

Webinar Q&A Report: Implantable Infusion Pumps: Insights for Your Next Animal Dosing Study



This report contains a summary of the questions (with corresponding answers) that were submitted during the webinar. For additional information, please visit our website at www.alzet.com, or contact Technical Services at alzet@direct.com or 800-692-2990.

PUMP PRICES

- ALZET pumps price requests: www.alzet.com/contact/request_prices.html
- iPRECIO Pump price requests: www.alzet.com/ipreciorequest.html

PUMP PERFORMANCE

Q: Have you ever looked out closer to the end-point of delivery? For example, with the ALZET Model 2006 (6-week pump), have you looked at delivery closer to the 6-week point?

Each lot of ALZET pumps is tested in DURECT laboratories to determine the exact pumping rate and reservoir volume and to ensure accurate compound delivery. These data are summarized and provided on the instructions found in each box of pumps. The release rate testing for the Model 2006 is performed beyond the 6 week point, after we see a drop off in the release rate. You should also know that all ALZET pumps can deliver for slightly longer than their specified duration. The average duration of pumping for a given lot of ALZET pumps can be estimated by dividing 95% of the average reservoir fill volume (μl) by the average pumping rate ($\mu\text{l/hr}$). This allows for a 5% residual volume which cannot be displaced from the pump. The value that results from this calculation is the actual duration of administration for the lot of pumps you are using.

Q: What is the maximal volume of liquid that I can perfused per day using an iprecio pump? How many times can I refill the pump?

The maximum daily volume of solution you can administer with the iPRECIO pump depends on which pump you are using. The iPRECIO SMP-300 pump can administer a maximum of 240 μl of solution per day when programmed at the maximum release rate of 10 $\mu\text{l/hr}$. Since the reservoir volume for this pump is 130 μl , you would need to refill the pump after the first 13 hours to continue delivery of the solution. The iPRECIO SMP-200 pump can administer a maximum of 720 μl of solution per day when programmed at the maximum release rate of 30 $\mu\text{l/hr}$. In general, the filling septum of iPRECIO pumps can withstand over 50 needle punctures for refilling, which is more than sufficient for most dosing studies.

Q: What is the compound molecular size limit for delivery with Alzet pump?

ALZET pumps can effectively deliver compounds of any molecular size, independently of their physical and chemical properties. Antibodies, hormones, liposomes, and steroids are all examples of high molecular weight compounds which have successfully been infused using ALZET pumps. To understand why molecular weight is not an issue, it is necessary to understand how the pump works and how its components interact to deliver the drug solution. The ALZET pumps have three concentric layers. The outer semipermeable membrane allows water to pass into the osmotic layer (sodium chloride). This osmotic layer swells as it gets hydrated, thereby compressing the inner drug chamber housing the drug solution. The drug solution does not pass through a semipermeable membrane, but instead is infused through a hollow metal tube, called the flow moderator. The inner diameter of the flow moderator is 500 microns, which is large enough to allow infusion of very large macromolecules commonly used in experimental research.

Q: What is the shelf-life for the ALZET osmotic pumps? Any physical indications that pump shouldn't be used?

The shelf-life for a properly stored ALZET Osmotic Pump is up to 7 years. That is to say, you can expect consistent and accurate performance for the duration of infusion, provided the pumps were stored in a dry, room temperature environment and were not exposed to direct heat or extreme conditions during the storage period. A lab drawer or cupboard is ideal. ALZET pumps should never be refrigerated or frozen. Unfortunately, there are no visible signs to indicate they should not be used. More information about shelf-life is available at:

http://www.alzet.com/resources/technical_tips.html#shelflife.

Q: Could you explain how to measure the residual volume in the pump?

Residual volume is the solution remaining inside the pump reservoir at the end of the infusion period. Measuring residual volume can be used as a qualitative assessment of pump functionality. A detailed description is available at:

http://www.alzet.com/products/guide_to_use/verifying.html.

Q: Can the osmotic pressure gradient be blocked by high intracranial pressure (i.e., 20-50 mmHg)?

The osmotic pressure of a saturated NaCl solution, such as that of the osmotic layer of the pump, is certainly high enough to pump against intracranial pressure and even arterial pressure. In fact, ALZET pumps are commonly used for delivery of agents to brain tissue in many experimental situations.

Q: Is there any difference of ALZET pump delivery when implanted SC vs. IP?

The performance of the ALZET pump will not differ depending on the implantation site. The in vivo environment has plenty of moisture (the quantity needed is extremely small) and the temperature is equivalent. There are differences in uptake and distribution of compounds depending on where they are delivered. Many studies have compared the sites. If a compound is cleared substantially by the liver, there can be a “first pass effect” following IP delivery whereby less reaches the systemic circulation because more is taken up via the portal vasculature and removed by the liver.

PUMP IMPLANTATION

Q: Can implantable osmotic mini-pumps be utilized while the animal is performing a behavioral task that takes place in water (e.g. Morris Water Maze, Forced Swim Test, etc.)?

Yes, the pumps have been used in many, many studies involving behavioral testing over the years. This is an advantage of this kind of completely implanted pump (as compared with a tethered infusion system, for example). Rodents have very loose skin on their backs and can accommodate the pump with no apparent discomfort. When implanted according to the recommended animal size guidelines, the pumps should not impose significant impediment on the animal to perform normal activity. Our database includes over 400 studies involving behavioral testing, and we can provide a list of citations on request.

Q: How many surgeries are safe for rodents in terms of inserting and taking out alzet pumps? How many exchanges are optimal?

We don't necessarily have a recommendation in terms of the optimal number of pump replacements. This is more likely dictated by the requirements of the study and your resident veterinarian recommendations. It also depends on the animal species and how spread apart the pump replacements are. I can say that multiple pump replacements are common during chronic dosing studies, and the subcutaneous pump replacement procedure can be performed fairly quickly in the anesthetized animal. We have many references describing multiple pump replacements. For instance, Ravindranath et al. used ALZET pumps for chronic administration of busserelin acetate to bonnet macaque monkeys for a period of 3 years, with pumps replaced every 3 weeks and up to 52 serial implantations. Banach-Petrosky et al. performs a 7 month study in mice with pump replacement every 4 weeks. Detailed information about pump replacements is available here:

http://www.alzet.com/resources/technical_tips.html#replacing_pumps.

Q: Which ALZET pump do you recommend for administration to sheep brain? We use a microinjection pump for 3-day infusion. Where do you recommend inserting the Alzet pump? Can the iPRECIO pump be used for infusion to sheep brain? How early iPRECIO can be fixed in animals?

All ALZET pump Models can be used for drug administration to the sheep brain. Since you are working with large animals, you will likely need to administer higher doses than those for rodents. Therefore, you may need to use the larger pump sizes (2ML sizes) since these will allow you to deliver larger volumes at higher flow rates. For a 3-day infusion, we have a 3-day pump (ALZET Model 1003D) with a release rate of 1 μ l/hr and a 100 μ l reservoir volume. If you cannot concentrate your total drug dose in a 100 μ l volume, you may need to use the Model 2ML1 (1-week duration) or Model 2ML2 (2-week duration), but you will need to remove the pump after the 3-day infusion that you require. With respect to the location of pump implantation, I'd suggest placing it in an area of the neck with enough catheter length to prevent catheter detachment as the animal moves. We can provide a list of references on the use of ALZET pumps in sheep. These publications might offer additional details about the experimental methods. For example, on publication #P9189, the authors implanted the ALZET Model 2ML2 and 2ML4 in the neck area of the sheep. You may also use the iPRECIO programmable pump to deliver solutions to the sheep brain in a similar way as with the ALZET pumps. You can implant the iPRECIO pump as early as needed since you can program the pump to start administering the drug solution at a later time point.

Q: Is there literature on how pumps could be angiogenic?

I am not aware of literature evaluating whether the pumps themselves could be angiogenic. We do have several hundred studies in which angiogenesis was a focus of the study, and using a variety of controls. In more than 200 of these studies, there was a control group that also received pumps (containing vehicle, for example).

Q: Where is the best place to implant the pump so animals don't itch (local corticoids) and destroy the pump?

The best place to implant the pump in rodents is on the back over the shoulder blades. When implanted correctly (i.e., sterile technique, appropriately sized SC pocket, right size animal for the pump model, etc.), a pump implanted here will not cause any problematic local reaction. If the solution in the pump is irritating, it might be better to either put the pump in the peritoneal cavity (if the animal is large enough) or attach a catheter such that a pump placed SC delivers the solution IP through the catheter.

Q: Can the pump be used for delivery of compounds directly to the intestine of mice? I would like to deliver metabolites or gut bacteria to intestine tissue in mice.

You can certainly use ALZET pumps for administration of metabolites or gut bacteria to the intestine of mice. You can implant the pump in the peritoneal cavity of the mouse with a catheter tip inserted in the intestinal location you wish to target. Our mouse IP catheter could be used for this application (catheter specifications are available at <http://www.alzet.com/products/catheters.html#MIPC>). We have two references in our database that describe intestinal administration in mice using ALZET pumps (more references are available for rats): P6355: Mrsny et al. Identification of heparin A₃ in inflammatory events: A required role in neutrophil migration across intestinal epithelia. PNAS 2004;101(19):7421-7426 P8774: Sugimoto et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. Journal of Clinical Investigation 2008;118(2):534-544

Q: Is the iPRECIO pump also available for mice that weigh less than 22 grams? Generally our female mice at 8 weeks of age weigh around 17-20 grams.

Unfortunately, a 22 g mouse is the smallest animal that the iPrecio pump can be used in. The SMP-300 pump itself is 25 x 15 x 7 mm and weighs just over 3 g, so it would not fit into a smaller mouse.

Q: Do you have any information regarding the frequency of skin lesions observed in animals with osmotic pumps?

Most users have no skin lesions when using pumps. Skin lesions are not expected. However, these can happen when the pump is too large for the animal (i.e., outside our weight guidelines), when the SC pocket is not made large enough for the pump, when the solution being delivered is very irritating or in the case of a post-surgical infection. We can provide information to help prevent any of these problems.

Q: Can I use either pump to infuse in the catheterized jugular vein?

You can certainly use either the ALZET Osmotic Pump or the iPRECIO Programmable Pump for administration of solutions via the jugular vein of mice and rats. We have various resources, including surgical implantation videos and protocols to assist you in using either pump for this application. You may access surgery and product videos at our vimeo channel at <https://vimeo.com/channels/alzet>. We also have various ALZET and iPRECIO-compatible catheters with customized tips for insertion to the jugular vein of rats and mice. Additional information about these catheters is available at: <http://www.alzet.com/products/catheters.html#MJC>

Q: Why you do not recommend to implant the Model 2006 pump IP?

ALZET pumps can be implanted IP in animals with sufficiently large peritoneal cavity. Depending on the size of the animal relative to the pump, IP implantation may disrupt normal feeding and exert pressure on the vital organs, which can adversely affect the health of the animal. We recommend against the use of the 200 µl size pumps (i.e., Model 2006) for IP implantation in animals under 150 grams. This is a very conservative recommendation to limit liability. However, we are aware of researchers that have ignored these recommendations and used them in their studies. We also know of publications that mentioned having used 200 µl size pumps for IP implantation in mice.

Q: How do I prevent the brain infusion kit from getting blocked during implantation?

Using a premade cannula, such as in the ALZET Brain Infusion Kit, is a good start. One should also follow the instructions when attaching the components (cannula-catheter-flow moderator-pump) and filling the assembly as a unit prior to priming. Lastly, priming the pumps, so that there is flow through the assembly upon implantation, will help ensure patency. The solution itself should be stable at body temperature so that it does not precipitate and obstruct flow. Additional information pertaining to verification of cannula placement/patency is available on our website at: http://www.alzet.com/resources/technical_tips.html#Verifying.

PUMP OPERATION

Q: Would the pumps still work if priming is not performed?

In some situations, not priming the pumps could cause problems. For example, catheter occlusion due to clotting blood would be a risk if using non-primed pumps for intravascular infusion. However, in most applications (such as SC delivery of an aqueous solution) it is not necessary to prime the pumps, and they would ramp up to steady-state infusion rates during the initial hours in the animal.

Q: What happens with air in pump after pump is on?

For accurate operation, it is essential that the ALZET pump is filled completely with drug solution and that correct filling is verified by weighing the pump before and after filling. With respect to the iPRECIO pump, you should also remove all air from the reservoir and fill the entire catheter with drug or vehicle solution prior to implantation. Air bubbles trapped within the reservoir of the pump will result in unpredictable pumping rate fluctuations.

Q: Do you have to explant the iPrecio-pump after battery life time is over?

Once the battery life runs out on the iPRECIO pump, drug administration will stop and the pump will become an inert object. Whether you remove the pump or not will depend on how long you plan to keep the animal in the study. For example, if the study is scheduled to terminate within a couple of weeks after battery runs out, it may not be necessary to remove the pump. However, if you plan to keep the animals in the study for weeks or months after, it might be better to remove the pump soon after the battery runs out.

SOLVENTS & SOLUTIONS

Q: How do the pumps respond if our suspension solution is ethanol/saline 50/50?

Ethanol can be used with ALZET pumps up to a maximum of 15% concentration. We recommend against using higher than 15% ethanol since it is incompatible with the reservoir material of the pump. Additionally, the lower viscosity of ethanol can cause random diffusion of the solution out of the pumps. Therefore, compound delivery may be independent of the actual delivery rate of the pumps.

Q: Is 50% DMSO 50% PEG solution preferable to 50% DMSO 50% saline solution?

The pumps will perform the same with either solution. If the former solution is viscous, you may need to prime the pump prior to implantation (soak the filled pump in 37C saline overnight before use – or longer depending on the pump model). From a biocompatibility standpoint, the more physiologic solution that is possible would always be preferred. So, if your drug is in solution in a 50:50 solution of DMSO and saline, I would suggest that option. Another factor influencing whether to choose one solution over the other would be the solubility properties of your compound. A 50/50 DMSO/PEG solution will have greater dissolving capacity compared to a 50/50 DMSO/saline solution. More information regarding recommendations for dissolving poorly soluble compounds is available here:

http://www.alzet.com/resources/technical_tips.html#comp_solvents_poor_sol.

PRODUCT USE

Q: Are both Alzet and IPRECIO compatible with MRI?

ALZET pumps can be used during MRI studies, but the stainless-steel flow moderator must be replaced with MRI-compatible materials (see answer below). However, the iPRECIO pumps cannot be used during MRI studies.

Q: How is sterility maintained for the outside of the pump prior to implanting?

ALZET pumps and iPRECIO pumps are supplied sterile, and we recommend that sterile technique be used during the filling and handling of the pumps and during the surgical implantation procedure. Inadvertent contamination of the solution or pump during filling may result in the growth of potentially activity-destroying microorganisms, tissue irritation, and erratic results. If the sterility of your solution is a concern, fill the ALZET pumps through a 0.22 µm syringe-end filter (e.g., Millex®-GV). During filling and implantation, ALZET pumps should be handled with surgical gloves. Skin oils may interfere with the performance of a pump if they accumulate on its surface. If a pump becomes contaminated, its surface may be wiped with an aqueous solution of 70% isopropanol immediately before use. Do not soak the pump in isopropanol.

Q: I would like to use the Alzet pump for intracranial infusion. Are all the parts MR-compatible? I will also be imaging during use.

ALZET Osmotic Pumps can and have been used successfully during MRI imaging studies. It is important to know that the flow moderator of ALZET pumps is not compatible with MRI equipment. However, this piece can be replaced with MRI-compatible materials for successful use. You can find detailed information about the use of ALZET pumps for MRI studies, including details for replacing the stainless steel flow moderator, in our website at: http://www.alzet.com/products/guide_to_use/mri.html.

Q: Is there a way to delay the release of the dosing for CNS delivery?

ALZET pumps can be adapted to allow for a recovery period following surgery, such as after implantation of brain cannulae. In this application, the ALZET pump is filled with drug solution and attached to a length of catheter tubing, which has been loaded with a control solution (no drug). If the control solution is such that mixing could occur between it and the drug solution, a spacer substance should be placed between the control and drug solutions. The spacer substance can be any liquid in which the drug solution is not miscible, such as oil or sterile air. Upon implantation, the ALZET pump begins releasing the drug solution from the pump reservoir

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into the catheter tubing, displacing control solution from the catheter tubing into the animal. Once all of the control solution is released, the drug solution reaches the end of the catheter tube and is then released into the animal at the pump's constant rate. Additional details about this procedure are available on our website at:

http://www.alzet.com/resources/technical_tips.html#DelayedCNS. If using an iPRECIO pump, the delayed delivery period can be easily programmed prior to implantation.

Q: Could you implant iPRECIO and ALZET pump in the same animal one for steady dosing and one for programmable purposes?

Implanting an ALZET pump together with an iPRECIO pump is also possible according to the same animal size guidelines as described earlier. In some cases, you would even be able to implant an ALZET pump in the peritoneal cavity, while the iPRECIO pump is implanted subcutaneously. I am aware of one publication where both, an ALZET pump and an iPRECIO pump, were used simultaneously (*Hamada et al. In vivo imaging of clock gene expression in multiple tissues of freely moving mice. Nature Communications Scientific Reports 2016:6 [Link to Publication].*)

Q: How many alzet pumps or iprecio pumps can be implanted in mice or rats for combinational therapeutic studies?

You may implant two or more ALZET pumps at the same time provided the animal is large enough to accommodate the additional size and weight of multiple pumps. We have many publications in the ALZET database documenting the use of multiple pumps simultaneously in a single animal. For example, up to three Model 1003D pumps have been implanted in mice (Kuroiwa et al. 2003), up to 4 Model 2002 pumps have been implanted in rats (Khan et al. 1983), and up to four 2ML-size pumps have been implanted in dogs, monkeys, and rabbits (Gilberto et al. 2002; Tarantal et al. 1994; Cellini et al. 2004). You can review additional information pertaining to this application at the following:

<http://www.alzet.com/downloads/ImplantingMultiplePumps.pdf>.

With respect to the iPRECIO pumps, I am not aware of any studies where multiple iPRECIO pumps are used simultaneously in an animal. However, you may also be able to implant multiple pumps depending on the size of the animal. This procedure is not possible in mice. In rats, however, you may only be able to use up to two of the iPRECIO SMP-300 pumps (smaller model). In larger animals, such as rabbits, dogs, or large primates, you may be able to implant two or perhaps more iPRECIO pumps (SMP-200 or SMP-300).

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Q: Can the wireless iPRECIO pump be controlled while in use (i.e., change the rate)?

Both iPRECIO pump models (SMP-200 and SMP-300) are primarily designed for pre-programmable dosing. However, since the SMP-300 pump is programmed by wireless communication, this pump offers more the possibility for reprogramming while in the animal. If reprogramming is required in the middle of the study, the user would need to access the infusion protocol in the iPRECIO software to cancel the protocol, then communicate this change to the pump, and program a completely new infusion protocol and download to the pump. The process is not instantaneous, and this is the reason why multiple reprogramming is not recommended. When reprogramming, the pump continues to deliver until the pump is receptive to accept the signal to abort the protocol. This depends on the communication interval that is chosen at the time of programming. The communication interval (how often the pump communicates to the base station) can be set to every minute, or every 2, 6 or 24 hours, or never. If you need the capacity to stop the infusion quickly at a specific time, you would need to program a communication interval of “every minute”. However, this setting consumes the battery life much faster than a communication interval of 6 or 24 hours (Refer to the table below for battery life information). If you program a longer communication interval, it takes longer to stop and reprogram.

Comm. Interval	Every Minute	Every 2 hrs	Every 6 hrs	Every 24 hrs	None
<i>Flow Rate (ul/hr)</i>	<i>Time (days)</i>	<i>Time (days)</i>	<i>Time (days)</i>	<i>Time (days)</i>	<i>Time (days)</i>
0.1	16	37	42	45	47
1.0	14	28	31	32	33
5.0	10	15	15	16	16
10.0	7	9	9	9	9.0

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