

PROTOCOL FOR GAS SAMPLING AND ANALYSIS IN STRANDED MARINE MAMMALS

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Method Article

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Abstract

Gas sampling in stranded marine mammals can now be performed in situ using the appropriate vacuum tubes, insulin syringes and an spirometer. Glass vacuum tubes are used for extraction of gas from cavities such as the intestine, pterigoyd air sacs, pneumothorax or subcapsular emphysema as well as for storage of the gas sample at room temperature and pressure. Insulin syringes are used for extraction of bubbles found in veins, then immediately injecting its content into the vacuum tubes for storage. Finally an spirometer is used to extract and separate the gas mixed with blood inside the heart. We have found that these are reliable tools for in situ gas sampling, storage and transportation without appreciable loss of gas and without compromising the accuracy of the analysis. Gas analysis is conducted by gas chromatography in the laboratory.

Introduction

Gas-bubble lesions have been described in cetaceans stranded in spatio-temporal concordance with military maneuvers^{1,2}. These authors suggested decompression like sickness as an explanation for the observed lesions. Decompression sickness is the disease caused by bubble formation due to gas phase separation in the body. Gas phase may arise from supersaturated gas tissues after decompression when the sum of the dissolved gas tensions (oxygen, carbon dioxide, nitrogen, helium) and water vapor exceeds the local absolute pressure^{3,4}. According to Bert (1878), the main gas “which would threaten life on being liberated would be exclusively the one the proportion of which was considerably increased in the blood”: nitrogen⁵. Gas chromatography has been demonstrated as a valid method to discriminate putrefaction gases from air embolism^{6,7}, and has been used as a forensic tool in humans for this purpose⁸. Indeed putrefaction gas is one of the problems that we might face when dealing with stranded marine mammals. We describe the step by step protocol that has been experimentally tested and verified for the reliable and consistent collection, storage and analysis of gas from different body compartments in stranded cetaceans. Furthermore, we have demonstrated by using this protocol that gases in acute and chronic gas embolism-affected cetaceans that were minimally decomposed had high or very high nitrogen contents in bubbles⁹.

Equipment

- 5-mL additive-free vacutainer (BD Vacutainer® Z. ref: 367624), vacutainer holders and double-pointed needle with a rubber barrier on the tube puncture side (ref: 360214) - Disposable insulin syringes (BD Plastipak U-100 insulin) - Spirometer (U201100896), tygon 2375 tubes, and an aspirator bottle with tubulation, 18 gauge needles. - Gas chromatograph equipped with a Varian CP7430 column and two detectors disposed after each other: a thermal-conductivity detector (TCD) and a flame-ionization detector (FID). **Equipment set up** **Spirometer set up** A tygon tube of approximately 1 meter long must connect the spirometer and the aspirator bottle. Another tygon tube of 50 cm should connect the spirometer with the needle. At the top of the burette another needle must be placed. All joints have to be

silicon and parafilm sealed. The whole system is filled with distilled water (Figure 1). **Gas chromatograph set up** TCD temperature at 80°C, filament temperature at 160°C. The temperature for the FID is fixed at 230°C. Samples are run for 25 minutes with an isothermal temperature of 45°C and an electronically controlled flux with a fixed pressure of 13.1 psi on the head column. Helium is used as the carrier gas.

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. Take photos of veins with bubbles.
4. Sample bubbles¹. **CRITICAL STEP**: If pneumothorax is suspected, gas sampling could be done by using the vacutainer®, inserting the double pointed needle in between the ribs². Do not open thoracic cavity!
5. Open first the abdominal cavity carefully (try not to cut medium to large size vessels).
6. Examine the mesenteric and renal veins as well as the lumbo-caudal venous plexus for bubbles.
7. Take photos of bubbles within vessels.
8. Sample bubble's content "in situ" using the insulin syringes¹.
9. Look for subcapsular emphysema.
10. Sample the subcapsular (gas) emphysema in situ using the vacutainer®².
11. Sample intestinal gases using the vacutainer². Preferably take at least three samples from different locations.
12. 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. **CRITICAL STEP**: Avoid cutting large vessels
13. Cut the pericardial sac dorsolaterally (making a "window") to have access to the pericardial veins, keeping the sac for filling up with water after first examination. **CRITICAL STEP**: Avoid making the pericardial sac window too large.
14. Examine the coronary vessels through the window.
15. Take photos of vessels and bubbles.
16. Sample bubbles¹.
17. Fill the pericardial sac with distilled water. **CRITICAL STEP**: distilled water is used to prevent atmospheric air contamination.
18. Sample the gas from the heart (right and left ventricles) using the aspirimeter³.
19. Sample the gas from the Aorta and the pulmonary trunk³.
20. 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 pteryoid sacs.
23. Sample pteryoid 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, pteryoid air sacs) and gas associated lesions (pneumothorax and subcapsular emphysema)

1. Couple the vacutainer® 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.

3 Gas sampling from the

heart cavities using the respirometer** 1. Fill up all the respirometer system with distilled water, including the needle on the top of the burette. 2. Remove any bubbles found in the system. ****CRITICAL STEP****: If the system is not completely filled with distilled water or if there are bubbles, this air will contaminate your sample 3. Move up the aspirator bottle to a higher height than the respirometer level and let water run out from the sampling needle (Figure 2). 4. Insert the sampling needle into the heart cavity. 5. Move down the aspirator bottle to a lower height than the respirometer level. 6. Wait for blood alone or blood mixed with gas to be suctioned (Figure 3). 7. Once no more gas is suctioned, clamp the sampling tube. 8. Move the aspirator bottle to the same level as the respirometer. (Gas will separate from the liquid and accumulate in the top of the burette) 9. To collect the gas, insert a vacutainer® in the needle at the top of the burette. 10. Open the stopcock. 11. Recover your sample. 12. Close the stopcock. 13. Remove the vacutainer®. 14. Repeat steps 10-13 as many times as necessary to collect all the gas. 15. Measure water temperature in the aspirator bottle. ****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. If samples need to be transported in a plane, they should travel within the passenger cabin to prevent dramatic changes in atmospheric pressure that might alter the vacuum tubes. ****Gas analyses and calculations**** 1. Analyze a minimum of three blanks per animal. 2. Calculate the detection limit for your gases based on the blanks as follows: $S_{min} = S_{blank} + 3S_{blank}^2$, where S_{min} is the minimum detectable signal, S_{blank} is the average signal for a given gas in the blanks, and S_{blank}^2 is the associated standard deviation. 3. Correct for deviations if the respirometer is used.

Timing

Time to collect samples varies depending on the presence or absence of bubbles, amount of bubbles and studied species. For a dolphin, maximum estimated sampling time is 30 minutes if the respirometer is used. If it is not used, sampling should take less time.

Troubleshooting

The evacuated tubes contain some atmospheric air, which we correct from our samples using the detection limit. We strongly recommend the use of BD 5ml additive-free vacutainer, because they contain low levels of atmospheric air and are made of break-resistant glass. If you don't have this tube available, use the smallest glass evacuated tube you can find, and take many blanks with your samples to correct for the standard deviation. Evacuated tubes do not resist too changes in pressure very well. If tubes need to be transported in a plane they should go into the passenger cabin. If you need to ship them, you should use a pressure resistant housing. Very small bubbles, won't give you signals higher than the detection limit. Sample the largest bubbles you find. Usually, bubbles larger than 0.5 mL give good results.

Anticipated Results

Gas embolism found in fresh animals might be composed of high or very high nitrogen, while gases produced by putrefaction might be composed of a mixture of nitrogen, hydrogen and carbon dioxide⁹.

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Figures

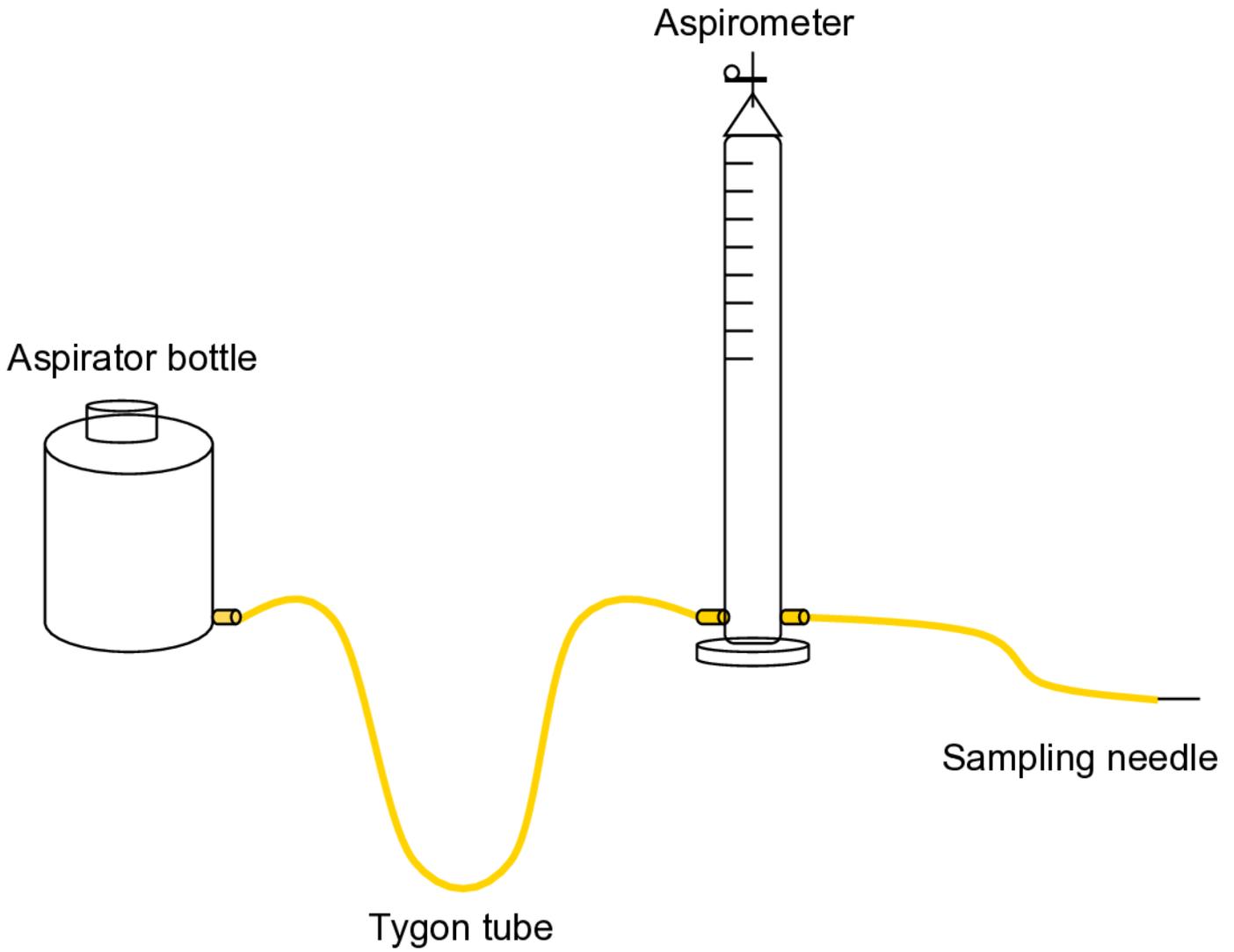


Figure 1

Aspirimeter display Schematic figure showing the different components of the aspirimeter.

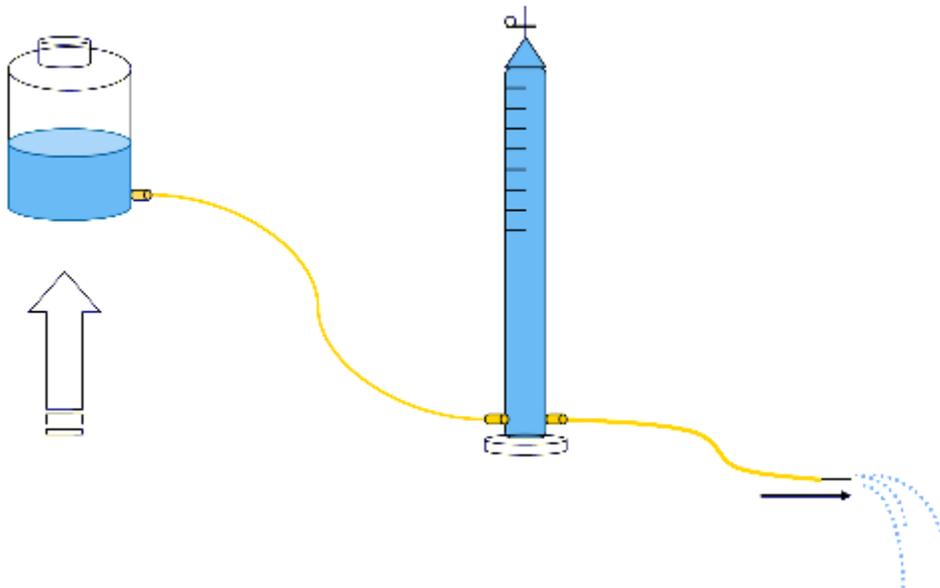


Figure 2

Sampling position At this position, the differences in height between the flasks provokes a difference in pressure. Liquid is moved from the aspirator bottle to the aspirimeter and from here to the sampling needle. Liquid is coming out through the sampling needle. There is no atmospheric gas even in the sampling needle.

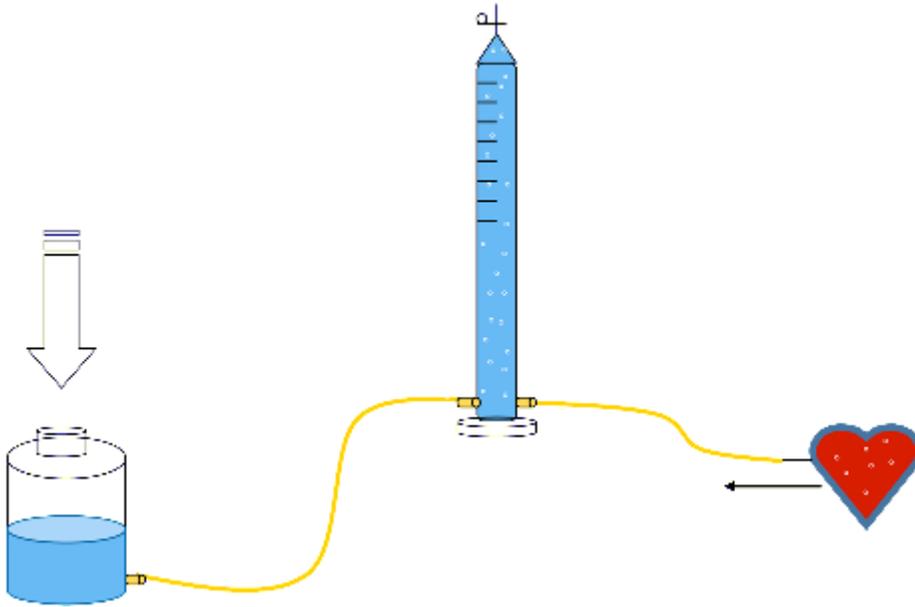


Figure 3

Gas extraction position At this position, the differences in height between the two flasks provokes a negative pressure, suctioning whatever is found inside the heart cavities.

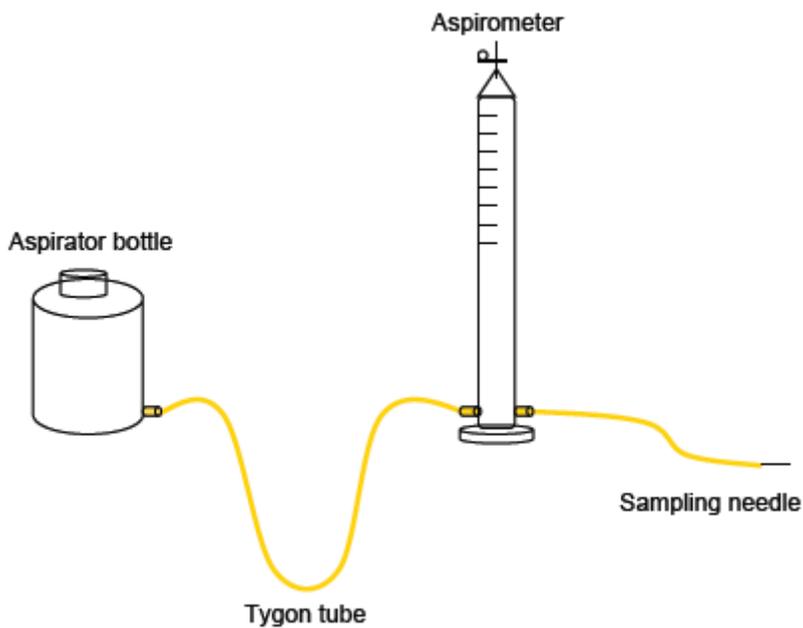


Figure 4

Figure 1 Aspirometer display Schematic figure showing the different components of the aspirometer.

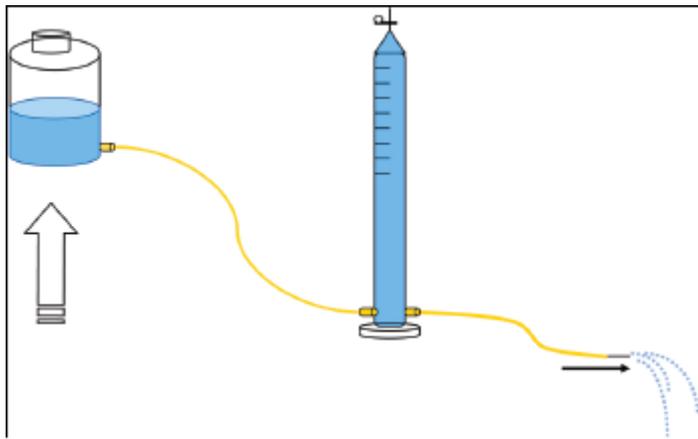


Figure 5

Figure 2 Sampling position At this position, the differences in height between the flasks provokes a difference in pressure. Liquid is moved from the aspirator bottle to the aspirometer and from here to the sampling needle. Liquid is coming out through the sampling needle. There is no atmospheric gas even in the sampling needle.

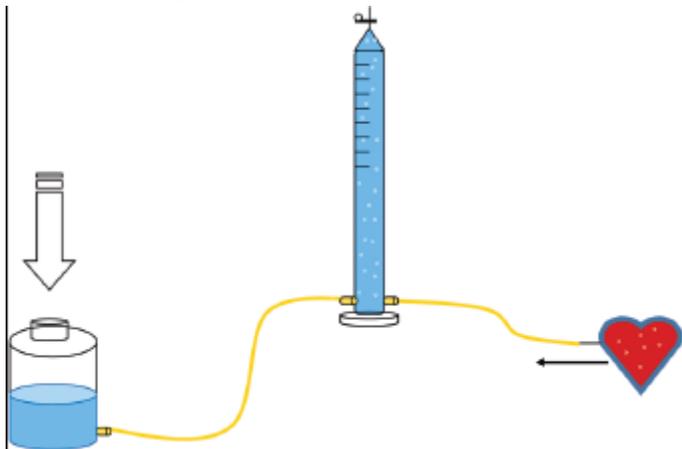


Figure 6

Figure 3 Gas extraction position At this position, the differences in height between the two flasks provokes a negative pressure, suctioning whatever is found inside the heart cavities.

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