

# Central Venous Catheterisation of the Gottingen Minipig by Jugular Vein Placement

## An Investigation of Potential Utility in Regulatory Safety Evaluation Studies.

Over recent years, the use of the minipig for non-clinical toxicology studies has expanded due to the model's anatomical, physiological and biochemical dispositions that allow extrapolation to man. This has resulted in the minipig becoming a viable non-rodent alternative that is accepted by regulatory authorities. Furthermore, as a wealth of background data accumulates and ethical concerns continue to be raised by the public over the use of other non-rodent species such as the dog, it seems there are very few factors that limit the minipigs' potential to become the primary non-rodent option.

One drawback of minipig use for non-clinical toxicology studies relates to the obtaining of serial blood samples. Most commonly, blood samples have been obtained from the cranial vena cava due to the veins easy access and the ability to obtain large blood samples on a single occasion. However, with the complexity of non-clinical studies increasing, the need for more extensive blood sampling is apparent.

Serial sampling from the vena cava over a short period of time can result in stress in the minipig, leading to animal welfare concerns and potential non-test item related mortality. This has required the development of surgically based blood sampling approaches such as installation of vascular access ports and, whilst this approach is certainly appropriate for some study types it is not ideal for use on routine, regulatory toxicity studies. A positive advancement in this area appears to be the previously described method for minimally invasive insertion of a central venous catheter into the external jugular vein, allowing the facilitation of multiple blood samples.

At Sequani, we have investigated the placement of a catheter into the external jugular vein. A small discussion on study design is detailed; however, with extensive technical details available in Ellegaard's catheter implantation educational package, the aim of this article is to share our first-hand experience with the technique and its potential use for future regulatory studies.



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## Study Design

The primary objective was to investigate the practicality of central venous catheterisation of the jugular vein, for potential use in regulatory non-clinical studies. Six minipigs were allocated between 3 groups with the intention to investigate insertion of a catheter for periods of time that would be useful in obtaining the toxicokinetic samples required in a 1 month regulatory study.

On all occasions, catheters (Arrow Gauge 14) were inserted using a Seldinger technique with the animal under anesthesia. The catheter was removed on the subsequent day following serial blood sampling for two groups and remained in-situ for an additional group to assess patency. Patency was assessed by visible signs of infection at the insertion site and leucocyte parameters analysed from the blood samples obtained. On the final occasion of catheterisation, a cut-down technique was employed for visualisation of the veins during the procedure. Blood sample quality and the ability to reinsert the catheter were also assessed.



## Discussion

### Catheterisation practicability and success

Insertion of a catheter into the jugular vein proved to be more successful as the technicians gained first-hand experience and became accustomed to the procedure.

In total, 17 catheterisations were attempted (excluding occasions where a cut-down method was applied), 11 were successful resulting in a catheterisation rate of 65%. The low success rate was a result of the first three catheterisations where none were successful. Excluding these occasions, where procedural experience was low, catheterisation rate improved to 79%.

The burden of the remaining unsuccessful catheterisations was on two animals where the first catheterisation attempt was unsuccessful and future attempts were made within a 7 day period. Surrounding vessels had been punctured causing swelling at the injection site, and insufficient recovery time was the likely cause of these unsuccessful attempts. It was found that animals could be successfully catheterised following a 14 day recovery period.

Experience with catheter insertion was associated with a clear reduction in the time taken to conduct each procedure; with later catheterisations being performed around 60% quicker than the initial ones.

### Patency and Re-insertion of catheters.

Patency was demonstrated for a period up to 22 days with no changes in related blood parameters or signs of infection. Longer patency is possible but could not be demonstrated further in this study due to one catheter becoming dislodged and another being damaged requiring replacement.

Self-inflicted removal of the catheter by one minipig resulted in no injury to the animal and future removal was prevented by adaptation of a neck collar to secure the catheter. This unintended removal allowed us to demonstrate the ability to reinsert the catheter on the day or day after removal. Reinsertion potential was also demonstrated on fortnightly occasions over a one-month period.



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## Discussion

### Blood sample quality

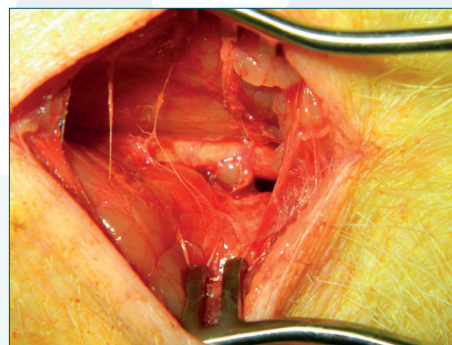
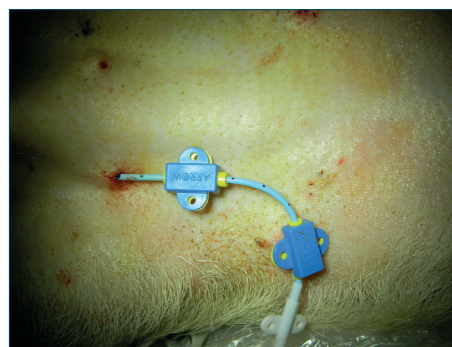
Blood sample quality was unaffected, with haematology and blood chemistry parameters comparable to background data collated from other collection routes. A number of dubious values were seen for standard coagulation parameters; however, this was attributed to heparin contamination from incomplete flushing of lines. This was resolved by more thorough flushing prior to blood sample collection and resulted in coagulation parameters within the expected range.

### Cut down method

On all occasions where the cut-down method was incorporated, insertion of the catheter was successful. Procedural times were longer than those where only the Seldinger technique was used; however, visual access of the vein resulted in easier catheterisation and allowed the insertion of a catheter into both the left and right veins simultaneously.

### Anaesthetic regime

Minipigs were anaesthetized using a regime of injectable anaesthetics/analgesics that comprised of, ketamine, medetomidine hydrochloride and butorphanol, in addition to isoflurane through a nose cone. The amounts of injectables were reduced on each catheterisation occasion in order to investigate the minimum required for a regulatory non-clinical study without compromising the state of anesthesia. Initially, vocalisation and agitation were observed for one animal on the administration of ketamine; this was subsequently removed from the regime with no adverse effects on the state of anesthesia. The reductions in the remaining injectable anaesthetics resulted in a shortening of recovery time following the procedure and subsequently resulted in less agitation for the animals as consciousness was obtained quicker.



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## Conclusion

Central venous catheterisation of the jugular vein looks a promising alternative for serial blood sampling in toxicology studies in the minipig. With experience, the insertion of a catheter is relatively quick and subsequently results in a reduction of manpower required for blood sampling. More importantly, a large reduction in stress was apparent resulting in an improvement in the welfare of the animal. Blood samples were obtained with ease on all occasions with the offering of a food treat removing the requirement of a sling or excessive handling.

These preliminary investigations suggest that the technique could be used on regulatory toxicology studies incorporating toxicokinetic bleeds that require serial blood samples. There are some issues around the amount of time that is needed to conduct the procedure on a study that may include 32 or more animals. Nevertheless, with well established procedures in place this could easily balance the work involved in repeated blood sampling using the traditional method. At present it would seem appropriate to insert the catheters for short periods only to cover each serial blood sampling session (usually at the beginning and end of the study). However, further investigative work into line patency could open the option to keep the catheters in place for a full 1 month regulatory study.

The appeal of this technique would be further enhanced by an adaptation of the anesthetic regime to enable administration of isoflurane through a nose cone only, without any injectable anaesthesia. This requires further investigation but could substantially accelerate the recovery time.

In conclusion, we intend to further develop and enhance the procedure before introduction to a regulatory study; however the progress made in this initial investigation will enable us to conduct further smaller scale studies using the technique and thereby develop proficiency and process efficiency to a suitable level. It should be noted that where less extensive blood sampling is required, bleeding from the cranial vena cava using the traditional method would still be considered most appropriate.

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