

ANIMAL HANDLING GUIDE V2

Abstract

This guide will show you how the animal handling material used in conjunction with MR Solutions' system are used. It will also give you tips and advice on animal handling.

Document history

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Introduction

This manual is designed to explain the use of the external components provided with the MRS systems.

Part 1 details the functions of the external components of the system.

Part 2 focuses on animal ethics considerations, handling and the safe application of different types of anaesthesia. Safety is considered from both the operator's point of view but also the animal's.

Users who are new to handling animal subjects for research purposes, and specifically for MRI scans, are recommended to read Part 2 before starting to operate the system. This manual provides documentation on the Preclinical Scan software package and the standard protocol library.

Please note

Reading this manual does not exempt users from the obligation to be aware of and to apply all regulations governing their activity. Users remain solely liable for all consequences arising from the use of this product. The aim of the recommendations set out below is simply to help users fulfil their obligations. They are not necessarily exhaustive. Users should ensure that they are not bound by other regulations or obligations, as these vary from country to country.

This document provides general advice. You should always check that the work you are planning on carrying is in accordance with your local laws & rules.

The MR Solutions' team can also provide you with quick guides on specialised procedure (Brain Imaging, Cardiac imaging, Quality control procedures etc). Please get in touch if you require assistance.

support@mrsolutions.com

Chapter 1. USING THE EXTERNAL COMPONENTS OF THE SYSTEM

1.1 Minerve: Universal bed for molecular imaging of small animals

Minerve systems allow the acquisition of high-quality preclinical images of small animals maintained under volatile anaesthetic in stable, reproducible physiological conditions.

It thereby minimises the variability of experimental and examination conditions during image acquisition.

Minerve systems are composed of the following sub-assemblies:

- Multi-station temperature regulation system (URT Multi-station) (1.1.1.1)
- Air circulating pump (PCA) (1.1.1.2)
- Temperature Sensor Unit body (TSU) (1.1.1.3)
- Technical beds for imaging (Mouse, Rat, Rabbit beds) (1.1.1.4)
- Preparation station (Preparation Holder) (Optional)

The technical supports for the imaging system and the animal beds are available in several versions, depending on the type of imaging system used or the animal species concerned.

Each of the sub-assemblies has its own identity plate with its name, model and/or version, and serial number.

The multi-station temperature regulation unit can also be used for other applications besides those linked to imaging.

Specific user manuals are supplied with the devices acquired by the user.

With these devices it is possible to:

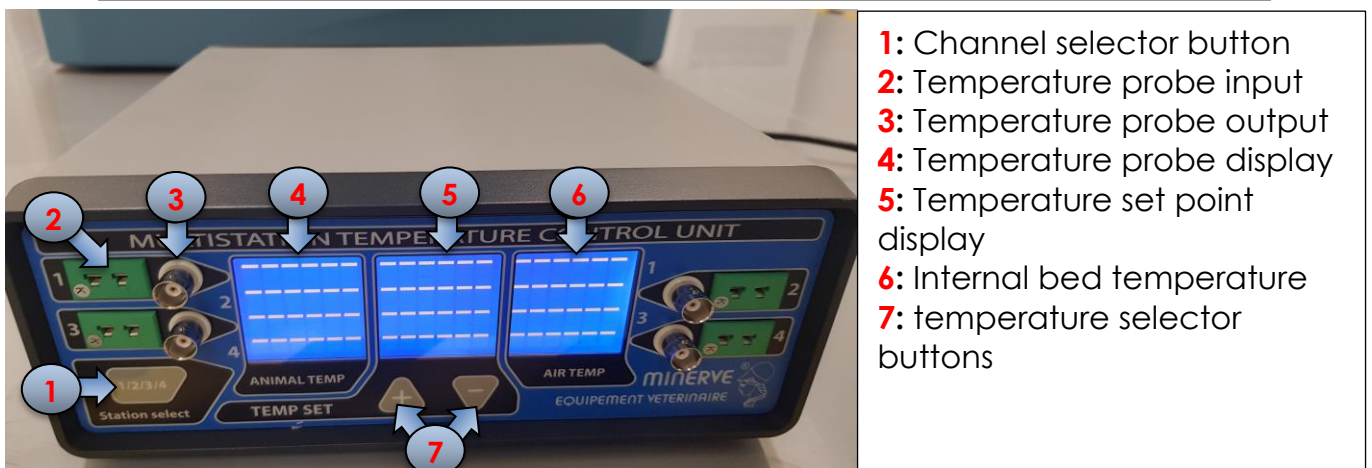
- Maintain animals such as rats, mice, guinea pigs, etc. under gaseous anaesthetic in a perfect state of immobility
- Isolate the animals from the surrounding environment
- Regulate their body temperature
- Adjust the position of the animals with a high degree of flexibility
- Easily install ECG, RESP, Temp probes on the animal
- Transfer the conditioned animal from a preparation zone to an examination zone while maintaining total isolation and without displacing the measurement probes
- Obtain good mechanical compatibility with most preclinical imaging devices
- Ensure low levels of attenuation of radiation used for imaging, thanks to the choice of materials used.

1.1.1 Common Minerve components

1.1.1.1 Temperature Control Unit (URT)

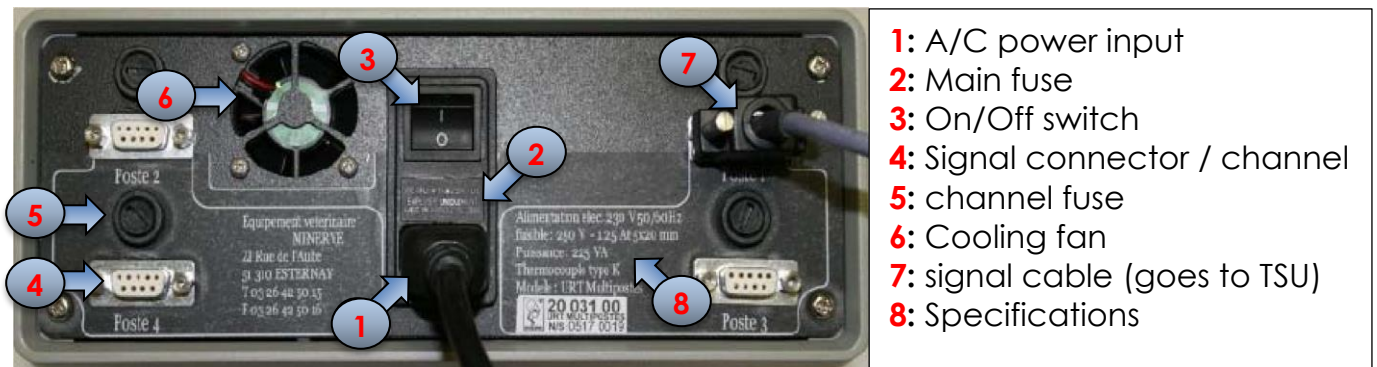
The URT controls the temperature of 1 to 4 accessories either directly (heating plate equipped with a resistance) or indirectly (circulation of air heated by heat exchanger then injected into a circuit to warm the animal).

Please note: It is strongly recommended to connect the MR system to output number 3, since it induces less noise in the system.



- 1: Channel selector button
- 2: Temperature probe input
- 3: Temperature probe output
- 4: Temperature probe display
- 5: Temperature set point display
- 6: Internal bed temperature
- 7: temperature selector buttons

Fig 1: Temperature Control Unit (URT) front



- 1: A/C power input
- 2: Main fuse
- 3: On/Off switch
- 4: Signal connector / channel
- 5: channel fuse
- 6: Cooling fan
- 7: signal cable (goes to TSU)
- 8: Specifications

Fig 2: Temperature Control Unit (URT) back

1.1.1.2 Air-circulating pump (ACP)

The air circulating pump (ACP) connects to one, two or three imaging beds. It circulates air through each of the beds connected to it.

Air introduced into the longitudinal channels of the beds is warmed as it passes through the heat exchanger connected to each imaging bed support (Preparation Holder and TSU).

Please note: Before using the ACP, read carefully the users' manual supplied with the equipment. Never connect this equipment to accessories other than those recommended by the manufacturer. Never wet the device or allow liquids to enter it.

The ACP has no impact on the ventilation of the animal being treated / anaesthetised. The warm air circulation circuit is totally isolated from the interior of the imaging bed.



Fig 3: Air-Circulating Pump (ACP)

1.1.1.3 Temperature Sensor Unit (TSU)

Devices for temperature regulation of the bed (URT + ACP) are connected by means of cable Ref: 2005141 (length 2.5 m) and tube Ref: 2005302 (length 2.5 m).

Connection to the anaesthesia system is by the Induct/Extract circuit (length as required). This coaxial circuit simultaneously delivers the anaesthetic and extracts polluted gases.

The Connection Kit Ref: 5145907 comprises a stop-cock for the anaesthetic circuit and a connector for the anaesthetic line.

Please note: Since temperature regulation of the Rat beds (Ref: 2006401, 2006410) and Mouse beds (Ref: 2006402, 2006411) is carried out by warming the air injected into the internal channels of the bed, users should ensure that beds are properly connected to the URT (cable ref: 2005141) and the ACP (tube ref: 2005302).

Never connect cable ref: 2005141 to the preparation holder if the ACP is not in operation. Temperature regulation will not function in the Rat and Mouse beds, and there is a risk of serious damage to the heat exchanger.

Check that the anaesthetic inlet valve is properly closed (cursor perpendicular to the axis of the valve) when no beds are being used with the preparation holder.

The bed connection and mounting systems are high-precision mechanical parts, so users should handle them with due care and attention. Avoid impacts that could cause damage leading to functional problems with the whole of the device.

If the TSU body is disassembled and stored, users should also take all necessary precautions to avoid mechanical shocks.



- 1:** Anaesthetic gas input
- 2:** Anaesthetic gas output (extraction)
- 3:** air in (for bed heating)
- 4:** Signal cable

Fig 4: TSU

1.1.1.4 Animal beds

The Minerve animal beds are designed to facilitate accurate temperature control of the bed extensions and to allow efficient delivery of anaesthetic gas.

Shown in Fig.5 below, the gas and heating section of the bed contains the framework for heating and circulation of gases. The air delivered by the air

pump is heated at the rear of the bed and then circulated through the extension to maintain a uniformly warm surface. Anaesthetic gas mixture is delivered via the inner hose and extracted through the outer hose.

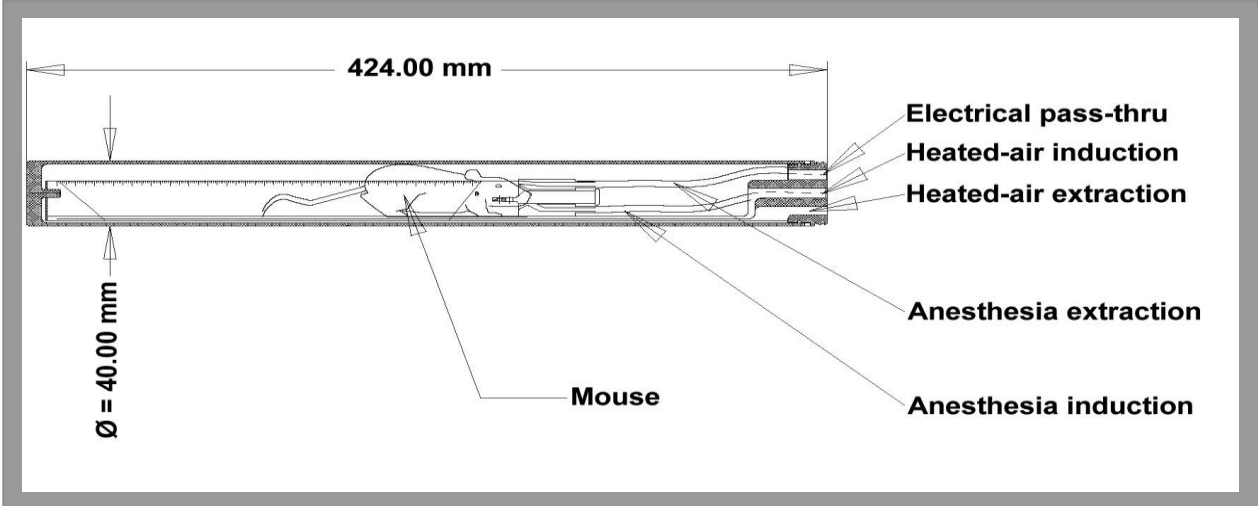


Fig 6: Mouse bed schematic

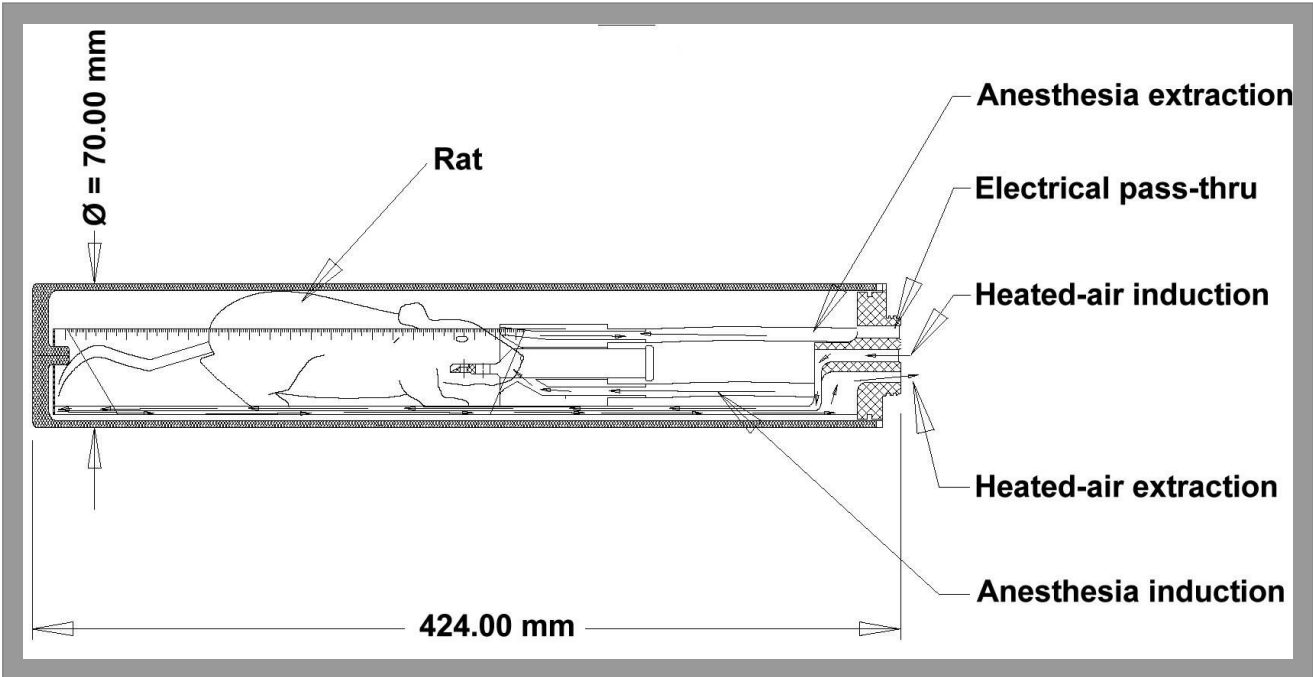


Fig 5: Rat bed schematic

There are several types of beds:

- The whole-body beds, where the animal can be positioned feet first or headfirst, prone or supine.



Fig 7: Rat Whole body bed

- The head beds, where the animal can only be placed headfirst (prone or supine). These beds have integrated stereotactic head holders, which are placed inside the animal's ears. These beds are specifically designed for brain imaging, but they can be used for whole body imaging as well.



Fig 8: Rat and Mouse head beds, and details of an opened stereotactic holder (right)

Minerve also offers what is known as the “mouse hotel”, which allows the anaesthesia and imaging of 3 mice at the same time. The mouse hotel is an insert that fits inside the rat bed.



Fig 9: Mouse hotel insert

1.1.2 Animal preparation

Use a dedicated area close to the place where animals are housed or arrive in transit.

To limit as much as possible the risks of cross-contamination, all accessories connected to the CTI (induction cage, thermo-regulated surgery table, preparation holder, and animal bed) should undergo prior decontamination. If necessary, install them in a micro-bacteriological safety cabinet or a glove-box.

1.1.2.1 Peripheral elements used during animal preparation

These would be all forceps, needles, gauze, catheter etc.

Please note: install these elements in a micro-bacteriological safety cabinet to limit as far as possible the risks of cross-contamination (animal manipulation behind a pathogen barrier).

1.1.2.2 Pre-anaesthesia (or induction) of the animal

The preparatory phase begins with pre-anaesthesia (the induction phase). Here, thanks to the induction cage connected to the anaesthesia station the operator can manipulate the animal according to rules of good laboratory practice (limiting stress and pain).

- Remove the animal from its cage.



Fig 10: example of induction cage

- Place it in the induction cage and close the lid.
- Adjust the flowrate of the carrier-gas to a value of between 1.5 and 2.5 litres/minute.
- Adjust the concentration of the anaesthetic agent (Isoflurane) to between 3.5 and 4.5 %.
- Observe the progressive slowing of the animal's breathing during pre-anaesthesia (generally speaking, it should not descend to a value lower than half the frequency observed when the animal is fully awake). By way of indication, the average time noted for pre-anaesthesia of rodents (Rat or Mouse) is about 2 to 3 minutes.
- Adjust the anaesthetic concentration to between 1.5 and 2% (values for Isoflurane).
To optimize the quantity of anaesthetic gas produced, adjust the flow-rate of the carrier gas to between 0.3 l/ min (maintenance of a mouse under anaesthesia) and 0.6 l/min (maintenance of a rat under anaesthesia) per animal.
- Remove the anesthetized animal from the induction cage.
- Place the animal on a thermo-regulated Minerve bed (connected to an available outlet of the temperature regulation unit).
- Before the system can be used with animals in place, a pre-heating time of approximately 5 /10 minutes is required for the thermo-regulated elements.

CAUTION: Failure to follow this procedure can lead to risks of hypothermia for anaesthetised animals.

- Monitor the physiological parameters of the animals and modify anaesthetic concentrations as necessary.

CAUTION: An excessive concentration of halogen gases could lead to apnoea and cardiac arrest.

Please note: Simultaneous procedures of pre-anaesthesia and maintenance under anaesthetic should not be carried out on the same anaesthesia station (anaesthetic concentration too high for animals maintained under anaesthetic).

1.1.2.3 *Installing the animal in the whole body imaging bed*

This operation is generally performed at the preparation station previously described. It requires the use of a preparation holder (optional) as well as the appropriate imaging bed for the planned application.

However the animal can also be transferred from the induction box straight the animal bed already fitted to the TSU of the animal imaging system.

- 1- Position the animal facing the anaesthesia mask of the bed.
- 2- Place the animal's incisors on the tooth bar provided.
- 3- Move the tooth bar backwards to position the animal's nose in the anaesthesia mask, then lock the mask by means of the knurled screw located on the mask.

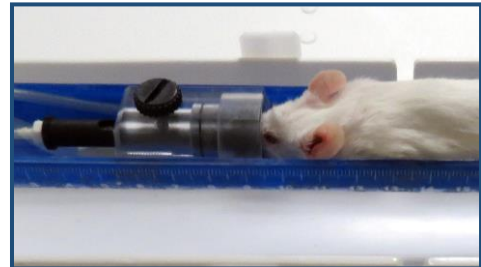
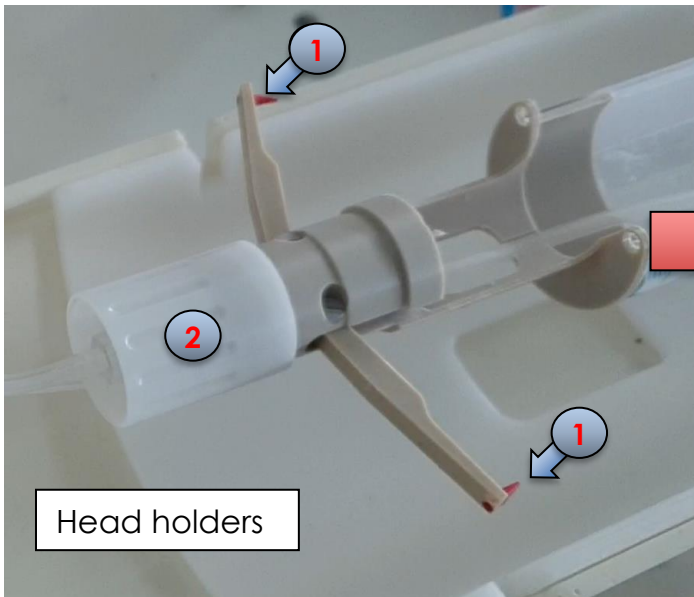


Fig 11: Animal positioned in mask

1.1.2.4 *Installing the animal in the head imaging bed*

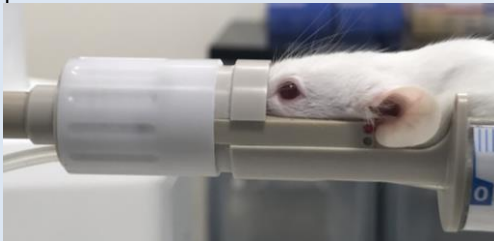
The animal must be placed in the bed in either supine or prone position, and its head must be secured to the head using the bed to avoid any movement during the scan.

- Place the head of the animal on the head platform
- Gently insert the head holders (1) in the ears of the mouse. If you are worried that the inserts are too long, you can trim the ends slightly to shorten them.



Once in positioned, screw in the white lid (2) to keep the holders in place.

Please note: The holders should fit whether the animal is in prone or supine position:



- Once the animal is setup on the bed, estimate the ruler value of where the brain would sit (Usually it is about -15mm).

Tips: Make sure that the head holders are flush with the side of the bed before screwing in the white lid of the bed. If it is not flush, then the animal is not well positioned.

If an eye is either closing or bulging out, this is a sign that the animal is not well positioned onto the bed (holder pressing against optic nerve). Adjust the position.

If the ears do not appear nice and round after inserting the holders, then the mouse is not well positioned.

Take your time, as you could pierce the eardrum of your animal if it is not set up properly!

1.2 Animal monitoring / gating

MR Solutions uses the SAll's monitoring and gating systems for the monitoring of the imaging subjects.

SAll is compatible with MR, PET, CT, SPECT and Optical imaging systems. Sensors measure ECG, respiration, temperature, pressure including invasive blood pressure (optional), oxygen saturation and end-tidal CO₂ in sedated mice, rats and other small animals (optional) undergoing imaging research. Systems continuously monitor animal physiological status while generating gate signals to synchronize scanner data acquisition with cardiac and respiratory motion.

MR-compatible heater and ventilator options are available to control the temperature and ventilation of mice and rats during MR or other imaging procedures.

Please Note: Please refer to the PC-SAM instruction manual for detailed instructions on how to use the software. You can also refer to MR Solutions' "Quick Guide_Cardiac Imaging" for detailed instructions on how to acquire a cardiac cine, and animal set-up.

1.2.1 Hardware setup

Please refer to the "Quick Guide_Connecting the gating system" document for instructions on how to set up the hardware.

1.2.2 Monitoring / gating hardware positioning

The animal's electrocardiograph (ECG) is obtained from two or three leads connected to sub-dermal needle electrodes, gold disk surface electrodes or pads.

The animal's respiration can be measured using a small pneumatic respiration pillow sensor.

Temperature is measured using a temperature probe in the animal's rectum (or sometimes by placing it between the animal and the animal bed).

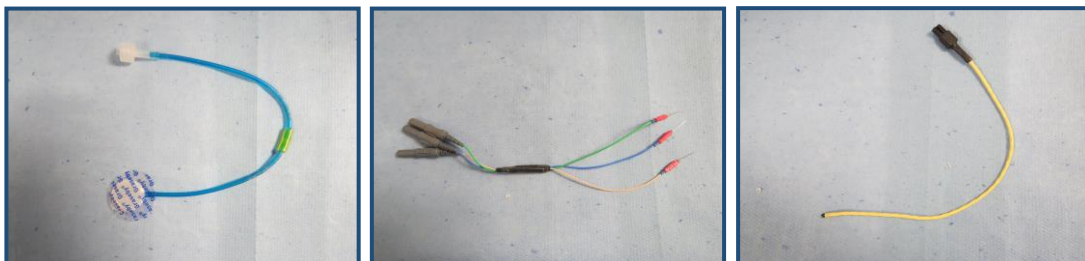


Fig 12: respiration pillow, ECG leads and temperature probe

The above monitoring probes are connected to the ERT module, which digitizes the signal after passing the signals sequentially through an RF filter, amplifier and pulse gradient filter.

That digitized signal is then transmitted out the magnet bore to the ERT Controller using a fibre optic cable.



Fig 13: ERT module

1.2.2.1 Positioning the ERT module

The ERT Module, powered by an external battery pack, is positioned in the magnet bore close to the animal. It may be positioned near the head or near the tail. A rectal probe temperature and respiratory pillow extensions cables are provided for use when the module is near the head.

Because the module can move during gradient activity, it is sometimes necessary to provide restraint by taping it to a stationary object.

If the module vibrates during data collection, artefacts (distortions on the waveform) will be present on the ECG waveform.



Fig 14: ERT module and battery positioned on a coil carrier

1.2.2.2 Connecting the Battery Pack Cable

The Battery Pack Cable attaches to the ERT module and Battery Pack using small Lemo connectors.

To make the connection, align the red dots and push the connector straight into the receptacle. To disconnect the sensor, grasp the connector with the thumb and index finger near the red dot and pull straight back. Do not turn or twist the connector housing.

1.2.2.3 Positioning ECG lead wires on your subject

ECG lead wires should be twisted with each other tightly and be as short as possible. This also applies to any extension:

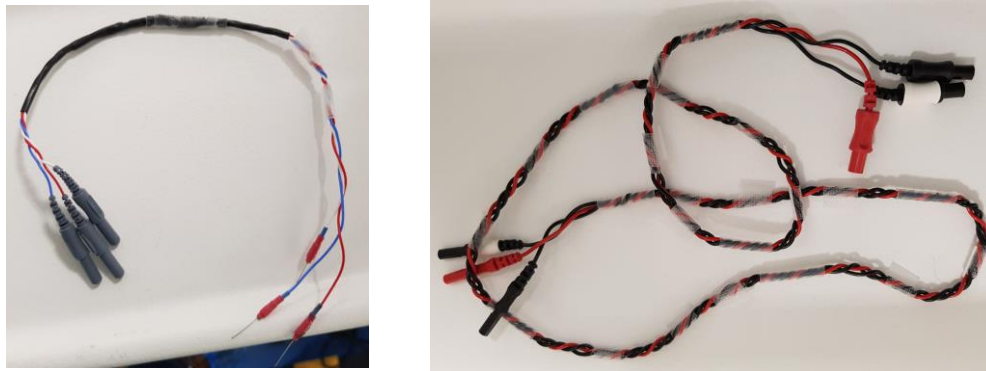


Fig 15: "twisted" wires

Excess wire should be wrapped in a neat coil and taped to collapse or eliminate the loop. Wrap excess wire around two or three fingers creating a loop, then pull the loop into a line and tape. The wire bundle should then be taped to the animal holder to eliminate movement from gradient vibration and/or air flow. Movement of the ECG lead wires will create artefacts in the ECG waveform.



Fig 16: "looping" of excess cable length

Caution: ECG lead wires should be twisted, and unnecessary loops should be avoided. The lead wires should be taped to eliminate unwanted movement from gradient vibrations and/or air flow. ECG lead wires can degrade tuning of the MR imaging coil. As a general rule, the lead wires should be routed as far away from the coil elements as practicable.

1.2.2.4 Positioning the thermistor temperature probe

The thermistor temperature probe will distort the magnetic field for a few mm around the tip, creating a small signal void in the MR image. This artefact is normally only a problem when imaging near the rectum. In that case the probe can be used as a skin probe near the animal's head.

Under some conditions it is possible for the thermistor temperature probe to heat from interaction with the MR scanner's RF coil. Observation of the measured temperature will indicate if this happens. If a temperature rise is observed when the scanner begins to acquire data and the temperature decreases when the acquisition is complete, reposition the ECG/T Module, Battery Pack and cable. A position near the centre line of the magnet bore is generally better than near the edge of the bore.

1.2.3 Fitting of the monitoring probes to the subject

1.2.3.1 ECG electrodes

2 or 3 needles can be used, depending on the signal obtained and your setup. The needles should be placed on the sides of the chest, and on one side near the left hind leg:



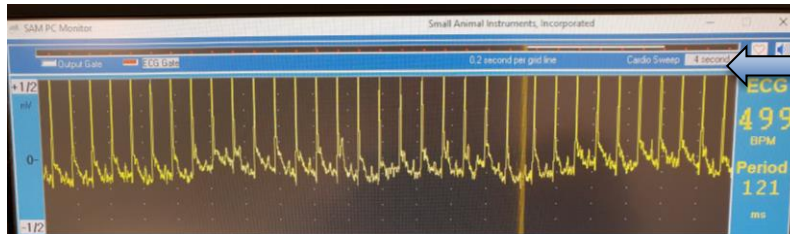
Fig 18: Positioning of ECG needles. The aim is to have the heart positioned between the red and white needles.



Fig 17: Mouse fitted with ECG needles.

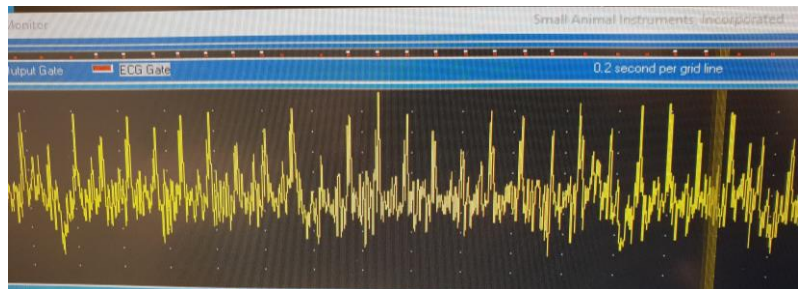
Make sure PC-SAM is on, and that you have a view of the signal obtained. The peaks you get should touch the edge of the Window, or even go out of it.

Example of good ECG signal:



The signal reaches the edge of the window

Example of poor ECG signal:



Important: Do not settle for a poor signal, as signal quality tends to degrade once you move your animal in the MRI FOV. If you do not have a good signal to start with, the gated image will not acquire well, and you will end up with blurry images.

Take your time!

Please note: if the peaks are inverted, you do not have to remove the needles from the animal. Simply switch the cables (red / black) at the plug level. Or work triggering the negative peaks (Please refer to the PC-SAM instruction manual for more info)

When you are satisfied with the signal obtained, make sure to tape the needles in place. After taping, double check that the signal is still strong.

1.2.3.2 Attachment of the respiration pneumatic pillow sensor

- Position the pneumatic pillow sensor near the animal's abdomen and secure it with tape. Try to avoid going up the chest close to the heart, as this may cause some cardiac signal to appear in the respiratory gate.
- Attach the sensor's tube to the ERT Module at the Luer connection. It may be necessary to use one of the Respiration Extension Tubes to make the connection.

The respiration circuit incorporates an automatic gain stage that adjusts the amplitude of the respiration waveform within pre-set limits. After attaching the pillow, small respiratory signals may take a few seconds to gain up and produce respiration gates and a respiration rate.

The respiration waveform exhibits a negative peak during inspiration. The gating algorithm detects the negative peak and produces a respiration gate during the peak. To gate on expiration, check the "invert" box for respiration in the GATING SETUP window.

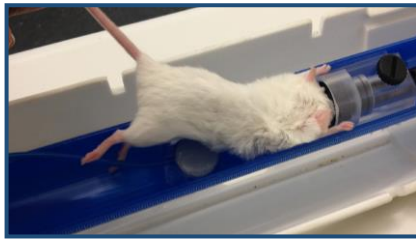


Fig 19: Pneumatic pillow positioning



1.2.3.3 Attaching the temperature probe

- Use a lubricant or lubricated probe cover on the tip of the temperature probe before inserting it into the rectum.
- Secure the probe lead by taping it to the animal's tail. The temperature probe extension cable can be used when the ERT Module is near the animal's head. Clean the temperature probe with isopropyl alcohol.

CAUTION: Temperature probes are fragile and should be handled with care. The tip of the probe can be broken or cracked if the tip is bent. Problems with the temperature probe can occur if it develops a crack.

Since the thermistor in the tip of the probe operates at 5V and a break lets conductive fluid from the animal contact with the thermistor wire, a small offset voltage can occur.

The offset voltage is displayed in the SYSTEM INFO window. It should be 2.5 V +/- 1.0 V for normal operation.

If it goes close to 0 V or to 5 V the ECG will be flat lined by the software and a Lead Off error message will be displayed.

An easy check to see if a large offset is due to the probe is to disconnect the probe from the ERT Module (no need to remove it from the animal). If disconnecting the probe removes the offset, the probe is at fault.

In an emergency a faulty probe will work if placed in a plastic or rubber sleeve (e.g. a thermometer cover or finger from a rubber glove). The sleeve provides electrical isolation.



Fig 20: Temperature probe positioning

1.3 MR Coils

This is a radio frequency coil that surrounds either the whole body, or one specific region, such as the head.

Volume coils have a better RF homogeneity than surface coils, which extend over a large area. The design is a "bird cage" coil; i.e. it consists of a number of wires running along the z-direction, arranged to give a cosine current variation around the circumference of the coil.

The volume coils are both transmitter and receiver coils.

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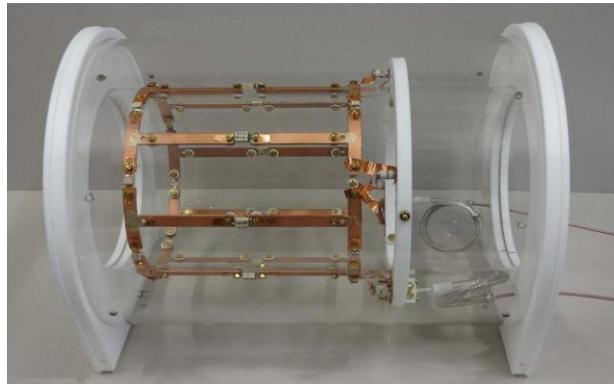


Fig 21: example of a "birdcage coil"

1.3.1 Volume Body Coil: Mouse/Rat

This coil involves the Minerve bed with the animal in it.



Fig 22: Mouse body bed and Volume coil

When the coil is placed in its dedicated slot on the coil carrier, it is automatically placed in the centre of the magnet once in the bore.

This is a coil with 2 channels, where each channel is connected to the exterior through the cables in the front. RIGHT cable corresponds to channel 1, LEFT cable is channel 2.

It is fundamental to ensure that the cables are screwed tightly, to ensure good image quality.

1.3.1.1 Positioning the subject / organ of interest in the centre of the coil

- After placing the animal on the bed and connecting the monitoring probes, you need to define the field of view (FOV) of interest.

The aim is to place the organ of interest as close to the centre of the coil / magnet as possible. To do so, modify the z position of your animal by moving the TSU forward or backwards:

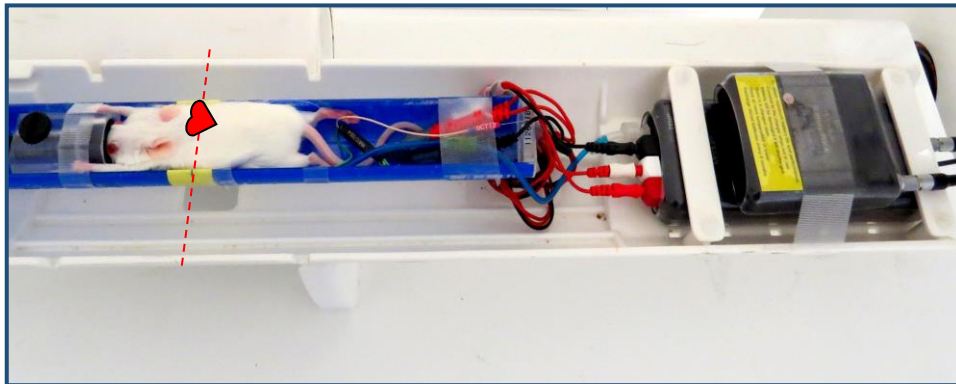
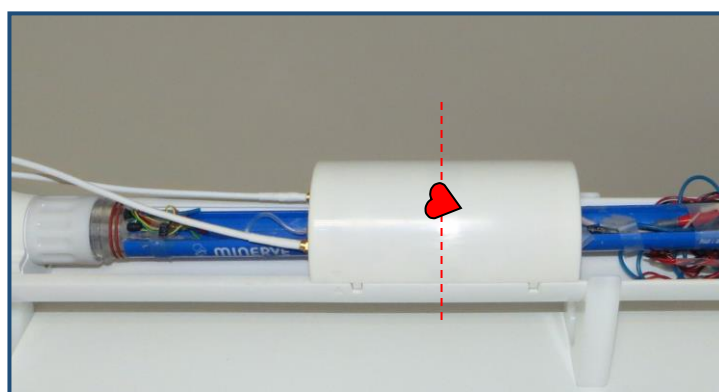


Fig 23: Mouse positioned on animal bed for cardiac imaging (outside the coil)



- To position the mouse in the notches of the coil carrier

Fig 24: Mouse positioned inside the coil for cardiac imaging (heart in centre of the coil)

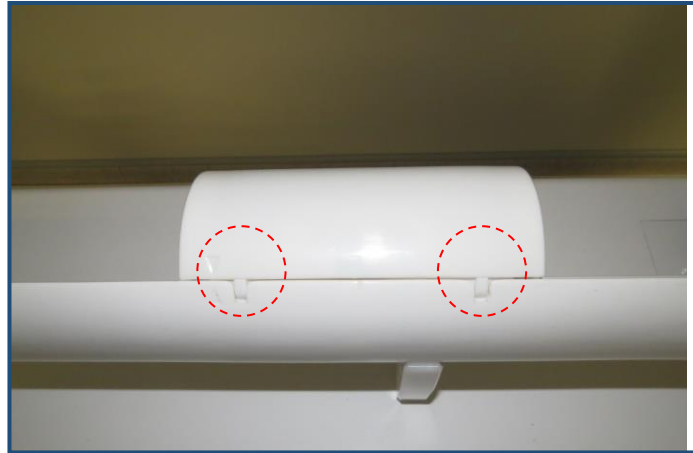


Fig 25: coil slotted in the coil carrier

- Attach the two white cables tightly to the coil.
- Make sure all is contained within the diameter of the coil carrier to avoid obstruction when inserting into the system bore. Use tape if required.
- Insert the coil carrier inside the bore of the system. If you encounter any resistance, repeat the step above.
- Connect the other ends of the white cables to the pre-amp.
- Check one last time that the monitoring signals of your subject are still optimal for the acquisition.

Please note: In some instances, you might struggle to slide the coil around the animal bed when it is connected to the TSU. In this case, you need to disconnect the bed, slide the coil around it, and reconnect the bed to the TSU.

This should happen quickly, so your animal is not left without anaesthesia or bed heating for too long.

This process should take no more than 10 seconds.

1.3.2 Volume Head Coil: mouse and rat

This coil has the same outward appearance as the body coil, but inside has a smaller diameter over about 2/3 of the axis of the coil for it to be close to the animal head. This allows 360° coverage of the head, plus a tighter read-out. For this purpose, use the dedicated Minerve head bed which adapts to this specific geometry.

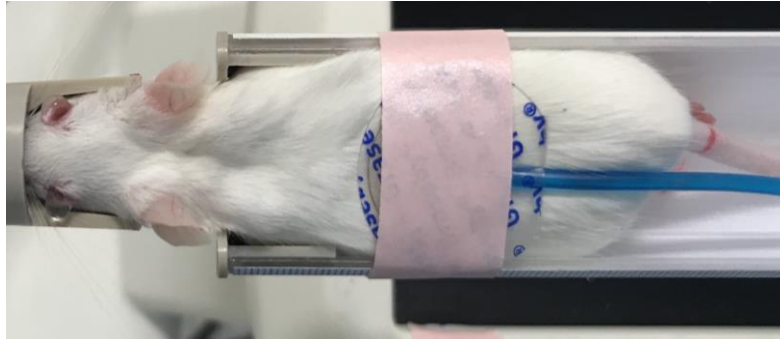


Fig 26: Minerve head bed

The anaesthesia system, air flow and heating, work as in the body beds.

Place the animal, define FOV, place the coil, insert in the bore and connect as mentioned above for the volume body coil.

The TSU should be all the way to the back. The only consideration is to make sure that the bed cannot slide freely inside the coil. The design is very accurate with the brain centred, allowing a few millimetres of adjustment depending on each animal.

Chapter 2. ANIMAL HANDLING

Animal legislation varies from country to country, but the considerations below tend to be a common trend.

2.1 General principles

The use of proper restraint and handling techniques reduces stress to animals and also to the researcher. Handling stress represents an experimental variable and should be minimized whenever possible. Animals can inflict serious injuries on humans and on themselves as a result of improper handling.

Shipping

Animals experience stress as a result of shipping. Acclimatisation periods of up to one week are recommended for all animals.

Handling

If a study involves significant handling of animals, it is recommended to acclimatise the animals to the handling. Prior to experimental manipulation, handle the animal on a regular basis in a non-threatening situation, e.g. weighing, petting, and giving food treats. Most animals, even rodents will respond positively to handling and will learn to recognize individuals.

Handle animals gently. Do not make loud noises or sudden movements that may startle them.

Handle animals firmly. The animal will struggle more if it sees a chance to escape.

Use an assistant whenever possible. Use restraint devices to assist when appropriate. Anaesthesia should be considered for any prolonged or potentially painful procedure.

2.2 Animal Imaging considerations

Animals have been used in research for decades.

Before conducting research on animals, scientists make certain animals are needed for their experiments. For more than 50 years, scientists have relied on the "3Rs" of **Replacement**, **Refinement** and **Reduction**. Although the use of animals in modern medicine and biology is essential, researchers should actively work to reduce the numbers used and improve on how they are used. Preclinical imaging, can assist in this, as it provides a non-invasive way to follow the same animal over time, reducing biological variation at the same time.

The dominant trend is combining all the available techniques together in the same animal, increasing the amount of information we get out of the subject, which is beneficial to the animals and beneficial to the science.

2.3 Animal anaesthesia

Animals are under general anaesthesia during imaging procedures, as scanning can last for an extended period of time, and requires the animal to be immobile.

During anaesthesia, it is imperative to:

- Carefully monitor and support mice body temperature, heart and respiratory rates, mucous membranes, and the degree of CNS depression
- After induction, position the animal on a heated platform or use a heating lamp to maintain body temperature above 35° to 37°. When using MRS systems, you are provided with the Minerve heated bed system, which will assure that your animal stays warm during imaging.
- Measure core temperature by rectal probe, monitoring respiration, and hearth rate and rhythm by electrocardiogram.
- Gently tape down the animal's limbs and monitor the delivery of oxygen by a nose cone placed over the muzzle
- Finally, apply an ocular lubricant to prevent corneal desiccation.

Most anaesthesia during imaging is gaseous, as it is easy to control, and is a safer option for weaker animal models.

Injectable anaesthesia is also usable, however it is more difficult to control, and dose varies from subject to subject. Animal model presenting liver or kidney disease should not be anaesthetised using injectable anaesthetics as they are metabolised by these organs.

2.3.1 *Injectable anaesthesia*

These anaesthetics are most commonly injected intra-peritoneally. They should not be used more than once a day per subject, as it can generate liver toxicity that could be fatal.

Most common agents are ketamine, barbiturates, and opioids. They all have their advantages and inconvenients, but as a general rule, animal imaging is carried out using gaseous anaesthesia, as it is easier to control, and the subjects recover a lot quicker from it.

2.3.2 Gaseous anaesthesia

CAUTION: Occupational safety is a serious concern. Inhalants must be directly vented out of the room, or, less reliably, adsorbed in a charcoal canister filter. Filters must be weighed and replaced before they reach target weight (usually an increase of 50g). Note that charcoal filtration is not accepted as a safe scavenging system by UBC Health, Safety and Environment.

The most common gaseous anaesthetic used is Isoflurane, which replaces the previously commonly used halothane. Sevoflurane is another option, less widely used.

Inhalation anaesthetics offer a wide margin of safety and allow the maintenance of a constant plane of anaesthesia compared with injectable ones. Absorption and elimination of inhalation anaesthetics occur through the lungs and allow rapid induction and recovery.

The basic anaesthesia machine consists of a source of oxygen, a flow meter, a precision vaporizer, a breathing circuit, and a scavenging system. In small animals, inhalation anaesthesia can be easily induced by placing the animal in an "induction chamber" and maintaining the desired depth with a face mask.

The concentration of an inhalation agent for anaesthetic induction and maintenance is usually expressed as the percent value of inspired gas mixture (Kohn et al. 1997).

Advantages

Rapid induction and recovery, with the ability to precisely titrate the level of anaesthesia.

*Isoflurane is recommended due to the fact that it is minimally metabolized (<0.17%) by the liver and therefore is less toxic to the animal's metabolism as compared to injectable anaesthetics.

Disadvantages

Disadvantages include the cost and logistics of using precision vaporizers; human exposure concerns; the risk of fatal overdose if an open system is used instead of a precision vaporizer; and depressed respiratory rate and decreased blood pressure. In addition, once animals awaken from gas anaesthesia, there is no residual analgesic activity.

Drug	Concentration for induction (%)	Concentration for maintenance (%)
HALOTHANE	4-5% + 0.8-1 L/min	1-2% + 0.8-1 L/min
ISOFLURANE	4-5% + 0.8-1 L/min	1-3% + 0.8-1 L/min
SEVOFLURANE	Individualized based on the mouse response + 0.8-1 L/min	Individualized based on the mouse response + 0.8-1 L/min

Fig 27: Recommended dosages for inhalation anaesthesia

2.3.3 Anaesthesia protocol for animal imaging

2.3.3.1 Material required

- Minerve anaesthesia system, equipped with a vaporizer, charcoal scavenger filters, induction chambers and nose cones / masks.
- Isoflurane anaesthesia gas.



2.3.3.2 Methods

- Weigh scavenger filter and record (saturation weight is indicated).
- Make sure that the level of isoflurane is sufficient by checking the level gauge (liquid should be located between the 2 arrows), turn the vaporizer to "0"/OFF and fill with isoflurane if necessary.
- Turn on O2 tank at 55psi.
- Set O2 flow meter to 1l/min.
- Place animals in induction chamber.
- Induce anaesthesia (see Table 1 above).
- Reduce anaesthesia to the maintenance concentration (imaging is non-traumatic only a low dose of anaesthetic is required to immobilise the animal; consistency of anaesthesia dosing procedures is recommended in order to reduce variability in anaesthetic effects).
- Cover the subject eyes with eye lubricant to prevent corneal dehydration (isoflurane inhibits blinking reflex).
- Transfer animals to imaging bed and place in front of individual nose cones.
- Make sure bed heating is active to protect animals from hypothermia.

2.3.3.3 Recovery

- Turn vaporizer dial to "0"/OFF.
- Keep O2 on for a couple of minutes to facilitate recovery.
- Animal(s) should recover within 1 min.
- Remove animal(s).
- Turn off O2 tank and flush anaesthesia system to remove caustic gas from system.
- Clean and sanitize equipment.
- Remain with the animal until they regain consciousness and are able to hold itself in an upright position. The time it takes for the animal to recover from anaesthesia will depend on how "healthy" it is, its age, the type of anaesthesia it had (gaseous or injectable), and how long it has been anaesthetised / dose of anaesthesia.

2.3.4 Anaesthesia tips

- Have all drugs and equipment ready before the animal is anaesthetised. You may not have time to look for things once the animal is under. Ideally, the animal should remain under anaesthesia for the shortest time possible.

- Reduce the respiratory tract secretions in some animals by premedication with atropine or glycopyrrolate (anticholinergics).
- For hairless animals, you can place some paper towels / blankets on their back to improve temperature control.
- Monitoring the animal (respiration monitoring / cardiac monitoring) during imaging can give you clues on how deeply anaesthetized your animal is. Monitoring allows you to keep control of the anaesthesia, and avoid the animal waking up unexpectedly.
- Before starting the imaging, you can check the level of anaesthesia of your animal by checking some reflexes:
 - *Palpebral reflex* - touching the eyelids causes blinking. If blinking, the animal has light anaesthesia.
 - *Toe pinch reflex* - pinching the toe or foot web will cause a pain response. If the animal withdraws the toe, anaesthesia is not deep enough. If it does not withdraw, it is not sensing pain.
 - *Corneal reflex* - touching the cornea of the eye with a tuft of cotton results in a blink. Once the animal has lost its corneal reflex, anaesthesia is too deep.
- Good anaesthesia is key to a successful imaging session. Too light or too deep can affect the results but also the well-being of the animal.

Chapter 3. COMMON ANIMALS NOMINATIVE VALUES

The following data will help you in determining the well-being of your animal throughout your experiments, and imaging sessions.

3.1 Mice

Lifespan	1-3 years
Adult weight	Males 20-30g, Females 18-35g
Birth weight	1-2 g
Heart rate	310 - 840 beats per minute
Respiratory rate	80 - 230 breaths per minute
Body temperature	36.5 - 38°C
Blood volume	7-8%, 1.5-2.5ml
Urine volume	0.5-1ml per day
Allergens	Dander, urinary protein

3.2 Rats

Lifespan	2.5 – 3.5 years
Adult weight	Males 300 - 500g, Females 250 – 300g
Birth weight	5 - 6 g
Heart rate	330 - 480 beats per minute
Respiratory rate	85 breaths per minute
Body temperature	36.5 - 38°C
Blood volume	7-8%, 50 - 70 ml/kg
Urine volume	3.3ml/100g bwt/day
Allergens	Dander, urinary protein

Chapter 4. FINAL WORDS

Should you encounter any issue or require technical support, please contact **MRS** Support in the United Kingdom.

Users finding errors or omissions in this document are requested to advise **MRS**.

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