Dear Colleagues,

As some of you already know, analyses of gas bubbles by gas chromatography has been able to show that gases in acute and chronic gas embolism-affected cetaceans that were minimally decomposed had high or very high nitrogen contents in bubbles (Bernaldo de Quirós et al., 2011). This technique, allows us to distinguish between putrefaction and decompression related gases. However, putrefaction gases are always produced postmortem, and may mask decompression related gases if the necropsy and gas sampling is not performed soon after death.

Until very recently, the method described by Bernaldo de Quiros et al. (2011) could not be applied in the US because of the impossibility of acquiring the right sampling materials. Now, we are pleased to announce that we have adapted our method for gas sampling in stranded cetaceans to materials available in the US, as well as the availability of a shipping method of the samples without altering the original gas composition of the samples. Therefore, we would like you to know that if you now face a gas embolism case you have the chance of collect the samples properly following the step-by-step protocol found in http://dx.doi.org/10.1038/protex.2012.002, as well as to ship to us the samples for doing the corresponding gas analyses if you don't have the possibility to do so in your lab. Below we described the new adaptations and shipping method that will enable you to sample and send us gas samples from stranded marine mammals:

- Adaptation to US material
  In our method published in Scientific Reports (Bernaldo de Quirós et al., 2011), we used 5ml BD vacutainers for sampling and storage of the gas sample. Unfortunately BD vacutainers are no longer produced but we have found an alternative that is commercially available in the US. This is the **MONOJECT™ Red Stopper (Silicone Coated) glass tubes of 2 mL (ref #8881301116)**. These tubes have successfully undergone the tests described for the 5ml BD vacutainers described in Bernaldo de Quirós et al. (2011).

- Shipping method
  We have acquired a plastic housing resistant to negative pressures. We would be pleased to send you the housing as soon as you let us know that you might have some gas samples that you would like us to analyze. This housing allows air shipping without pressure changes in its interior, assuring that your samples won't suffer modifications because of changes in pressure during the flight, as well as enabling a fast transportation for immediate analysis of the gas samples in our lab.

In addition, we could send you all the necessary material for gas sampling together with our housing if requested. However, we encourage you to acquire the material in order to do the necropsy and gas sampling as soon as possible. As previously mentioned, post-mortem time is crucial if you would like to use the gas analyses as an additional diagnostic technique. Thus ideally you should buy the materials for sampling, to be ready for a case where there are bubbles of potential interest. They are all useful for more than just bubble sampling.

Please find bellow a list of materials and a brief description of the protocol.

MATERIAL LIST: (This is the basic material you might need):

- Tubes: **MONOJECT™ Red Stopper (Silicone Coated) glass tubes of 2 mL (ref #8881301116)**. It has to be this exact tube. Price according to Fishersci: Pack of 100 for $20.61
  https://www.fishersci.com/ecomm/servlet/Search?storeId=10652&langId=-1&nav=0&keyWord=monoject%208881301116&store=Scientific
- One Use Tube Holders: BD Vacutainer (ref # 36481), or equivalent in other brand. Price according to Fishersci: Case of 1000 for $50.09
  https://www.fishersci.com/ecomm/servlet/Search?keyWord=one+use+holder+364815&store=Scientific&nav=0&offSet=0&storeId=10652&langId=-1
- Double pointed needles: BD Vacutainer Blood Collection Needles (ref # 367210), or its equivalent in other brand, but the tube puncture side of the needles must have a rubber barrier covert. Price according to Fishersci: Pack of 100 for $20.88
  https://www.fishersci.com/ecomm/servlet/fsproductdetail?storeId=10652&productId=662238&catalogId=29104&matchedCatNo=0266524&fromSearch=1&searchKey=367210&highlightProductsItemsFlag=Y&endecaSearchQuery=%23store%3DScientific%23nav%3D0%23rpp%3D15%23offSet%3D0%23keyWord%3D367210&xrefPartType=From&savings=0.0&xrefEvent=1337092772056_0
- Disposable Insulin syringes: **BD Plastipak U-100 insulin (ref # 329651)**. It has to be this exact syringe. Price according to Fishersci: Pack of 100 for $35.93.
  https://www.fishersci.com/ecomm/servlet/fsproductdetail?storeId=10652&productId=4755080&catalogId=29104&matchedCatNo=148292B&fromSearch=1&searchKey=329651&highlightProductsItemsFlag=Y&endecaSearchQuery=%23store%3DScientific%23nav%3D0%23rpp%3D15%23offSet%3D0%23keyWord%3D329651&xrefPartType=From&savings=0.0&xrefEvent=1337093415853_0

PROTOCOL

- The dissection should be done very carefully in order to avoid cutting large vessels. No organs should be removed before gas sampling.
- Sample up to 5 bubbles from each of these locations: mesenteric veins, renal veins and coronary veins. Take extra samples if you observe bubbles in other locations of interest according to your opinion (each case might be different). Sample the bubbles using the insulin syringe and injecting promptly the content into the monoject tubes.
- Sample intestinal gas from the following locations: duodenum or nearby intestine sections, middle intestine and rectum. Do so by using the monoject tubes together with the double pointed needles coupled to the one-use tube holders.
- Sample subcapsular gas (emphysema) if found. A location of interest is the peri-renal area. Sample the subcapsular gas as described for the intestinal gas.
- Store tubes at room temperature, preferably with the stopper downwards. Store blanks together with the samples (a minimum of 3 is required).

You can find a step by step protocol for gas sampling in the following link: http://dx.doi.org/10.1038/protex.2012.002. The Protocol Exchange is an open resource where the communities of scientists pool their experimental know-how to help accelerate research. It enables researchers to more readily reproduce or adapt the methodology described. Protocols Exchange is organized around the concept of Laboratory Groups. All Protocols are associated with a Lab Group and authors cannot

share protocols except as members of a Lab Group. This protocol belongs to an "open" lab Group. It is open under invitations but also upon request. We will be happy to answer all your questions by email if required, but we encourage you to use this tool, since your questions might be helpful to other groups. Therefore if you wish to participate, or make comments or suggestions concerning this protocol, you should request to become a member of the following lab Group, where this protocol belongs: "Institute of Animal Health, University of Las Palmas de Gran Canaria". However, we would be glad also to answer your questions in a more individual way if preferred. Questions should be addressed to the following e-mail address: ybernaldodequiros@whoi.edu

If you wish to send us samples, you could contact Yara Bernaldo de Quirós on the following phones: working hours 508-289-3651, emergencies 508-274-5964 and we will discuss personally how is the best way to proceed on each specific case. We recommend you to contact us as soon as possible in case you need specific material to be sent to you, if you need some recommendations in your specific case or simply to send you the housing chamber as fast as possible. Gas samples should not be stored longer than a week; therefore we encourage you to send the samples the day after the necropsy as latest. Thank you for your cooperation.

Sincerely,
Yara Bernaldo de Quirós Miranda, PhD Postdoctoral Investigator
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REFERENCES

Bernaldo De Quirós, Y., González-Díaz, Ó., Saavedra, P., Arbelo, M., Sierra, E., Sacchini, S., Jepson, P.D., Mazzariol, S., Di Guardo, G., and Fernández, A. (2011). Methodology for in situ gas sampling, transport and laboratory analysis of gases from stranded cetaceans. *Scientific Reports* 1.