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Background

Pigs share many physiological, anatomical, and metabolic similarities to humans making them a great model. Recently, there has been an increased demand to use miniature pigs as a non-rodent species for pharmacology and toxicology studies as part of pre-clinical drug safety testing. Typically, these tests require more than 8 blood collection time points over a 24 hour period and the same pig may be used to evaluate multiple dose levels or for multiple studies resulting in many blood collections per animal. Additional blood vessel access may also be required for test articles administered intravenously (IV).

Collecting Blood from Pigs Can be Challenging.

- Pigs have few superficial vessels.
- Pigs are intelligent quickly develop aversions to needle puncture.
- Pigs are large so they can be difficult to handle when stressed posing safety risk to animal and staff.

Although there are many solutions available for blood collection and IV dosing in pigs, each of the systems presents its own unique challenge. Below are some pros and cons of some of the more common devices used. This list is not all inclusive, and it does not include peripheral catheter devices.

Device	Pro	Con
VAP	<ul style="list-style-type: none"> • If properly maintained can last for years • Staff can train animals to tolerate access but regular positive reinforcement training needed • Right angle Huber needles can be left in place for short periods of time to allow for repeat access without needle puncture • Catheter-in-catheter VAPs can be used for longer periods 	<ul style="list-style-type: none"> • Access requires skin puncture (painful, risk of skin thinning over VAP site with repeat access) • Good aseptic technique extremely important in order to reduce infection risk. • Pigs can develop aversion to needle puncture even with topical analgesia. • VAPs can be expensive depending on the type and number of VAPs used • Placement of catheter-in-catheter into specialized VAPs can be challenging especially on awake pigs. • Catheter-in-catheter systems require very good placement of catheter to use the system for blood collections. • If Huber needle is left in place or catheter-in-catheter system is used, the insertion site needs to be covered with bandage and/or jacket to protect the insertion site and prevent access device from coming out.
External Jugular Catheter	<ul style="list-style-type: none"> • Easy, pain-free access • Lower cost than VAP • If Seldinger technique mastered (difficult), placement is less surgically invasive • Typically only one surgical site 	<ul style="list-style-type: none"> • Higher risk of infection • Short duration of use (~10-14 days) • Requires neck bandaging to prevent the animal from pulling out/damaging catheter
Direct Needle Puncture	<ul style="list-style-type: none"> • Low risk of infection 	<ul style="list-style-type: none"> • Stressful and potentially painful to pig • Technically difficult and more challenging for staff if not animals not trained, can quickly develop aversions • Limited number of superficial vessels

Modified Rat Vascular Access Buttons in Miniature Pigs

Recently, the use of Rat Vascular Buttons™ (Instech Technologies, Inc.) experimented with as a means for sample collection in Göttingen miniature pigs (*Sus scrofa*). This is a transcutaneous device that permits quick, aseptic access to a catheterized vessel (with up to three channels) without needle puncture of the skin. The PinPorts™ allow access using a syringe fitted with an injector and require minimal site preparation. A cap fits snugly over the top of the button to protect the PinPorts™. The cap on the pig buttons has been modified to have two magnets to keep the cap in place. The design of the device makes vascular access easy (limited site preparation), painless (no needle puncture of the skin), and does not require the animal to have a neck bandage or jacket to protect the device.

Due to the transcutaneous nature of this device, it was assumed that these devices would develop high rates of infection and have a shorter range of use, more similar to an external jugular catheter. However, studies done by Ellegaard (Adrian Zeltner) demonstrated that Rat Vascular Access Buttons™ can be used successfully to sample blood in miniature pigs. In their studies, buttons had a lower rate of infection than expected (25% within 4 weeks of surgery) and 41.7% of catheters remained patent over a three month study period (95% patency in the first month).

Due to the potential benefits of using such a device (reduced pig pain/distress, ease of use, cost, etc.), Frontage Laboratories, Inc. started investigating whether or not Rat Vascular Buttons™ could be a feasible alternative for blood sample collection in miniature pigs on pharmacokinetic and toxicological studies.

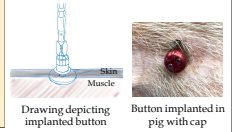


Are Buttons a Good Alternative for Repeat Blood Sample Collection in Miniature Pigs?

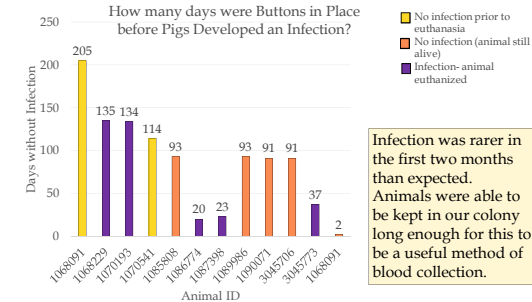
Frontage Laboratories, Inc. is a full service contract research lab that specializes in pre-clinical drug testing. The nature of our work requires that we get multiple blood samples from miniature pigs efficiently and ideally with as little stress to our animals and technicians as possible. Modified Rat Vascular Access Buttons™ seemed to offer the perfect solution. Since the beginning of 2019, we have surgically implanted buttons in 12 male Göttingen miniature pigs with double (N=11) or single (N=1) channel buttons. Double channel buttons had one PinPort™ used for dosing (white) and one for bleeding (red). Below we present the results of our preliminary work.

Questions of Interest:

1. How prevalent is infection when buttons are used in miniature pigs? *We predicted to see a high rate of infection within the first couple of months.*
2. Can buttons be used successfully to collect blood for pharmacokinetic studies? *We predicted that buttons would be able to collect for pharmacokinetic studies successfully.*
3. Does the use of buttons make blood sample collection easier and/or less distressing for the animals and animal technicians? *We predicted that pigs would be less distressed by the blood collection and technicians would appreciate the ease of access.*



How prevalent is infection in miniature pigs with buttons?



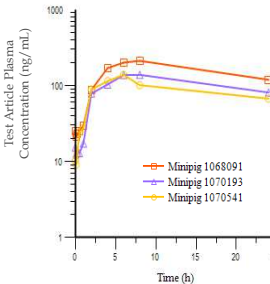
Infection was rarer in the first two months than expected. Animals were able to be kept in our colony long enough for this to be a useful method of blood collection.

Five out of twelve pigs have developed button site infections (purple), but two of these animals only developed infections after over 130 days. The three animals that developed an infection within 40 days of surgery had surgery performed within a 10 day period of each other. At this time, we do not know the reason these 3 animals developed infections sooner than the other animals. Animals were terminated soon after infection was identified. Two animals were terminated prior to developing infection due to limitations of use for the animals (yellow). The remaining five animals are still alive in the colony without any signs of infection (orange).

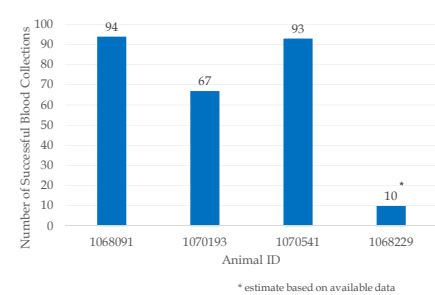
Can buttons be used to successfully collect blood for pharmacokinetic studies in pigs?

Four of the animals with buttons have been used on multiple pharmacokinetic (PK) studies. Button ports provided adequate blood sample collection volumes needed (1-2 mL) quickly and samples yielded good pharmacokinetic data (left). Only one out of the four animals had their port stop being functional for blood collection (but still flushed) shortly after start of use. The buttons on the rest of the animals remained functional for over 60 blood collection time points (right). Animal 1068091 port was still functional for blood collection at the time of euthanasia.

Example of Pharmacokinetic Data Collected using Pig Buttons



Number of Blood Collections performed on each Miniature Pig Using Buttons

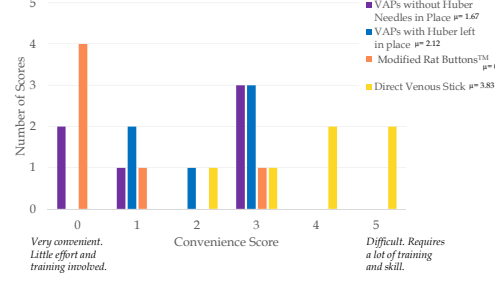


Many blood samples of a sufficient volume were able to be collected from buttons. Blood samples collected using buttons for PK data yielded good results.

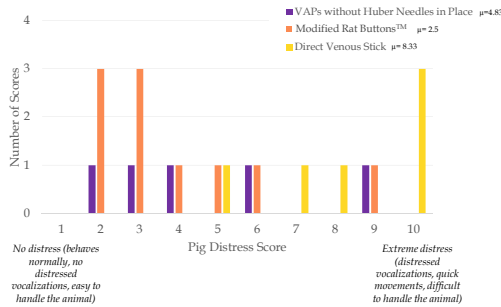
Does the use of buttons make blood sample collection easier/less distressing for the animals and technicians?

Technicians started verbally requesting that the Frontage surgical team preferentially implant buttons shortly after the first button was placed. Technicians stated that buttons were easier use because the pigs reacted less negatively. Accessing the buttons required fewer materials and less site preparation. To confirm this feedback, technicians working with pigs were asked to fill out an online survey on their experiences bleeding miniature pigs using the different devices. Technicians were asked to not respond to questions if they were unable to answer the question based on their experiences. Six animal technicians responded to the survey. This survey only provides a subjective assessment of pig responses to the use of buttons that was based on past experiences with the different types of devices. Future work will involve a more in depth behavioral and physiological measurement pig response to button use compared to other devices.

How Convenient is it for you to collect blood using each type of device on a scale of 0-5?



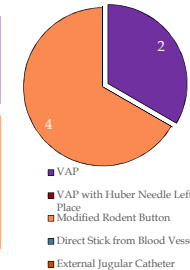
How distressed do miniature pigs seem when you are collecting blood using each device on a scale of 1-10?



Which method of blood collection do you prefer for miniature pigs?

VAPs: "Why do you prefer this method?"
-Easiest, most reliable method
-Most efficient

Buttons: "Why do you prefer this method?"
- Ease of Access (no needle puncture, less aseptic preparation needed)
-Works reliably



Most technicians prefer to use buttons over other blood collection methods. Most technicians perceive pigs to be less stressed during blood collections when buttons are used.

Conclusions and Future Work

Rat Vascular Access Buttons™ provide a good alternative to other devices used to collect repeat blood samples in miniature pigs. Our results have provided sufficient evidence that buttons remain infection-free for long enough to meet study objectives, that blood samples obtained from buttons provide good PK data, that most technicians prefer buttons over other devices, and that subjectively pigs appear to be less distressed when using buttons compared to other devices.

Buttons have a similar cost and ease of use to jugular catheters, but they have the added benefit of lasting longer and not requiring additional bandaging to protect the device. Buttons may not be able to last as long as vascular access ports (although we were not able to access catheters beyond 205 days), but they do provide a much easier site preparation and potentially less painful or distressing vascular access than VAPs based on technician feedback.

Future work will include a histological evaluation of the button insertion site of infected and non-infected pigs as well as a histological evaluation of the tissues near the end of the catheters. We also hope to perform some more in-depth objective investigations to miniature pig responses to the use of these devices. Finally, we plan to further refine our techniques to continue to reduce infection rates and improve functionality of Rodent Vascular Access Buttons in miniature pigs.

Acknowledgements

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