Practical Guide to Gas Anesthesia in Rodents

The anesthetic machine dispenses the gases that are necessary to induce sleep, provide immobilization and prevent pain to animals during surgical procedures or other potentially painful manipulations.

The basic anesthetic delivery system consists of a source of oxygen (O_2), a pressure regulator, an O_2 flowmeter, a precision vaporizer, a patient breathing circuit, and a scavenging device.

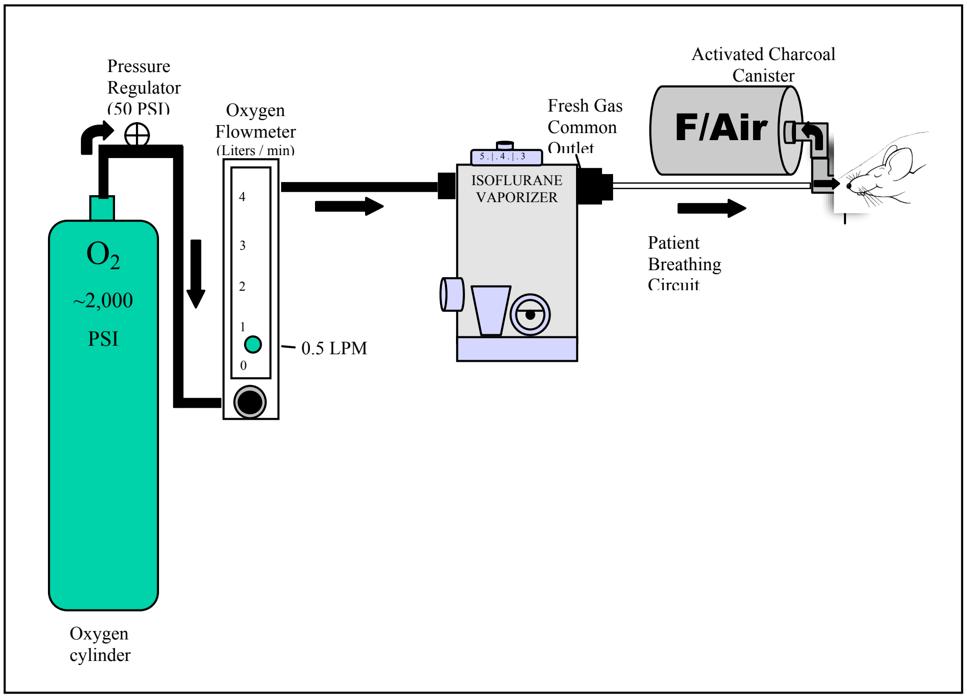

Figure 1. Components of the gas anesthetic machine for use on rodents.

The O_2 source provides a vehicle for transporting the anesthetic gases into the patient's lungs; the pressure regulator reduces the gas pressure coming from the O_2 tank; the flowmeter reduces the pressure further and allows for precise adjustment of gas flow into the patient; the precision vaporizer converts the volatile anesthetic liquid into a gas form and delivers a precise amount of anesthetic vapor; the patient breathing circuit consists of the tubing and nose cone connecting the vaporizer to the patient; and the scavenging device removes excess anesthetic gases that may pollute the room and may lead to health problems in animals as well as in humans.[1]

During delivery of gas anesthesia to the patient, O_2 flows through the vaporizer and picks up the anesthetic vapors. The O_2-anesthetic mix then flows through the breathing circuit into the patient's lungs, usually by spontaneous ventilation (respiration). Occasionally, it is necessary to use assisted

[1] The purpose of gas-scavenging systems is to eliminate waste anesthetic gases (WAGs) from the work area to minimize breathing by personnel. WAGs may be eliminated via an active (i.e., vacuum driven) or passive (outside wall, activated charcoal F-Air® canister) system. Substantial room pollution with WAGs may occur (1) during chamber induction if the chamber is improperly scavenged; (2) maintenance of anesthesia by a loose facemask or nose cone, (3) discharge of waste gases from a breathing circuit into the room, and (4) spillage or vapor escape when filling the vaporizer.

ventilation, especially when opening the chest cavity. Assisted ventilation is accomplished by use of a ventilator or respirator.

OXYGEN SUPPLY. At UCLA, DLAM will provide oxygen (O_2) in large (size "H") pressurized cylinders attached to the wall next to the workstation in the procedure or surgery rooms, or in small portable "E" tanks. "H" tanks hold approximately 6,900 liters of O_2 at very high pressure (2,200 pounds per square inch [PSI]). "E" tanks hold 660 liters at about 2,000 PSI. Since such high pressure would damage the animal's respiratory tract, a pressure-reducing valve or regulator is used to reduce this pressure to about 50 PSI. Further reduction in pressure is necessary, and this is achieved by adjusting the flowmeter control knob for safe delivery of gas to the patient.

When used at a gas flow of 0.5 liters per minute (LPM), an "H" tank provides up to 230 hours of anesthesia time (an "E" tank gives up to 20 hours at this flow rate). Either size tank must be replaced when the pressure gauge reads 500 PSI or less.

OXYGEN FLOWMETER. This device uses an adjustable needle valve to deliver the desired flow in liters per minute (LPM), not PSI, to the patient circuit. Flows of around 0.5-1 LPM of O_2 are commonly used in rodent anesthesia. Flow is read from the middle of the float (indicator metal ball) on the graduated scale (see Figures 1 and 2). The flowmeters provided with the DLAM machines are specific for O_2 (Please do not connect any other gas sources).

ANESTHETIC VAPORIZER. Precision vaporizers produce an accurate gaseous concentration out of a volatile liquid anesthetic. They are specific for each anesthetic agent; in our case, **isoflurane** (<u>Please do not fill the vaporizers with any other anesthetic agents</u>).The dial or knob on the vaporizer can be adjusted so as to deliver a precise percentage of anesthetic gas to the patient. Modern vaporizers, such as those provided by DLAM, are accurate at O_2 flow rates as low as 0.3 LPM and automatically adjust the anesthetic concentration to compensate for ambient temperature fluctuations. These precision instruments must be serviced and calibrated every 3 years.

The Portable Anesthetic Machine (PAM), consisting of O_2 flowmeter, vaporizer, and common outlet for rodent anesthesia is depicted in the Figure 2.

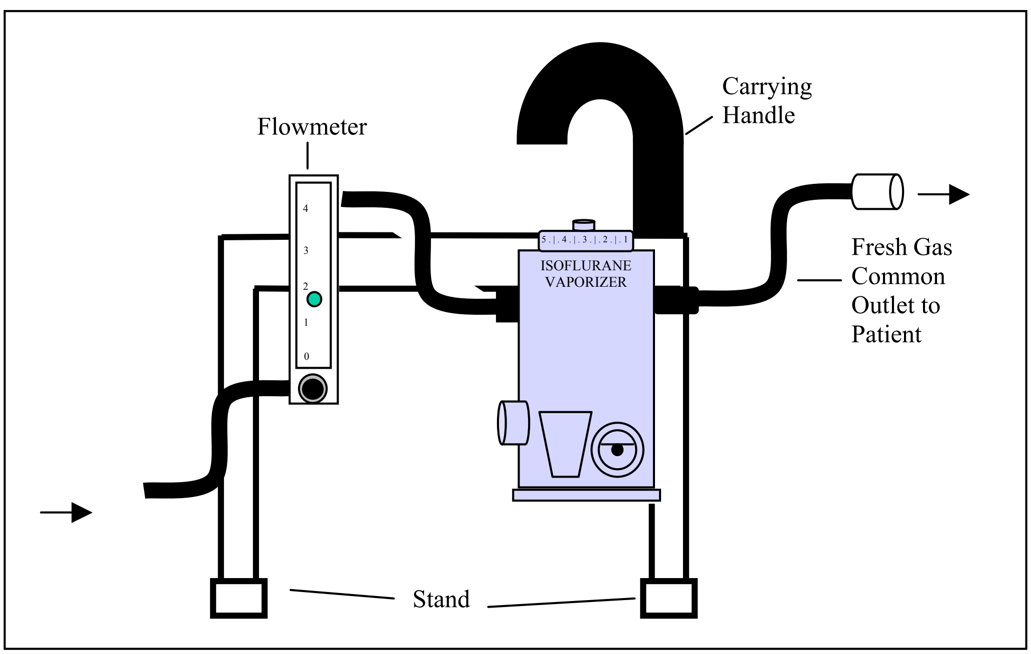

Figure 2. Components of the Portable Anesthetic Machine (PAM). See text.

PATIENT BREATHING CIRCUIT. The patient breathing circuit is the channel for delivery of O_2 or anesthetic gas to the patient. The goals of an anesthetic breathing circuit are to:

A. Deliver O_2 to the patient
B. Deliver anesthetic to the patient
C. Remove carbon dioxide (CO_2) that is produced by the patient
D. Provide a method for assisting or controlling ventilation, if needed

The non-rebreathing system commonly used in rodent anesthesia (Figure 1) uses fresh gas flows that are high enough to both deliver anesthetic to the animal and wash out exhaled CO_2. This setup connects the exit port of the nose cone to a scavenging device, e.g., "F/Air®" activated charcoal canister[2], which absorbs excess volatile anesthetic agent.

SCAVENGING SYSTEMS. These systems are designed to absorb or eliminate waste anesthetic gases (WAGs) to minimize room pollution. WAGs may be exhausted to the outside or may be directed to absorbent materials, such as activated charcoal. The rodent anesthetic machines provided by DLAM may be equipped with either system.

IN SUMMARY, GAS FLOWS IN THE FOLLOWING DIRECTION:

O_2 CYLINDER → REGULATOR → FLOWMETER → VAPORIZER → PATIENT BREATHING CIRCUIT → SCAVENGING SYSTEM

POTENTIAL PROBLEM WITH NON-REBREATHING CIRCUITS
Hypothermia and Dehydration. Because there is no rebreathing of exhaled air, these systems deliver a relatively high flow of dry, cool gas to the patient, which may cause significant body heat and humidity loss. Small rodents are prone to becoming hypothermic and dehydrated in prolonged procedures under these conditions.

Be aware that anesthetized animals usually have impaired thermoregulation. As a guideline, if the procedure will last longer than 10 minutes, the rodent should be placed on a warming surface. When using a heating blanket, the operator must ensure that the animals are not exposed to excessive heat, which may reduce arterial blood pressure to dangerous levels (shock) and/or cause skin burns. Please consult with a DLAM veterinarian if you are unsure about what temperature setting to use.

With procedures lasting over 1 hour, especially if blood loss is anticipated or has actually occurred, it is advisable to provide the animal also with **supplemental fluids (lactated Ringer's solution: 1-2 ml SQ or IP in mice; 5-10 ml SQ or IP in rats).**

[2] The weight of the F/Air® canister must be recorded upon installation and the canister should be replaced after its weight increases by 50 grams; this indicates that its capacity to absorb halogenated anesthetics has been exhausted. Halogenated anesthetics include isoflurane, halothane, enflurane, desflurane, sevoflurane and methoxyflurane. Please note that F/Air® canisters do not absorb other anesthetic gases, such as nitrous oxide (N_2O). When in use, F/Air® canisters should be placed in a position so as not to occlude the bottom, e.g., on a wire holder or on their side.

GETTING STARTED – INDUCING AND MAINTAINING ANESTHESIA IN RODENTS

Anesthesia may be induced in a single rodent, or in several animals simultaneously by use of a properly scavenged induction chamber.

For all procedures:

1. Verify that there is sufficient O_2 in the tank for the planned duration of the procedure. Have the tank replaced if the pressure gauge reads < 500 PSI.
2. Verify that there is sufficient isoflurane in the vaporizer by checking the fluid level through the glass window. Replace as needed.

Anesthetizing a single mouse

1. Scruff the mouse in the usual fashion as for manual restraint
2. **Turn on the gas flow rate to 1.0-1.5 LPM and set the vaporizer at 5% isoflurane, depending on age and physical condition of the rat.**
3. Hold the mouse's nose firmly but gently against the opening of the anesthetic nose cone *(Note: since rodents are obligate nose-breathers it is not essential to include the mouth in the nose cone; just make sure the nose is firmly inside the tubing or nose cone)*
4. When the mouse relaxes and its breathing slows down and becomes rhythmic, turn the vaporizer down to 2%
5. Verify that the toe-pinch reflex is negative prior to beginning any surgery or other procedures.

Anesthetizing a single rat

1. Wrap the rat in a towel or blue pad such that only the rat's head sticks out. Be sure to cover the front legs to prevent the rat from pushing off the nose cone. It is a good idea to cover the ano-genital area of the rat to avoid getting feces and urine on you – not uncommon during induction.
2. Turn on the gas flow rate to 0.5 LPM and set the vaporizer at 3-5% isoflurane, depending on age and physical condition of the mouse.
3. Hold the rat firmly but gently against either your chest or the work surface. Gently expose the rat's nose to the opening of the nose cone and observe respiration – rats may hold their breath in response to the pungency of isoflurane *(Note: since rodents are obligate nose-breathers it is not essential to include the mouth in the nose cone; just make sure the nose is firmly inside the tube or nose cone)*
4. When the rat has relaxed and its breathing slows down considerably, remove and cap the conical tube, turn on the gas flow rate to 0.5 LPM set the vaporizer to 2% isoflurane.
5. Immediately connect the rat's nose to the nose cone at the end of the patient's breathing circuit.
6. Verify that the toe-pinch reflex is negative prior to beginning any surgery or other procedures

When using a properly scavenged induction chamber:

1. Check all connections and make sure that the inflow to the chamber (from the vaporizer's common outlet) and its outflow (to the F-Air canister) are in the open position. Make sure the back of the patient breathing circuit is connected to a second F-Air canister.
2. Place the animal inside the chamber and close the lid tightly.
3. Turn on the O_2 source and set the fresh gas flow into the chamber to 2 LPM using the flowmeter control knob.
4. Turn on the vaporizer and adjust the isoflurane concentration to 4-5% (use lower % for sick, very old or very young animals; when in doubt, use 3-4%).

5. When the animal is in a moderately deep plane of anesthesia (lying on its side and breathing rhythmically), evacuate the chamber.
6. Remove it from the chamber and close the lid immediately.
7. Connect its nose to the nose-cone adaptor and turn on the gas as above.

Monitoring anesthesia

Animals must be monitored throughout the procedure, i.e., from induction to full recovery. An animal is considered to have recovered from anesthesia when it is fully awake and able to rest on its belly (sternal recumbency).

It is essential to develop your powers of observation when monitoring anesthesia. The effectiveness of monitoring will depend on the physiologic parameters chosen. Do not rely on a single parameter.

We are somewhat limited in the number of parameters available to monitor in rodents. Commonly used variables include: respiration rate and pattern, heart rate, color of the footpads, and response to toe-pinch reflex and painful manipulations. Respiratory rate and pattern change from fast during induction to more slow and rhythmic as the depth increases. Heart rate follows a similar pattern.

Learn to interpret the monitoring parameters. For example, a positive toe-pinch reflex (leg withdrawal) indicates that the animal has not yet reached a surgical plane of anesthesia. However, an absence of withdrawal could be seen in an adequately anesthetized animal or in one that has been dangerously overdosed. The safest alternative is to use a combination of all the above parameters.

Recovering from anesthesia

1. When finished with the procedure, shut off the isoflurane vaporizer and O_2 flowmeter.
2. Rodents recovering from anesthesia should be placed alone or with other similarly anesthetized animals in a clean cage lined with paper towels to prevent injuries and accidental inhalation of bedding material, which may lead to aspiration pneumonia.
3. Shut off O_2 flow at the tank valve
4. Check the flowmeter to verify that the O_2 flow has ceased and turn the flowmeter control knob to the OFF position (fully closed clockwise; do not over-tighten)
5. Observe the animal(s) until fully recovered
6. **Depending on the temperature of the rodents, it maybe wise to add a heating pad under the recovery cage.**

2. Anesthetizing a group of rodents

Several rodents may be anesthetized as a group in an induction chamber and maintained individually on a nose cone for the duration of the desired procedure. One animal at a time may be removed from the induction chamber and connected to the breathing circuit. By use of a dual diverter valve the anesthetic flow may be diverted to either the chamber or the individual animal, or be allowed to flow in both directions simultaneously.

1. Turn on the O_2 source and set the fresh gas flow into the chamber at 2 LPM for induction
2. Place the rodents inside the chamber and close the lid tightly
3. Turn on the vaporizer and adjust the isoflurane concentration to 4-5% or lower, if indicated
4. When the rodents are moderately anesthetized evacuate the chamber and remove one animal
5. Immediately close the lid and restore gas flow to the chamber

6. Connect the rodent's muzzle to the nose-cone adaptor as above and open the gas flow to the nose cone
7. Maintain gas flow at ~1-2 LPM to supply both the nose cone and the chamber
8. Adjust the vaporizer setting to a maintenance level of 2% isoflurane
9. Recover each animal by shutting off the flow of anesthetic to the nose cone and allowing the animal to breathe room air. Return the animal to its cage when awake
10. Continue to remove one animal at a time from the chamber and proceed as described above for the first one
11. At the end of the procedures, shut off the vaporizer and close the valve to the breathing circuit
12. Evacuate the chamber to minimize atmospheric pollution
13. Shut off the O_2 at the tank valve and wait until the flowmeter reads 0 (zero)
14. Close the flowmeter without over-tightening the knob

The main problem associated with chamber induction is the potential for room pollution and personnel exposure to inhalation anesthetics. This occurs when the chamber is opened to introduce or remove animals without properly evacuating the anesthetic from the chamber.

Troubleshooting the anesthetic machine

The most common issues with anesthetic machines are leaks. Leaks commonly occur around tubing connections, flow valves and O_2 yokes. It is imperative that any leaks be corrected since they can waste gas and/or expose the operator to high levels of anesthetic vapors.

Calculations

Calculation of Oxygen left:

1. An oxygen **E tank** (small), when full, contains 660 L at 1,900 psi service pressure. Multiplying gauge pressure (in psi) x 0.3 = approx liters of O_2 left in the tank

   Example: When the pressure gauge reads 1,000 psi there will be approx 300 L left (1,000 x 0.3). If you are flowing 0.5 L/min oxygen, the tank will give you 600 minutes (= 10 hr) of continuous flow. In practice, when you reach 500 psi, you replace the tank

2. An oxygen **H tank** (large), when full, contains 6,900 L at 2,200 psi service pressure. Multiplying gauge pressure (in psi) x 1.7 = approx liters of O_2 left in the tank.

   Example: When the pressure gauge reads 1,000 psi there will be approx 1,700 L left (1,000 x 1.7). If you are flowing 0.5 L/min oxygen, the tank will give you 3,400 minutes (= 56 hr) of continuous flow. In practice, when you reach 500 psi, you replace the tank.

Calculation of how long isoflurane will last:

A 100-ml bottle of isoflurane will give you 32 hr of continuous anesthesia when you are flowing 0.5 L/min oxygen at a vaporizer setting of 2.0%.

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IMPORTANT

- DLAM will purchase and maintain the anesthetic machines and will also supply the O_2 source.

- To minimize cross-contamination among users' animals, each lab will be responsible for purchasing, cleaning and maintaining their own patient breathing circuits and any induction chambers.

- In order to ensure the quality and compatibility of the different components DLAM will purchase the required breathing circuits and induction chambers, and recharge the labs interested in acquiring these devices.

- The liability and complexity of the anesthesia machine require the skill of a knowledgeable operator for proper maintenance. Periodic servicing of this equipment should be left to the manufacturer or reputable service company. DLAM will arrange for their maintenance.

- If you have questions, please contact the **DLAM veterinary clinic (x54505)**