PREPARATION AND IMPLANTATION ALZET OSMOTIC PUMPS (SOP-9)

Osmotic pumps manufactured by ALZA Corporation (Palo Alto, CA) are designed for constant rate delivery of test solutions over a period of days or weeks. Pumps are implanted SC or IP for systemic delivery. Drugs can be delivered to the central nervous system (ICV or intraparenchymally), by attaching the delivery port of the pump (implanted SC) to a centrally implanted cannula via a small length of vinyl tubing.

The principal of pump operation is straight forward. An inner reservoir made of a biologically inert and impermeable synthetic elastomer is filled with the solution to be delivered. The outer wall of the pump comprises a rigid semipermeable membrane. Between the inner reservoir and the outer wall of the pump is an 'osmotic sleeve' which expands as water passes into it, compressing the inner reservoir and displacing the drug solution. The inner pump reservoir is compatible with most drugs and drug diluents, except aliphatic and aromatic hydrocarbons and natural oils. Kits are available from the manufacturer to test chemical compatibility with pump materials. Additional information on the operation and applications of osmotic pumps can be found in the manufacturer's Technical Information Manual (Appended)..

PUMP SPECIFICATIONS

AlZET pumps are available with a variety of delivery rates (0.5 - 10 μl/hr) with delivery durations of between 3 days and 4 weeks, e.g.:

Pump Model #	l003D	l007D	200l	2002	2ML1	2ML2	2ML4	
Delivery rate	1.0	0.5	1.0	0.5	10.0	5.0	2.5	(μl/hr)*
Duration	3	7	14	14	7	14	28	(d)
Reservoir	100	100	200	200	2000	2000	2000	(μl)

*Note: The above nominal specifications are performance targets set by the manufacturer. The actual performance of individual lots of pumps will vary somewhat with respect to this nominal performance target. The actual mean volume and pumping rate (measured in vitro in 0.9% NaCl at 37˚C) of each lot are listed on the instruction sheet included with each box of pumps. Keep this variability in mind when designing experiments and always record the lot # and specifications of the pumps actually used.

FILLING THE OSMOTIC PUMPS

Sterile technique should be used during all stages of filling and handling of the pumps, as well as during surgical implantation procedures. It is particularly important that surgical gloves are worn, as the presence of skin oils on the pump

surface may interfere with proper pump performance. If a pump becomes contaminated, it can be wiped with 70% isopropanol immediately before use (do not soak the pump in isopropanol). All solutions loaded into pumps should be sterile. In particular, any non-sterile drug diluent solution should be filtered through a 0.22 μm filter, e.g., Millex-GV, Millipore Corp.

- When filling the pumps, all solutions must be at room temperature.
- Draw the solution into a disposable hypodermic syringe fitted with a blunt-tipped filling tube (supplied with each package of pumps). Make certain that the syringe and attached tube are free of air bubbles.
- With the flow moderator removed and the pump in an upright position, insert the filling tube all the way into the pump (to the bottom of the pump reservoir).
- Slowly depress the plunger of the syringe. When solution appears at the pump outlet, stop injecting and slowly remove the filling tube.
- Still holding the pump vertically, slowly insert the flow moderator until the cap is flush with the top of the pump (this should displace a small amount of fluid from the filled pump). Wipe off the overflow. Verify that the flow moderator is fully inserted into the pump.
- To ensure accurate operation of the pumps, it is essential that each pump is filled completely with solution and that air bubbles are not trapped inside. This can be accomplished in one of two ways. The first is to weigh the pump and moderator before and after filling. The second is to record the volume displaced from the syringe into the pump. In either case, **the recorded fill volume should be at least 90% of the reservoir volume**. If not, evacuate the incompletely filled pump and refill as described above

ATTACHMENT TO CATHETERS OR CANNULAE FOR DIRECTED DELIVERY

The output port of the pump can be attached to a catheter in order to direct the delivery of a drug solution to a particular site; e.g., veins, arteries, the cerebral ventricles or directly into the brain or any other organ or tissue.

For intravenous or intra-arterial administration, catheters should be constructed of medical grade polyethylene tubing (e.g., PE 50 or PE 60) or similar material (microenethane tubing). Medical grade vinyl tubing (V-50 or V-60) has superior flexibility and attachment properties. These properties have distinct advantages in certain applications, for example the connection pumps to cannulae implanted into the brain or ventricular system. This tubing is available from Bolab, Inc. (Lake Havasu City, AZ). Silastic tubing is not recommended, as it allows diffusional exchange of materials.

Regardless of the material selected, all catheters and cannulae should be 'sterilized' before use. Most catheter materials should not be autoclaved (e.g., PE, microenethane). . To 'sterilize' catheters made of these materials, expose them to UV light in the hood prior to use. Vinyl tubing may be autoclaved, although this will result in shrinkage. Segments of vinyl tubing (V3, Bolabs) ~ 30

mm in length are used to attach pumps implanted in the intrascapular region to cannulae mounted on the skull. To obtain final size, the tubing must be cut in 40-45 mm lengths to compensate for shrinkage in the autoclave.

When pumps are used in conjunction with cannulae and catheters, all elements should be filled and assembled prior to equilibration and implantation in the animals. This is accomplished by attaching a filled pump to a catheter which is also filled with the drug solution. The juncture between the pump and the catheter should be sealed and reinforced with a cyanoacrylate adhesive. In cases where the opposite end of the catheter is to be attached to a cannula or other device (E.g. for ICV administration), the cannula should be fixed to the catheter before filling. The entire, filled assembly should then be equilibrated as described below.

EQUILIBRATING AND IMPLANTING THE PUMPS

Equilibrating the Pumps

If after filling and assembly osmotic pumps are implanted directly into animals, the rate of drug delivery will not reach steady state for several hours. If immediate pumping at a constant rate is required by the experimental design, pre-filled pumps should be placed in a sterile 0.9% NaCl solution at 37˚C at least 4 hrs before implantation. Equilibrating the pump in this manner is particularly important when the pump is used with a catheter, and should be done as a matter of routine.

Implanting the Pumps

Osmotic pumps should always be implanted using aseptic surgical technique. Pumps can be placed either subcutaneously (SC) or intraperitoneally (IP). Any substance administered IP is absorbed principally via the hepatic portal circulation rather than via capillaries that enter the systemic circulation directly. Therefore, the IP route should be avoided with drugs having a large first pass effect. The preferred site for SC implantation is on the back, between or slightly posterior to the scapulae. After the animal has been appropriately anesthetized and prepped, a small incision is made at the base of the neck and a subcutaneous pocket to receive the pump is created by blunt dissection with a hemostat or other suitable instrument. The pocket should be deep enough to allow some free movement of the pump, but not so large that the pump moves excessively or slips down onto the flank of the animal. The pump should not rest immediately beneath the incision. Other implantation sites may be used provided that there is sufficient space to accommodate the pump, that the animal's ability to breathe and move freely is not impeded and that pressure is not put on vital organs.

When selecting the ALZET pump for use in a particular study, it is important that the animal size guidelines provided by the manufacturer are followed. For example, in adult mice only the 1000 series pumps can be

implanted IP, while pumps of the 1000 or 2000 series can be placed SC. In adult rats, SC or IP implantation is acceptable for all pump models.

EXPLANTING THE PUMPS AND RECOVERY OF DRUG SOLUTIONS

Water continues to enter the pumps, even after the reservoir has emptied completely. Over time, excessive swelling of the osmotic agent may cause the pumps to leak a concentrated salt solution resulting in a potentially severe local irritation. Therefore, after their “pumping lifetimes” have ended, ALZET osmotic pumps should be explanted from the animal. They should remain in place no longer than half again the specified pumping duration, e.g. a pump rated for 14 days should be removed from the animal by the 21st day post-implantation. As for their implantation, pumps should be explanted using aspectic surgical technique.

In some circumstances, it is useful to recover the pumps to verify their proper function and/or to evaluate the bioactivity of any residual drug solution. When pumps are to be recovered at the termination of an experiment they should be removed after the animal is appropriately anesthetized but prior to perfusion or decapitation in order to avoid loss or contamination of drug solution. There may be some hypertrophy of connective tissue surrounding the pump. After freeing the pump, cut the catheter (if present) as far from its connection to the pump as possible. Clear any remaining tissue from the pump and remove the flow moderator. Draw up any fluid remaining in the pump using a ‘filling tube’ (supplied by the manufacturer) or hypodermic needle of equivalent size that has been cut flush and sanded smooth. Record the volume recovered. Place the recovered drug solution into a sterilized vial marked with the date and animal #, or otherwise coded for later identification. Place samples on ice until all pumps have been emptied. Store samples at -70˚C if they are not to be assayed immediately.