STEP-BY-STEP PROTCOL FOR GAS SAMPLING IN MARINE MAMMALS

First considerations:

- The gas sampling and analysis **cannot** be done if you cut the head first.
- The analysis of the gas samples is time sensitive. Ideally we should run the samples within one week from sampling.
- Samples are affected by low pressure when shipped by aircraft. We have pressure resistant housing to transport the samples that we can ship to you. Please let us know as soon as you have a stranding and you decide to do gas analyses, since the transport of the housing to you, and then the transport of the samples to us is going to take a couple of days, and as explained before, gas samples are time sensitive!
- Gas analyses can be run at Woods Hole Oceanographic Institution, MA, USA, or at the University of Las Palmas de Gran Canaria, Canary Islands, Spain. We strongly recommend that you choose the closest location, so we can run the samples as soon as possible.
- Contact information:

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The following instructions are a summary of the "protocol for gas sampling and analysis in marine mammals" with some updated information. For further information please visit the link to this article:
http://www.nature.com/protocolexchange/protocols/2299

Material you need:

- 2-mL additive free glass tube (Kendall Monoject™ blood collection tube, ref: 301116)
- BD vacutainer® one use holder (ref: 364815)
- Double pointed needle with a rubber barrier on the tube puncture side (BD vacutainer® eclipse™ blood collection needle, ref: 368607).
- Disposable insulin syringes (BD Plastipak U-100 insulin ref: 329651).

PROCEDURE

Dissection

1. Carefully remove the skin and blubber minimizing damage to the major subcutaneous veins.
2. Examine the visible and larger subcutaneous veins for bubbles.
3. Score the amount of bubbles in the subcutaneous veins (see data sheet).
4. Take photos of veins with bubbles.
5. Sample bubbles*[1].

CRITICAL STEP: If pneumothorax is suspected, gas sampling could be done by using the vacutainer®, inserting the double pointed needle in between the ribs*[2]. Do not open thoracic cavity!

6. Open first the abdominal cavity carefully (try not to cut medium to large size vessels).
7. Examine the mesenteric and renal veins as well as the lumbo-caudal plexus for bubbles.
8. Score the amount of bubbles in the lumbo-caudal plexus (see data sheet).
9. Take photos of bubbles within vessels.
10. Sample bubble's content "*in situ*" using the insulin syringes*[1].
11. Look for subcapsular emphysema.
12. Sample the subcapsular (gas) emphysema *in situ* using the vacutainer®*[2] .
13. Sample intestinal gases using the vacutainer*[2]. Preferably take at least three samples from different locations.
14. Open thoracic cavity. If desired, ribs could be disarticulated except the first 3 or 4 cranial ones. These ribs should be cut at 1/3 from the vertebral articulation.
13. Examine the coronary vessels.
15. Take photos of vessels and bubbles.
16. Score the amount of bubbles in the coronary veins (see data sheet).
14. Sample bubbles*[1].
15. Follow up with routine necropsy protocol.

CRITICAL STEP: do not cut any systemic vein or sample organs until this step is reached.

21. Separate the head from the body.
22. You might disarticulate the mandible to have a better access to the pterygoid sacs.
23. Sample pterygoid sacs using the vacutainer®*[2].

CRITICAL STEP: do not open the sinuses before gas sampling.

*[1]Gas sampling from bubbles in veins

CRITICAL STEP: place the vein under water whenever possible to avoid atmospheric air contamination.

1. Sample each bubble with a new dispensable insulin syringe (BD Plastipak U-100 insulin)
2. Inject the content immediately into a new vacutainer® each time.
3. Label the vacutainer® with volume recovered and location of the bubble.

CRITICAL STEP: Use one new syringe and one new vacutainer for each bubble.

*[2] Gas sampling from cavities (intestine, pterygoid air sacs) and gas associated lesions (pneumothorax and subcapsular emphysema)

1. Couple the vatuainer® plastic holder to the double pointed needle
2. Insert the needle into the cavity
3. Push the vacutainer® against the other end of the needle
4. Leave for a few seconds
5. Remove the vacutainer®
6. Remove the needle

CRITICAL STEP: If any of these steps is not done following this sequence, atmospheric air contamination will occur.

CRITICAL STEP: If steps from 3-13 are not done carefully following this sequence, air contamination will occur.

Storage and transport

1. Store the samples at room temperature and atmospheric pressure.
2. Store blank tubes with the samples; one blank per sample or a minimum of 3 blanks per animal.
3. Samples should be ground transported (please remember that samples are time sensitive!), or shipped by plane inside our pressure resistant housing. Please contact us as soon as possible so we can ship you the housing.