General Protocol For Blood Flow Experiments

BioPAL's stable-labeled microspheres can be used in a wide range of research protocols — from blood flow measurements to particle deposition studies. Microspheres are commonly used in cardiac research. The following outlines some general procedures for sample collection and preparation for shipment.

1. **The Injection:** To accurately measure blood flow in myocardium, the suggested minimum dose of microspheres can be calculated from the following equation (dose = $1.2 \times 10^6 + 1.9 \times 10^5$ times the mass of the subject in kg). This dose may need to be increase, if you are measuring flows in other organs, see next page.

2. **Organ Collection:** Organs should be either retro-perfused with sansSa*Li*ne™ to remove interstitial sodium and chloride or tissue samples should be rinsed in sansSa*Li*ne to remove surface blood and other contamination. This procedure may improve the signal-to-noise ratio. Each tissue sample is then placed into a BioPAL sample vial. Label the vial and cap using a permanent ink marker (1,2,3, etc. plus other markings as needed). *PLEASE do not label vials with tape or wrap vials with parafilm and PLEASE do not code your vials, for example A1*.

3. **Histochemical Stains and Dyes:** If histochemical stains or dyes are to be used, we strongly suggest substituting BioPAL's sansSa*Li*ne™ for normal saline or PBS. SansSa*Li*ne is sodium- and chloride-free thereby increasing assay sensitivity following neutron activation.

4. **The Blood Sample:** SansSa*Li*ne should be used to aid in the transfer of blood samples to a BioPAL blood sample vial. Label the vial and vial-cap using a permanent ink marker (1,2,3, etc. plus other markings as needed). For best results, centrifuge the blood sample in the blood sample vial for 1 minute at 2,000 RPM. Aspirate the supernatant to a level safely above the visible pellet. Resuspend the pellet in sansSa*Li*ne and repeat the centrifugation and aspiration steps.

5. **Drying Sample:** All samples (both tissue and blood) should be dried *via* a warming-oven (70 C overnight) or *via* a heat lamp. This step will avoid potential leakage during shipment to BioPAL. (Warning: Direct and/or prolonged exposure to a heat lamp can melt vials.)

6. **Bagging Your Samples:** After drying, place each blood sample into a separate zip-lock bag, which is provided with the blood sample vials. Fill-out the label on the bag. For each experimental subject, place all tissue samples and bagged-blood samples into a separate large sample bag and fill-out the bag label. Five large sample bags have been provided.

7. **Forms:** Fill out all information on the ASSAY REQUEST FORM. (Note: Customer numbers have not been assigned.) One ASSAY REQUEST FORM should be used for each experimental subject. Place the completed ASSAY REQUEST FORM in the large sample bag with its corresponding tissue and blood samples.

8. **Ship the samples, *via* a courier of your choice, to the following address:**
   BioPhysics Assay Laboratory, Inc.
   80 Webster Street
   Worcester, MA 01603
   Phone: (508) 770-1190 Fax: (508) 770-1191

9. **Outside the USA:** If shipping into the United States, please review the enclosed important shipping information notice (see page 8).

How Many Microspheres Do I Need To Inject?

Regional blood flow can be estimated with hematogenously delivered microspheres **(1)**. When appropriately sized microspheres are used, regional blood flow is proportional to the number of microspheres trapped in the region of interest **(2)**. A number of excellent review articles describe and validate the use of microspheres for the measurement of regional organ perfusion. A classic review by Heyman, *et al.* **(1)** contains many details for radioactive microsphere use that apply to stable-isotope labeled microspheres - the only difference being that the assay of the microspheres is performed by BioPAL, which uses neutron activation technology for measuring microsphere content.

The number of microspheres injected must be calculated to assure a sufficient number to accurately determine blood flow to the organ of interest. A minimum of 400 to 600 microspheres is needed per tissue segment for a 95% confidence interval on a blood flow measurement **(3-5)**. As a result, we recommend the following equation to estimate the minimum total number of microspheres needed per injection,

$$\mathbf{Y} = 1.2\text{x}10^{6} + 1.9\text{x}10^{5}\mathbf{X},$$

where Y is the minimum number of microspheres needed for injection and X is the mass of the subject in kg (i.e., 1.5 million for a rat, 2.5 million for a rabbit, 5 million for a small canine, 7 million for a large canine, 9 million for a swine). This equation is designed for an average myocardial study. Therefore, studies involving other organ systems may require an adjustment to microsphere dose.

For example, if a researcher were studying regional myocardial perfusion using a 20-kg animal model, we would recommend a minimum of 5 million microspheres per injection. Assuming the heart receives 4% of the cardiac output, one would expect 200,000 microspheres to be uniformly distributed in the myocardium. If the researcher subdivides the 200-g heart into one-gram segments, there would be 1,000 microspheres per segment, which is safely above the 400-600 microsphere cut-off. However, if the researcher anticipates that some segments would have a 75% reduction in blood flow due to an experimental intervention, then only 250 microspheres will be present. As a result, we would recommend that the researcher increase the injected number of microspheres to 10 million to ensure statistically valid blood flow measurements in low-flow zones. If other organ systems are being studied, we recommend the researcher perform this thought experiment to estimate a required dose for their specific application.

A frequently asked question by researchers new to the microsphere technique is: will large numbers of microspheres affect the physiological state of the model? The answer is NO. The microvascular system is so vast that what may seem to be a large number of microspheres is still a trace concentration (6-8). Estimates suggest that several grams of microspheres are needed to induce physiological effects.

References

1. *Prog Cardiovasc Dis* **20**:55-79 (1977)
2. *Circ Res* **66**:1328-1340 (1990)
3. *J Appl Physiol* **31**:598-604 (1971)
4. *Basic Res Cardiol* **80**:417-429 (1985)
5. *Jpn Heart J* **30**:695-708 (1989)
6. *Am J Physiol* **246**:H418-H434 (1984)
7. *Am J Physiol* **254**:G275-G279 (1988)
8. *Circulation* **83**:974-982 (1991)