

Ordering Information

MD-2100	Shunt Microdialysis Probes, 25 mm membrane window, 3/pkg.
MR-5314	Veterinary Bonding Glue, 3 mL
MR-5313	Introducer Needle
MF-5164	FEP Teflon Tubing, 0.65 mm OD x 0.12 mm ID, 1 meter (clear)
MD-1510	Flanged Tubing Connectors (clear), 20/pkg.
MD-1508	UniSwitch Syringe Selector: changes perfusion fluid without stopping flow
MF-5365	Surgical Instrument/Accessories Kit

Warranty

Shunt Probes are warranted to be free from manufacturing defects and viable for a single use. Reuse of probes after insertion into tissue or handling during in vitro calibration studies is not guaranteed since this is wholly dependent on the skill of the individual user. BAS is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date. BAS will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of the probe. This warranty does not cover damage to membranes or cannulas through improper preparation, inappropriate connections or faulty handling by the user. The foregoing warranty is in lieu of all other warranties expressed or implied including but not limited to the implied warranties of merchantability and fitness for a particular purpose.

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SHUNT PROBE

USER'S GUIDE

7/3/97 Update

A-1375

Introduction

Microdialysis sampling was originally developed in the neurosciences for use in brain tissue. In the decade since the introduction of commercial probes, the applications of this technique have expanded. A variety of peripheral body tissues, as well as cell suspensions and other biological samples, are now routinely monitored by microdialysis.

The use of microdialysis sampling in pharmacokinetic research has offered significant benefits such as more frequent data points, clean samples, no loss of body fluid, targeting of a specific tissue, and consumption of fewer experimental animals per study.

A microdialysis probe consists of a short length of hollow, semi-permeable membrane attached to narrow-bore inlet and outlet tubes. An aqueous solution (called the perfusion fluid), which closely matches the ionic composition of the surrounding extracellular fluid, is pumped slowly through the probe. Low molecular weight compounds, such as the analyte of interest, diffuse into (recovery) or out of (delivery) the probe lumen. Large molecules, such as proteins or analytes bound to proteins, are excluded by the membrane. The molecules entering the lumen of the probe are swept along continuously at a constant flow rate by the perfusion fluid. They exit the probe when the dialysate is collected for analysis.

Inserted into the bile duct, toward the liver

From bile duct into small intestine

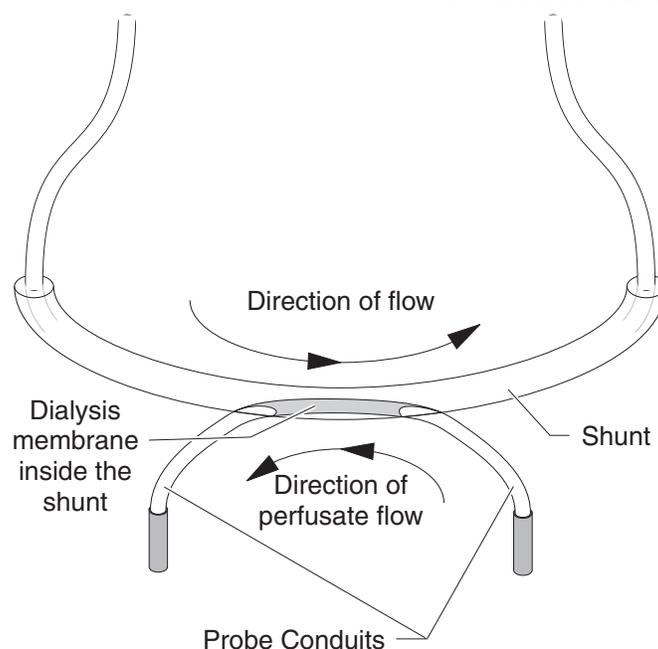


Figure 1. The BAS shunt microdialysis probe.

Design

The BAS shunt probe (see Figure 1) is designed for sampling from the bile duct of awake, freely moving rats without diversion of the bile. The shunt, which carries the bile flow, is made of biocompatible tubing of appropriate dimension for implantation into the bile duct of adult rats. A linear-style microdialysis probe with a 25-mm membrane window is suspended inside the shunt and continuously samples the flowing bile. **Flow within the dialysis probe is counter to the direction of bile flow to maximize recovery.** Fittings on each end of the probe conveniently connect to BAS tubing connectors, which in turn attach the probe inlet and outlet to devices with a 23-ga. cannula such as a liquid swivel, syringe, liquid switch, or sample collection device. Since bile flow is not impeded by the sampling and continues after probe implantation, bile salts are not depleted and normal enterohepatic circulation is maintained.

Probe Preparation

Pores within the dialysis membrane are coated with a protective layer of glycerol. Until removed, this glycerol may interfere with assay results or affect recovery. To remove the glycerol for use in vitro, the probe can be perfused at 2 $\mu\text{L}/\text{min}$ for 30 minutes with water or Ringer's solution while the shunt is perfused in the opposite direction at 20 $\mu\text{L}/\text{min}$ with the same solution.

For in vivo studies, the probe is usually implanted without flushing. In bile, glycerol will be flushed from the membrane during the first several hours after implantation, which is normally during the animal's recovery period. Pretreatment of the probe is not recommended for in vivo studies because once wetted, the membrane becomes soft, more delicate and susceptible to damage. Once wetted, a probe membrane must always be kept wet.

Probe Efficiency

Microdialysis sampling is not typically performed under equilibrium conditions, so the concentration of analyte within the lumen of the dialysis fiber does not exactly match that in the surroundings. The perfusion flow rate usually sweeps the sample through the probe too rapidly for equilibrium to be established between the probe lumen and the surrounding sample matrix. The dialysate concentration of the analyte relative to the concentration in the sample matrix (the bile) may be thought of as extraction efficiency.

Some of the factors that affect extraction efficiency are:

- membrane surface area
- temperature
- perfusion flow rate
- nature of the analyte

Since the dialysis membrane for the shunt probe is 25 mm in length, high extraction efficiencies may be expected for many analytes.

The need to determine in vivo extraction efficiency in a specific probe depends on the nature of the information desired from the study. Studies of endogenous compounds usually compare changes in concentration to a pre-perturbation basal level. For many pharmacokinetic applications it is not necessary to determine probe extraction efficiency since the probe will provide a reliable reflection of the free analyte concentration changes in the bile. If the actual analyte concentration in bile must be obtained, probe calibration may be necessary.

Probe Implantation

For experiments in which the animal will recover from anesthesia and be maintained in an awake animal system, aseptic procedures should be followed. In describing the implantation procedure, it is assumed that the experimentalist is familiar with aseptic techniques.

If appropriate to the experimental protocol, withhold food from the animal for 12-24 hours before the implantation surgery to clear the stomach and upper intestinal track, providing easier access to the bile duct.

Suggested Instruments and Supplies:

- 5-0 monofilament or braided non-absorbable suture [without needle] for ligating the shunt into the bile duct
- Suture and needle for closing incisions
- Surgical introducer (MR-5313)
- Bowman retractor
- Stainless steel micro-spatula
- Probe or explorer: fine, angle tip with slightly blunt point
- Spring scissors
- Suture-tying forceps (2 pairs)
- Vessel cannulation forceps
- Other instruments as desired

Shunt tubing preparation

1. Select the end of the shunt tubing corresponding to the probe conduit with the green connector. Using a scalpel or razor blade to make a clean cut, shorten one end of the shunt tubing to about 2 cm and bevel it slightly. This end will be inserted into the bile duct toward the liver.
2. Using MR-5314 VetBond glue, or a biocompatible cyanoacrylate, apply a small dot or thin ring of glue around the tubing 5-7 mm from the cut end and allow it to cure.
3. Prepare a similar dot or ring 1-2 cm from the other end of the shunt. If aseptic surgery is planned, this preparation should be made before sterilizing the probe.

Surgical Procedure

1. After the animal is anesthetized, shave a small area at the back of the neck and the abdominal area.
2. At the back of the neck, make an incision through the skin only. The incision should be about 1 cm long and

perpendicular to the midline of the body. Once the shunt is ligated into the bile duct, probe conduits will be tunneled under the skin and externalized through this incision.

3. With the animal on its back and its head to your left, make an incision through the abdominal skin along the midline of the body. This incision should begin at the xiphoid process and extend about 3 cm toward the tail. (The xiphoid process is a white cartilage extension of the sternum, but it won't be visible until the muscle wall is opened.)
4. Carefully loosen the skin from the underlying muscle along the edges of the incision.
5. Using forceps with tissue grips, lift the muscle away from the internal organs. Carefully make an incision through the muscle wall using iris scissors or a scalpel. The placement of this incision should correspond to the one in the skin. Hold the incision open with a Bowman retractor.
6. You may find it helpful to place a bolster (tightly rolled paper towels or a plastic tube about 1.5 cm in diameter) under the rat's thorax to arch its back. The internal organs will then fall away from the liver, making location of the bile duct easier.
7. Gently move the liver lobes toward the far side of the abdominal cavity. Small pieces of lint-free tissue moistened with saline solution (0.9% by weight NaCl) may be used to hold the liver and small intestine out of the way. Keep exposed liver and intestinal tissue moist during the implantation procedure to minimize damage to these organs.
8. Locate the hepatic portal vein and bile duct. They run beside one another in a mesentery and will be below the liver. A saline-moistened cotton swab works well as a probe to find the vein and duct. Gently separate the bile duct from the surrounding mesentery and support it over a microspatula. Isolate the anterior-most 1 to 2 cm of the bile duct from the mesentery. Work as close to the liver as possible to avoid damaging the pancreas. Be careful not to damage the hepatic portal vein.
9. Place three lengths of nonabsorbable suture under the supported bile duct (5-0 braided silk works well). Loosely tie the one proximal to the liver and lay it over the rat's chest. Clamp the loop with a small hemostat to hold it in position if necessary. Tie the distal suture loosely and lay or hold it in position toward the tail. Center the middle suture between the others and spread it to the sides. The ligatures should not put tension on the bile duct.
10. Use spring scissors to make a nick in the bile duct about 1 cm distal to its juncture with the liver. Insert the shortened end of the shunt into the duct, toward the liver. The tubing should slide in as far as the glue dot or ring. Secure the proximal suture anterior to the glue dot, making sure that it ties down anterior to the nick.

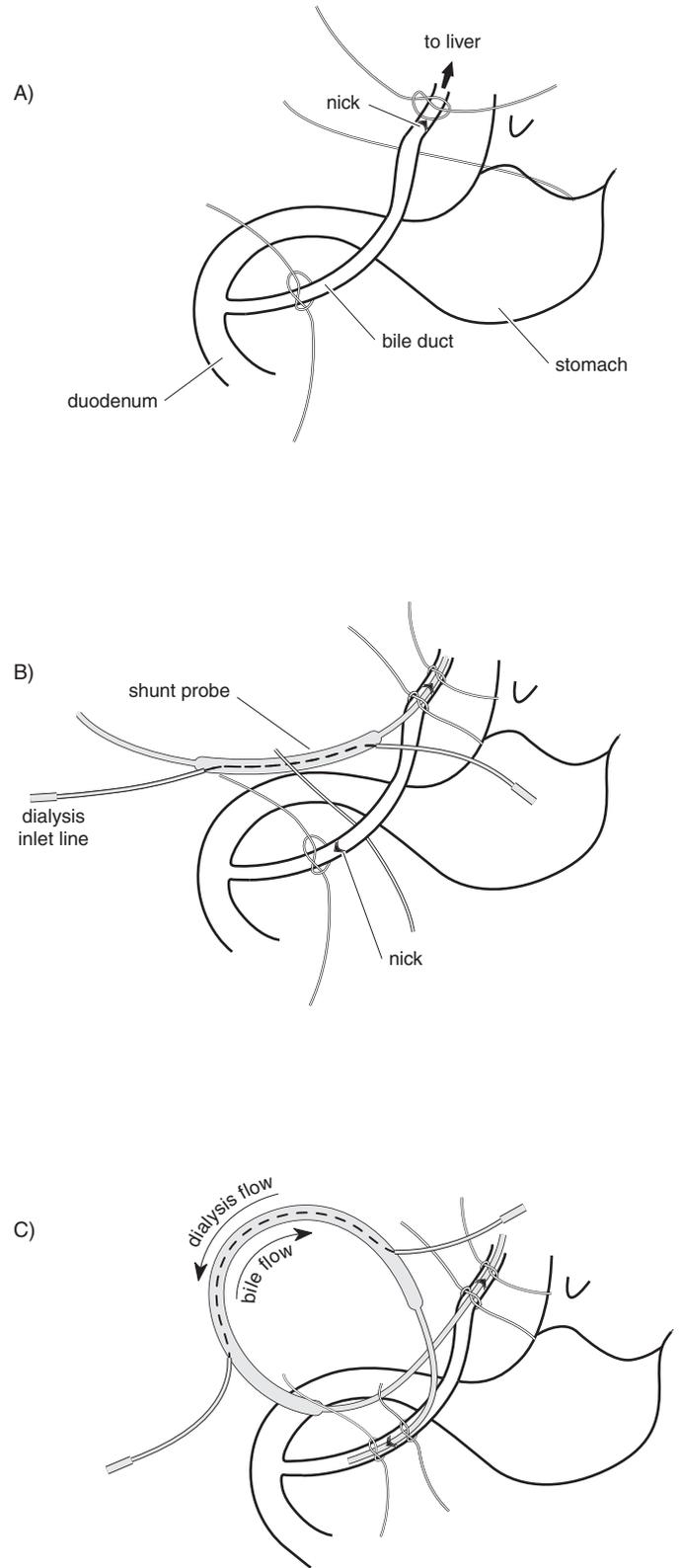


Figure 2. Procedure for implanting a Shunt Microdialysis Probe into the bile duct of an adult rat.

CAUTION: The ligature should be tight enough to prevent bile from leaking around the shunt tubing but not so tight as to obstruct flow through the shunt.

11. Bile should fill and flow through the shunt very quickly. Be sure bile flow is established through the shunt before continuing to secure the probe. Tie a second knot in the proximal suture. Now tie the middle suture around the bile duct and the shunt tube close behind the glue dot. Cross-tie one end of the proximal suture with one end from the distal suture.

CAREFULLY arrange the probe conduits so they do not become entangled with the ligatures as the second end of the shunt is inserted.

12. Working at the isolated region of the bile duct distal to the liver, position the remaining (posterior) suture about 6 mm from the center suture. **Adjust this loop so it is close to the bile duct but do not tighten it.**
13. Make a nick in the bile duct about 3 mm anterior to this ligature.
14. Now make a loop with the shunt and insert the free end of the tubing into the duct toward the small intestine as far as the glue dot allows. Secure the posterior ligature around the duct and shunt tubing posterior to the glue dot. Tighten the ligature to prevent bile from leaking but do not restrict flow through the tubing.
15. Using the ends of the center suture, tie around this second end of the shunt to hold the probe into a loop. Make a second knot in these two sutures and cross-tie as before.
16. Being careful not to cut the probe or shunt, trim the ends of the sutures, leaving them about 5 mm long.
17. Remove the support from under the bile duct. Carefully remove any pieces of tissue used to hold the liver or intestine aside. Remove the bolster and gently tuck the shunt into the body cavity.
18. The probe conduits will extend through the incision. Make sure the liver and small intestine are correctly repositioned with no twists or kinks in the intestine.
19. *If the animal is to remain anesthetized:* Prevent the internal organs from drying by closing the incision in the muscle and skin [as described below] or by covering the organs with a saline-moistened gauze or tissue pad. Remoisten the pad as needed throughout the experiment. To establish dialysis flow counter to the bile flow, use the yellow connector as the inlet.
20. *If the animal will recover from the anesthesia:* Suture the abdominal muscle wall closed with the probe conduits exiting through the incision at the anterior end. With a surgical introducer (MR-5313), tunnel under the skin exiting at the incision at the back of the neck.
21. Thread the probe conduits into the introducer and pull the introducer out of the neck incision to externalize the conduits. Adjust the conduits so they are not pulled tight or bent sharply.

22. Use tissue glue (MR-5314) to close the neck incision and hold the probe conduits. Close the abdominal incision using sutures, tissue glue, or tissue staples. Use the yellow connector as the inlet to establish dialysis flow counter to the bile flow. See the BAS BeeKeeper and Return User's Guide (A-1816) for details about tethering an awake animal.

Sample Handling Precautions

Microdialysis samples are particle free, protein free, and ready to be injected onto a liquid chromatograph for immediate analysis. If you are loading the sample into an on-line injector, there will be no delay between the time of collection and time of analysis and there is nothing else to consider. If you are collecting samples into glass or plastic vials for later analysis, there are a few precautions that should be taken.

Microdialysates may contain the analyte you wish to study, but they will also be loaded with nutrients which have diffused out of the sample tissue (glucose, amino acids, lactate, vitamins). This makes microdialysis samples an ideal growth medium for microbes. It takes surprisingly little time for an airborne spore to land in your sample, multiply at a logarithmic rate, eat nutrients in your sample, and pollute it with metabolic waste.

Retard bacterial growth by refrigerating or freezing samples as they are collected. After use, clean all parts of the system, including tubing, swivels, syringes, etc. We recommend an antibacterial wash (e.g., Kathon rinse, CF-2150, diluted to 0.005%) followed by thorough flushing with distilled water.