals 1 to 2 weeks before euthanasia of sentinel animals so they can be in contact (direct or indirect) to increase the likelihood of pathogen transmission.
- Keep one of the old sentinels alive until the negatives results are received.

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SOP updates		
Version 2	April 16, 2015	Addition of hamster and guinea pigs. Clarification of swabs used for microbiology culture.

Version 3	August 15, 2016	Reduction of number of sentinel needed when EZ-Spot or Opti-Spot are used.
Version 4	May 25, 2017	Addition of HemaTIP. Elimination of IDEXX Radil tests. Update of testing required to match the exclusion lists. Clarification of the notes under the tables.