ELLEGAARD
GÖTTINGEN MINIPIGS

Care and maintenance of Göttingen Minipigs with implanted venous catheters and vascular access ports

Advice and guidance notes for customers

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Introduction

Maintenance of long-term implanted vascular catheters in Minipigs has a significant impact upon the ultimate performance of the device, as well as animal health and welfare. Meticulous attention to detail will help to ensure success. Many of the complications of vascular catheters in Minipigs are virtually impossible to eliminate successfully, once established, making prevention of problems paramount in any catheter maintenance program.

Prior to implantation, time should spend acclimatizing Minipigs to their working environment and handling. This is an essential step in facilitating good maintenance procedures - by ensuring the animal's co-operation.

This datasheet provides essential information and advice on the care and use of Gottingen Minipigs with venous access catheters and subcutaneous vascular access ports (VAP).

There are many stakeholders in the care, use and welfare of surgically prepared animals who need to be both informed and educated to minimize the occurrence of problems. Ensuring good communication and planning among the responsible individuals and functional roles is crucial to making sure everything goes well.

2. March 2015 AZ/TW

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Why vascular catheters?

Acute or short-term venipuncture is associated with some procedural stress in the Minipig. Repeated venipuncture (e.g. for serial blood sample) will result in additive stress, especially if sampling frequency is frequent. Good handling by competent, well-trained personnel and animal training or habituation will help to reduce this animal stress. Surgically implanted vascular catheters provide access to the blood stream for blood sampling or administration of compounds into the blood stream on a constant basis. The stress of frequent blood sampling is therefore reduced and the possibility of administration of compounds by infusion over long time periods is facilitated. It may also facilitate some of the systems for automated blood sampling or sampling from a distance if an extension is used.

Why vascular access ports?

Long term surgical access to deep blood vessels was originally achieved by leading catheters through skin to the exterior where the catheter was temporarily sealed (e.g. by a 3-way tap or plug in a Luer connector). In this case, there is an ever present risk of infection gaining access where the catheter exits the animal's body or via the open end of the catheter.

The external part of the catheter is always at risk of damage and may need protection in a jacket worn by the pig, or small pouch sutured to the skin. Pigs are particularly noted for their tendency to rub and scratch on walls or pen fixtures. The external connector, however well sealed, always remains a potential portal through which infection may access the catheter lumen. Group housing of pigs with exteriorized catheters is inadvisable as animals may interfere with each other's implants.

Vascular access ports and attached catheters are totally implanted which significantly reduces the incidence of catheter related infections that develop. The incidence of catheter damage is much reduced with total implants and it is possible to group house animals with ports when they are fully recovered from surgery and healing is complete.

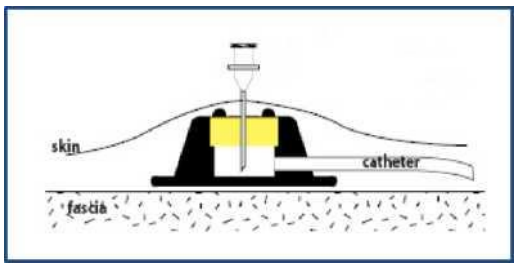
Vascular access ports

The vascular access port (VAP) permits intermittent access to a catheter using a chamber connected to the catheter lumen. The chamber consists of a metal or plastic casing with a connecting stem for the catheter and a thick, self-sealing septum, which can be punctured with a needle.

The port is implanted at a subcutaneous site so that a suitable needle inserted through the skin and septum accesses the lumen of the port-catheter system. Once

healing of the surgical incision is complete, the port and catheter are surrounded by intact skin and connective tissue so the likelihood of an implant infection arising from the animal's skin is unlikely.

The greatest risk of infection is during access of the port by needle puncture, but with good aseptic technique, the risk of catheter sepsis is much lower than with externalized catheters. As with all vascular catheters, careful prevention of biofilm accumulation and thrombosis is necessary to prevent thrombotic occlusion of the catheter system and the development of a substrate which favors sepsis.



Responses to implantation

A complex interaction occurs from the time of implantation of a vascular catheter between the materials from which it is constructed and the animal body into which it is implanted. Nearly all materials acquire a biofilm that influence the long-term behavior of the catheter and the presence of the foreign material incites responses in the body.

Understanding of these interactions is a valuable starting point for appreciating the principles of catheter design, implantation and maintenance. The materials used in construction of the catheter will influence the type of response mounted at the surface of the catheter and by the surrounding tissues.

Locking and flushing the VAP and catheter

The presence of blood within the lumen of a catheter contributes to biofilm formation and thrombosis.

When not in use, the lumen of the vascular catheter and VAP are filled with a solution which prevents blood entering the system and giving rise to biofilm or thrombus. The solution is referred to as a lock solution and the process of filling the catheter and VAP with lock

solution to exclude blood is called locking the catheter. In addition to occupying the space within the lumen to exclude blood, the lock solution is also important in prevention of thrombosis and prevention of infection.

Key desirable features of lock solutions are:

- Anticoagulant
- ease of sterilization
- compatibility with catheter materials, compounds and vehicles
- haemocompatible
- low toxicity

Commonly used lock solutions are generally isotonic although hyperosmotic solutions may be encountered. Use of high viscosity lock solutions (e.g. glycerol or high molecular weight polyvinylpyrrolidone - PVP) can be helpful in helping to prevent blood ingress into the catheter but consideration must be given to the ease of removing the lock solution.

Solutions based on sterile physiological saline (0.9% sodium chloride) with heparin are the most commonly used but have no antimicrobial effect. Hypertonic solutions (e.g. 40% dextrose) provide an antimicrobial environment.

A variety of different lock solutions have been described (see Appendix 2 - locking solutions for vascular catheters)

Procedure for accessing a VAP

It is necessary to access VAPs for regular maintenance (flushing and locking) in addition to experimental use. Typically, we suggest that routine flushing and locking be performed at weekly intervals. With experience, the interval may be taken out to two weeks.

When the port is accessed for experiments, it will be flushed and then locked as with routine maintenance. Frequency of maintenance flushing/locking is a balance between risk and benefit. If performed too frequently, there may be problems associated with increased chance of infection, skin irritation due to frequent needle punctures and cumulative damage to the septum. If the interval between routine maintenance is too great, then the risk of thrombosis at the catheter tip and microbial colonization increases. The nature of the locking solution selected will also influence the frequency of flushing and locking.

When the VAP is accessed and the catheter flushed, there is always a possibility that some pyrogenic material or microbial contamination may enter the minipig's blood stream, giving rise to adverse clinical reactions (pyrexia, bacteremia, septicemia or distant infection). We therefore recommend that, where possible, catheter flushing and locking be performed early in the working day to allow time for observation of the animals after flushing. It is also a good idea to avoid these procedures close to a weekend or holiday when animals may have less frequent observation.

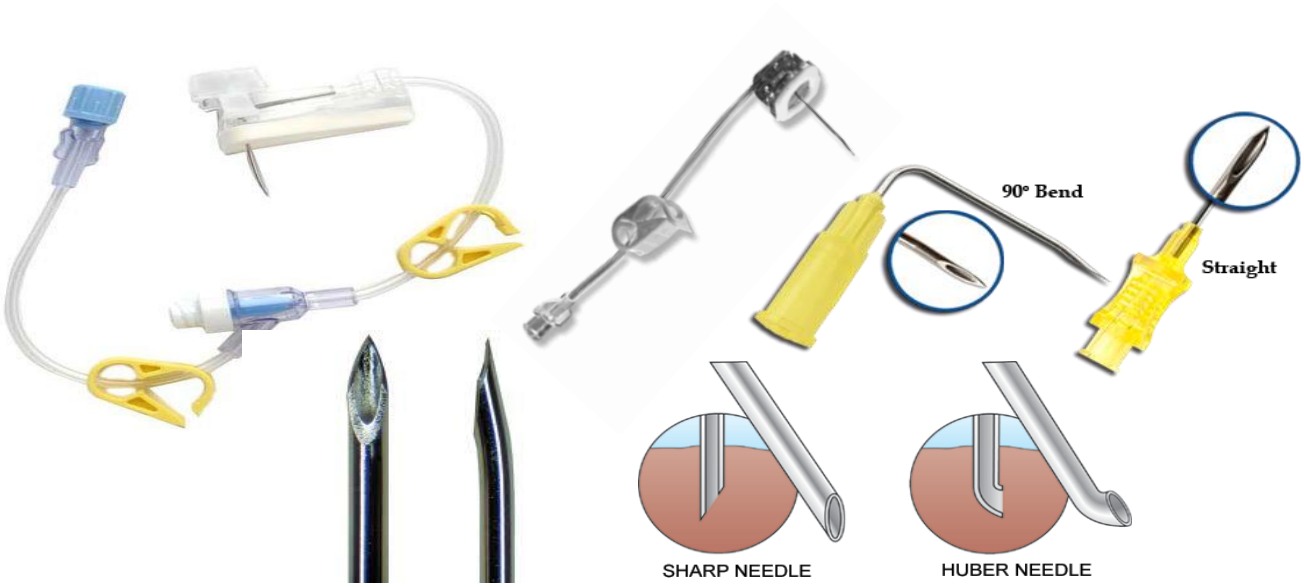
Accessing a VAP involves the following stages:

- Puncture of the VAP
- Aspiration of old lock solution until blood flows back
- Sampling or dosing
- Flushing to remove all traces of blood from the lumen of VAP and catheter
- Instillation of lock solution
- Removal of the needle

Puncture of the VAP

Modern vascular access ports have a robust septum which is capable of withstanding many repeated needle punctures and then effectively sealing again. To ensure this function, special non-coring Huber needles are used. The point configuration prevents "coring" or “cutting of a plug” of the septum material. Please consult the manufacturer's recommendation for the needle diameters which may be used for particular ports.

Huber needles are available straight or right angled. The use of right angled needles permits easier long term access as the needle and connectors lie flat against the animal's skin and can be secured into position. Huber needles are also available with either standard Luer connectors or an attached flexible giving set tube which may have a clamp and side arm attached.



Prior to accessing the VAP, the site of the VAP and track of the catheter to the vessel should be visually inspected for signs of abnormality. The implants should then be palpated to locate them and ensure integrity and further check for signs of abnormality.

Bristles and hair over the port and a surrounding margin are clipped occasionally to keep them short and reduce chance of infection. Frequent clipping or close shaving may give rise to dermatitis and is best avoided. After clipping, all loose bristles are removed by swabbing with a sterile gauze swab moistened with sterile 0.9%saline.

The port should be carefully palpated through the skin to determine the location and the center of the septum. It may help to mark this with an indelible marking pen or surgical skin marking pen. In other species (dog, primate, and rabbit) where skin is thinner, the port is easier to palpate that in pigs so it is worth spending some time to confidently locate the port septum.

Once the septum has been located, the skin is cleaned and disinfected using an appropriate cleansing agent such as povidone-iodine scrub or chlorhexidine scrub (these are the same as preparations used in "scrubbing up" of the hands prior to surgery). The agent should be used at the manufacturer's recommended concentration and skin gently wiped with a sterile gauze swab moistened with the cleaning solution, working away from the point of intended puncture. It is best to wipe in a spiral movement and cleanse an area about 10 x 10cm. When the swab reaches the outside of the area to be prepared, it should be discarded and a fresh swab used to start again at the centre. This process should be repeated until there is no sign of hair or loose skin or dirt on the swab and the area around the port puncture is clean.

Following cleansing, the skin is disinfected using an antiseptic solution (similar to the final

skin dressing used prior to surgery). Povidone iodine or chlorhexidine tincture are recommended. The solution should be allowed sufficient time to dry on the skin. Contact time is important in ensuring efficient disinfection. Also, all traces of alcohol should evaporate to avoid any associated puncture pain due to its use. Following the final skin dressing, do not touch the prepared area.

Using a strict "no touch" technique, stabilize the rim of the port with one hand. With the other hand, insert the needle in one thrust through the skin and underlying septum until the needle is felt to contact the internal base of the chamber. The needle should be perpendicular to the port. It is recommended that a 2ml syringe containing 1ml of saline be attached to the needle and the dead space of the needle and any connecting tubing be filled with saline to exclude any air bubbles. Some parts of the venous system may actually be under negative pressure and the possibility of air being aspirated should be avoided.

Aspirating from the port

Once the port has been punctured satisfactorily, gently aspirate using the syringe attached to the needle. Excessive suction may result in collapse of the catheter or suction of the catheter tip against the vessel wall and failure to aspirate the old lock solution. The old lock solution should flow into the syringe (the volume to be removed will be known from the dead space of the port and catheter assembly). Following withdrawal of the old lock solution, continue to aspirate until about 1 ml of blood has been withdrawn. This will ensure complete removal of old lock solution. If a blood sample is to be taken, a fresh syringe can be attached and the desired sample withdrawn before proceeding to catheter flushing.

Flushing the port and catheter

The syringe used to aspirate should be rapidly replaced with a 5ml syringe filled with flushing solution. This is usually 0.9%sterile saline. The port and catheter are flushed by brisk injection of saline to flush all traces of blood back into the circulation. A slightly rhythmic variation in pressure on the plunger can be used to provide a pulsatile flushing to help displace blood from the lumen of the VAP/catheter assembly. Avoid excessive pressure during flushing as this may potentially damage or disrupt connections in the implant assembly and could cause discomfort for the animal around the catheter tip.

Flushing should be performed as rapidly as possible after aspiration of blood to minimise the contact time of blood with the lumen of the VAP and catheter and reduce the tendency to biofilm or thrombus accumulation.

Locking the catheter

A suitably sized syringe (1 to 2ml) is filled with a slight excess of lock solution. Replace the syringe used for flushing with the syringe containing the lock solution. A volume of lock solution equivalent to the dead space is then injected slowly. *Once this volume is reached, the needle withdrawn while the excess volume in the syringe is being injected.*

This procedure is called “positive pressure locking” and ensures that no blood enters the tip of the catheter, which would contribute greatly to the blockage of the system.

Study design considerations

There is great potential for physicochemical and biological interactions between the materials of the port and catheter, the animal in which the devices are implanted, flushing and locking solutions, materials injected and vehicles used to dissolve compounds administered. External infusion systems (syringes, reservoirs and tubing sets) may also need to be considered.

In planning an infusion study, we recommend that stability of compounds to be infused be evaluated in vitro by simulating the infusion equipment (e.g. in a laboratory incubator at 38°C). Loss of compound may occur due to instability (e.g. hydrolysis in solution) at body temperature, crystallization (which may occur in catheter tubing) or binding to materials in the infusion assembly.

Design the infusion system with as few connections as possible since each connection may increase risk of accidental disconnection, introduction of infection and act as a focus for compound crystallization.

Infection - catheter related sepsis (CRS)

CRS is an ever present hazard of long term implanted vascular catheters. The catheter forms a direct portal to the blood stream through its lumen and the potential space around the outside of the catheter wall (the catheter track) is a second (and often overlooked) portal of infection.

Direct exteriorization of a catheter through the skin poses additional risks of infection over totally implanted devices (vascular access ports, VAPs). Once bacterial colonization has occurred on the catheter, the complex reaction between bacteria and the biofilm provides an environment, which is highly resistant to attempts to eliminate the contaminants.

Provided that catheters are inserted and maintained with suitable aseptic technique, the most likely source of infection is from the animal's own flora - either the skin surrounding the catheter exit site, or a distant focus of infection within the body. Use of Dacron™ velour cuffs deep to the dermis has proved an effective barrier to the ingress of bacteria.

Once CRS is established, it is effectively impossible to eliminate using antibiotic and other therapy. Experience in some laboratories of removing infected catheters and then delivering a course of appropriate systemic antibiotic is that the original infection will recur at the site of implantation of a fresh catheter. Prevention is therefore of the utmost importance and the value of adherence to strict aseptic technique cannot be over emphasized.

Thromboembolic complications ("fibrin cuffs")

The pig seems particularly prone to formation of thrombophlebitis associated with vascular catheters. Correct anatomical placement of catheters and optimal construction are important factors in preventing this.

For central venous catheters, insertion through a jugular vein with location of the tip just inside the right atrium is our preferred approach. Use of the femoral vein and vena cava have shown a high incidence of thrombosis and "fibrin cuff around the catheters, significantly reducing longevity.

If minor, these complications may not interfere with compound administration but will cause problems with blood removal via catheters.

Thrombolysis of vascular catheters

Where a thrombus or fibrin flap is confirmed or suspected at or around a catheter tip, the use of thrombolytic therapy may be considered. A variety of agents are available: historically, urokinase was used and more recently streptokinase and tPA are available.

One problem of introducing a thrombolytic solution into a catheter lumen is inability to infuse due to obstruction. In this event, intermittent positive and negative pressure can be applied either by using a three way tap connected to the port puncture needle. Quite strong negative pressure is applied deliberately to collapse the catheter and withdraw content, before adjusting the tap position to allow injection of the enzyme solution. Repeating the process will gradually allow flow of the solution to the catheter tip. Alternatively, the port can be punctured with two needles - one for the suction syringe, the other for the infusion solution.

Catheter thrombolysis is most likely to be effective for recent or freshly formed thrombus. It is less effective or ineffective as thrombus ages.

Use of thrombolytic solutions within the catheter lumen will not, of course, influence fibrin flap or cuff arising in the vessel wall around the catheter tip. In these cases, system thrombolysis could be considered but is likely to be limited in effect.

Special problems of continuous and long term infusions

Where compounds in solution are infused continuously through a vascular catheter, it is not uncommon to encounter problems associated with chemical incompatibility between the catheter and the compound or vehicle. The particular conditions inside the catheter and connecting tubing may predispose to crystallization of dissolved compounds which may potentially block the catheter. Such studies should be carefully planned with due care and attention given to thorough investigation of these compatibilities.

Necropsy

We recommend that a detailed necropsy be performed in animals with VAPs and vascular catheters to document the appearance of local and distant body tissues.

The appearance of the implants should be noted and any sites of damage recorded.

Information obtained at necropsy is valuable to help retrospective interpretation of data obtained in the animal's lifetime and also for refinement of future surgical procedures and implant design.

Appendices

Appendix 1 - Complications and troubleshooting

	Normal	Possible findings/Cause	Action
Animal - general condition and symptoms - daily observations by husbandry and other staff	The animal should show normal signs of health and well	• Changed behaviour, abnormal vocalisation, abnormal appearance of skin, eyes. • Anorexia • Respiratory signs (tachypnoea, dyspnoea or cough)	General signs of ill health may indicate complications due to catheter related sepsis or pyrogens. Body temperature should be monitored and veterinary consultation sought.
Overlying skin and surrounding tissues - visual inspection	Overlying skin should be normal in appearance	Redness or other discolouration of the skin, discharging sinuses, scaliness, ulceration or thinning of the skin are all abnormal. They are likely to be indicative of of implant infection; however, pigs as a species are more prone to foreign body reactions and are prone to rub the site of subcutaneous implants.	Veterinarian consultation
	Following post surgical healing, the skin and surrounding connective tissue should surround the implants	• Accumulation of fluid around the VAP is abnormal. • Thickening of surrounding soft tissue should be absent or minimal - excessive fibrosis around the port is abnormal	Veterinarian consultation *Although fluid or soft tissue reactions around the implant are usually the result of infection or foreign body reaction, they could also arise if the catheter was fractured, kinked or detached from the port with leakage of blood or lock solution.*
Implants - visual inspection and digital palpation.	The port should be palpable; the connecting stem point in the direction in which it was inserted and the catheter should follow its original track.	Port inversion is possible but very unlikely in pigs due to the low mobility of skin (compared with other species) Displacement of the port may result in the connecting stem pointing in the wrong direction	If available, radiographic examination may add additional diagnostic data to help decision-making. Some catheters have a radiopaque marker incorporated to they are readily visible on

2. March 2015 AZ/TW

		Kinking of the subcutaneous portion of the catheter may be palpable as a sharp angle or "knot". Discontinuity of the catheter: modern catheters are very robust and unlikely to fracture. Breakage or splitting is most likely to occur where the catheter makes sharp changes in direction and especially at the point of attachment to the metal connecting stem of the port.	radiographs. Where this is not the case, injection of a water soluble contrast medium will opacify the catheter *provided* it is still patent and not occluded. Depending upon the circumstances of the case, it may be possible to continue to use an animal with slight malposition of implants or alternatively, the possibility of surgical revision should be carefully evaluated.
Problems puncturing the VAP	A non-coring needle of appropriate diameter and length should quite easily be inserted in one thrust through the skin and septum of the port so that the tip makes contact with the base of the port chamber.	Excessive tissue reaction (fibrosis, surrounding fluid or skin thickening) may make puncture of the port difficult or impossible with the length of needle normally used A longer than normal needle may be required if swelling or other reactions have occurred.	If there is suspicion of infection around the port, there will be a risk of introducing this into the port and catheter lumen during
	With good technique and correct needle in good condition, puncture should cause little or no reaction by the animal.	Pigs may resent puncture if they have had previous bad experience of the procedure! A fresh needle should be used for each puncture to avoid discomfort from damaged needles and also damage to the port septum from damaged needles. • Operator experience and training is important	Ensure animal is well trained and acclimated, suitably restrained. Operators should be suitable trained and experienced. Use new, good quality needles for each puncture - take care to avoid excessive pressure of the needlepoint against the base of metal ports Consider options for local analgesia of overlying skin for puncture (consult

			veterinarian).
Difficulty aspirating lock solution and blood	Following puncture, the lock solution should be aspirated easily and then blood flow obtained.	Has needle fully penetrated the septum with point well into port chamber?	Check needle placement. Consider using longer needle.
		Excessive suction may cause catheter to collapse and/or suck the catheter tip against the vessel wall (or fibrin flap if any are present)	Apply very gently suction while aspirating. Use pulsatile suction.
		Transient positional effects may influence catheter function.	Move the animal slightly (limb positions, head and neck flexion/extension etc) in an attempt to reposition catheter tip within central lumen of vessels.
		Small, soft thrombus in catheter tip	Maintain gentle pulsatile aspiration to detach and aspirate thrombus into catheter lumen - when flow of blood is achieved, aspirate a relatively large volume of blood (5-10ml) to ensure as much small thrombus as possible is aspirated into the syringe. If all attempts fail, consider thrombolysis (see below)
		Fibrin flap at catheter tip	As above.
		Kinking or disruption of catheter	If the catheter is kinked, it will be impossible or difficult to aspirate. The same will apply if the catheter is fractured - especially at the site of attachment to the port

			where it may become disconnected.
		Obstruction due to crystallization from solutions	Test compounds of low solubility may crystallize out of solution in implanted catheters and may give rise to obstruction. Concentrated sugar solutions (sucrose or dextrose) could also do the same.
Initial attempts to aspirate fail	If all attempts to aspirate fail, cautiously attempt to inject a small volume of saline solution	If saline can be injected, this suggests that a fibrin flap may be present acting as a one-way valve or a small soft thrombus at the tip was present. Repeat aspiration attempts after flushing in. If blood can be aspirated, this suggests a thrombus has been displaced or a fibrin flap temporarily displaced.	Pulsatile alternate positive and negative pressure may help to clear a catheter. Where saline has been injected without removal of lock solution, animals should be carefully observed afterwards for signs of adverse event.
Unable to infuse	There should normally be free flow of infusion into a VAP and catheter	•Physical damage/obstruction of VAP /catheter assembly •Extensive fibrin flap (fibrin cuff) Well established thrombus in catheter tip/lumen	Radiographic evaluation (if available) may help with injection of contract medium to opacify fibrin flaps or cuffs. Consider thrombolysis

Appendix 2 - locking solutions for vascular catheters

This list is not exhaustive

0.9% saline	Provides filling of the catheter without exogenous chemical matter. Provides no thrombosis prevention and no antibacterial environment. Cheap and easily obtained.
0.9% saline with heparin (100-500 iu/ml)	The most widely used material, with heparin providing thrombosis prevention. Selected heparin concentration is rather empirical …. For more frequent access (say every one to two days) 100iu/ml is sufficient but for weekly flushing/locking then a more concentrated solution (500iu/ml) is recommended. This solution provides no antibacterial action.
40% dextrose (glucose)	The high osmolarity provides a hostile environment to bacterial colonisation and multiplication. The high osmolarity will also contribute to clot prevention/disruption in a non specific manner - for additional thrombus prevention, addition of heparin (100-500iu/ml) is possible. The viscosity of the solution helps to prevent blood accessing the catheter lumen (and makes the lock solution slightly more difficult to withdraw!) Crystallisation is a risk. The material is naturally occurring in blood and rapidly metabolised. Available cheaply as a pharmaceutical formulation.
50% sucrose (saturated)	As for 40% dextrose - cheap and readily obtained - usually prepared and sterilised in the lab by autoclaving (so risk of contamination/pyrogens!)
Glycerol	High viscosity, high osmotic pressure. Can be difficult to aspirate.
Polyvinylpyrrolidone (PVP)	High viscosity, high osmotic pressure. Can be difficult to aspirate.
Sodium citrate	Cheap and simple antithrombotic where heparin is contraindicated. Heat labile - therefore cannot be sterilised by autoclave. No antibacterial action.
Taurolidine citrate solution 6.7% TauroLock™ TauroLock™-Hep500 TauroLock™-U25.000	Taurolidine is an antibiotic and use of citrate salt provides specific antithrombotic action. Supplied commercially, it is effective and convenient but relatively expensive. Ellegaard recommends TauroLock™-Hep500, catheterized Minipigs are supplied with this lock solution in the catheter at delivery. TauroLock™-U25.000 is helpful if there are thrombi as it contains urokinase.

2. March 2015 AZ/TW