

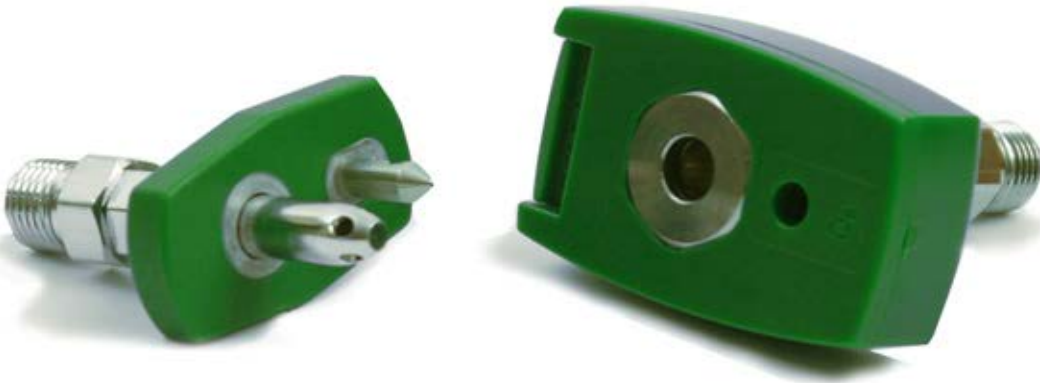
SOP for AVS anesthesia machine usage
7/15/09 MW, re: 9/29/11, 6/10/15MW, 3/4/19
IACUC Approved: 3/21/19

The anesthesia machines are provided by AVS for use of the PIs. The machines are set up for following these SOP's and at no time are these machines to be adjusted other than described in the following SOP. If you have any questions or wish to use the anesthesia machine in a different manner, please see Dr Wong.

*There are inherent risks in using gas anesthesia. Please read the Isoflurane MSDS for risks to individuals.

*Note individual machines may vary, please see your user guide if you are not using an AVS owned anesthesia machine for any questions.

1. For usage at Kakaako, attach quick release attached to anesthesia machine to quick release on counter top. The regulator in Kakaako is already set to 50 PSI.

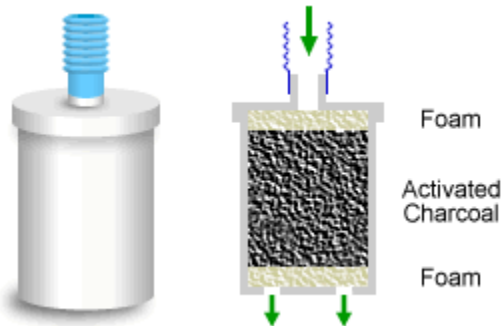


For usage at Manoa, attach oxygen regulator to oxygen tank. Make sure it is attached properly and tight. You should not hear any leaking/hissing of air. The prongs on the regulator should match up with the oxygen tank. The tank must be secured properly (standing upright and secured to a mount so that it will not fall). Open the regulator to 50 PSI. The anesthesia machine will not run properly if the PSI is not set to 50.



*Note that oxygen availability in a gas cylinder is proportional to the PSI of the tank. Since pressure is pushing the oxygen into the anesthesia machine, as the pressure in the oxygen tank decreases the amount of oxygen available to use decreases. This means that the flow to the animal will remain the same but the amount present in the tank will reduce at a faster rate, i.e. the lower the PSI in the tank is from the starting PSI of a new tank, the less time you will have to do anesthesia.

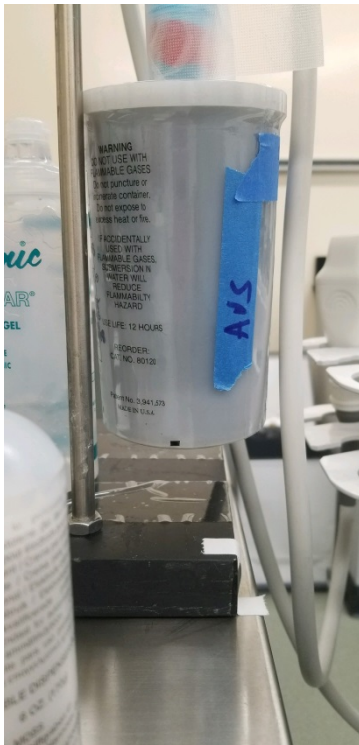
2. Insert rodent into anesthesia chamber.



Make sure that there are competent F/Air canisters attached to each blue hose (2 required, purchased and maintained by the PI). It is the responsibility of the PI to monitor the weights of the F/Air canisters, usage time, and to properly dispose of them through EHSO. Please read the information that comes with the F/Air canisters to determine

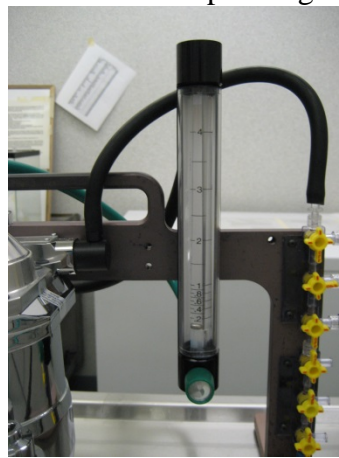
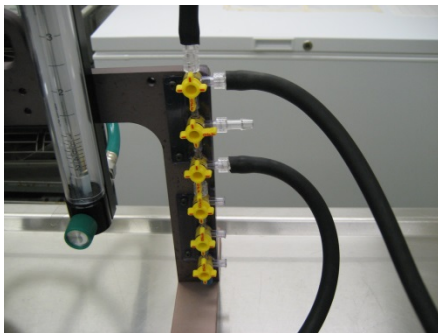
when they are no longer useable. Please contact the Lisa Johns at EHSO Kakaako or Tim O’Callaghan at EHSO Manoa for disposal.

F/Air canisters must also be vertical, so that the gas exhaust flows from the top of the canister to the bottom. The bottom openings must be uncovered to allow the scrubbed air to pass through. Do not use F/Air Canisters on their sides (horizontally) as this can lead to the gas exhaust bypassing the charcoal filters. Do not block the bottom of the canister when in use as this will block exhaust from coming through.

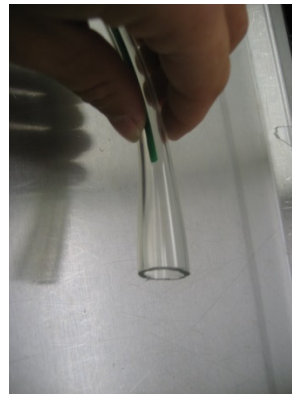


Note that at Kakaako, anesthesia machines can also be exhausted down into the downdraft table or into a hard ducted Biosafety Cabinet. Please contact Dr Wong, if you wish to use this method for exhausting anesthesia.

3. Adjust the 1st 3 way stopcock so that it is closed arrow is pointing down.



4. Open oxygen flow meter to 1 liter/min.
5. Adjust vaporizer setting to induction levels, 5% isoflurane.
6. Once rodent is anesthetized, turn off the oxygen flowmeter on the anesthesia machine. This will discontinue any anesthesia from being moved through the anesthesia machine. (Note even if the vaporizer setting is at 5, without the oxygen flowmeter running, there is no oxygen passing through the vaporizer and thus no anesthesia) Remove the animal from the induction chamber and place on a countertop or table and place rodent bain over its face (One size fits all)



7. Turn the close arrow on the 1st 3 way stopcock to the right, closing the line to the induction chamber. Do not at any time move the other stopcocks positions. The 2nd stopcock should have the closed arrow pointing down.
8. Turn on the oxygen flowmeter to 1L/min and adjust the vaporizer setting to between 2-3% isoflurane. Use less isoflurane if the animal is very deep and more isoflurane if the animal is light. (toe/tail pinch response, movement other than breathing, or other signs commonly used when monitoring depth of anesthesia)
9. Proceed with the surgery.
10. When the surgery is approximately 1 min from finishing, turn the vaporizer off. This will allow the rodent to continue to breathe fresh oxygen but remove the excess isoflurane from its body. This will also decrease recovery time from anesthesia. Place the animal in a cage for recovery.
11. If at Manoa, close the regulator attached to the oxygen e tank. Allow the pressure to drop and then close the oxygen flowmeter on the anesthesia machine and disconnect the regulator from the e-tank. If at Kakaako, disconnect the quick releases.



12. Spray the inside of the anesthesia chamber with the disinfectant present in the room. Please remember these are shared with other PI's so please keep them clean. Do the same with the rodent bain, disinfecting the inside and the outside of the rodent face mask.

Monitoring Rodents Under Anesthesia

1. After induction, position the animal on a heated platform (such as a hot water bed) or use a heating lamp to maintain body temperature above 95 to 99°F.
2. Gently tape down the animal's limbs and monitor the delivery of oxygen by a nose cone placed over the muzzle.
3. Apply an ocular lubricant to prevent corneal desiccation.
4. Anesthesia is considered adequate when the animal stays still quietly, is unresponsive to external stimuli, and has constant heart and respiratory rates. In rodents the absence of the palpebral reflex suggests a fair anesthetic depth.